PETITORIO

Industria y Comercio
SUPERINTENDENCIA

02 102451 00

SUPERINDUSTRIA Y COMERCIO
Radicacion : 02102451 00000000 Folios: 480
Fecha (AMD): 2002-11-12 17:29:16
Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC cación
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL

(1) SOLICITUD DE :

[X]	Patente de Invención	[ ]	Patente de Modelo de Utilidad
[ ]	Capítulo I.	[X]	Capítulo II.

(2) SOLICITANTE (71)

Nombre: PFIZER INC.

Dirección: 235 East 42nd Street, New York, NewYork 10017, Estados Unidos de América.

Nacionalidad o Domicilio: ESTADOUNIDENSE

Lugar de Constitución: New York, Estados Unidos de América.

Teléfono: Fax:

E-Mail

IDENTIFICACION

C.C. [ ] NIT [ ]

C.E. [ ] Otro [ ]

Cual

Número

(3) REPRESENTANTE O APODERADO

Nombre: CARLOS R. OLARTE

Dirección: Avda 82 No. 10-62 Piso 6

Teléfono: 634-1500 Fax: 376-2211

E-Mail: patents.bogota@bakernet.com

IDENTIFICACION

C.C. [X] NIT [ ]

C.E. [ ] Otro [ ]

Cual

Número 79.782.747 Btá TP 74.295

(4) INVENTOR (ES) (72)

Nombre: Christopher Gordon Barber, Andrew Simon Cook, Graham Nigel Maw, David Cameron Pryde, Alan Stobie.

Dirección: Ramsgate Road, Sandwich Kent, CT13 9NJ, Reino Unido.

Nacionalidad o Domicilio: Reino Unido

(5) PCT (85)

Solicitud Internacional No. PCT/IB01/01205 Fecha Julio 2, 2001

Publicación Internacional No. WO 02/02513 Fecha Enero 10, 2002

(6) Título (54) DERIVADOS DE CICLOPENTIL-GLUTARAMIDA COMO INHIBIDORES DE ENDOPEPTIDASA NEUTRA.

(7) Clasificación Internacional (51) C07C 237/22 // A61K 31/19

(8) Prioridad

SI [X] NO [ ]

(33) País de Origen	(32) Fecha	(31) Número de Solicitud
UK	JuLio 6, 2000	0016684.3
UK	Enero 22, 2001	0101584.1

(9) Comprobante de pago No. 83,154 **Fecha** 23-09-02

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página

(10) ANEXOS

- [X] Comprobante de pago de la tasa de presentación de la solicitud
- [ ] Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica
- [X] Poderes, si fuere el caso
- [X] Documento de cesión del inventor al solicitante o a su causante
- [ ] Declaraciones
- [X] Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- [X] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- [X] Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen preliminar (IPEA)
- [ ] Traducción del examen preliminar internacional efectuado por la IPEA
- [ ] De ser el caso, copia del contrato de acceso
- [ ] De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas
- [ ] De ser el caso, certificado de depósito del material biológico
- [ ] Arte final 12 x 12
- [ ] De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud
- [ ] De ser el caso, modificaciones efectuadas a la solicitud internacional.
- [X] Otros: Búsqueda Internacional, Documentos de Prioridad junto con sus traducciones.

(11) FIGURA CARATERISTICA

(12) Solicito la concesión de la patente,

NOMBRE: CARLOS R. OLARTE

FIRMA:

C.C. 79.782.747 de Btá T.P. 74295

Instrucciones para la presentación de los anexos, ver reverso de esta página

3

Industria y Comercio

SUPERINTENDENCIA

NIT : 800176089-2

SUPERINDUSTRIA Y COMERCIO
Radicacion : 02102451 00000000 Folios: 480
Fecha (AMD): 2002-11-12 17:29:16
Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 02 - 63,154
FECHA : SEPTIEMBRE 23 DE 2002

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
JULIAN ANDRES VARGAS	CONSIGNACION	BANCO POPULAR	050-00110-6	3135146	23/09/2002	38,825,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO		TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1	TRAMITES DE SOL. DE PATENTE DE IN	378,000.00
			TOTAL : $	378,000.00

SON: TRESCIENTOS SETENTA Y OCHO MIL PESOS

RESPONSABLE : _____

REC[redacted]E CAJA APLICADO AL EXPEDIENTE No._____

Carrera 13 No. 27-00 Pisos 5, 7 y 10
Conmutador 382 0840
Fax 350 5220
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

Traducción oficial del inglés por Marlén Neira Castro, resolución 2117 del Ministerio de Justicia de Colombia.

PODER DE PFIZER INC.

CONSULADO DE COLOMBIA EN LONDRES

Fechado el 22 enero, 2001 certifica que Kenneth Greenwood, ejerce funciones de notario de Egham, Surrey Inglaterra, cuya firma fue reconocida por J.M.A. Mason, de Legalizaciones del Ministerio de Relaciones de Gran Bretaña; Que J. Trevor Lumb es el asesor de patentes de Pfizer Inc. sociedad establecida en Nueva York, Estados Unidos. Firma del cónsul: Jairo Berrio.

CERTIFICACION NOTARIAL

El 8 de enero del 2001 el Notario Público da fe:

1.compareció J. Trevor Lumb, domiciliado en Estados Unidos, quien suscribió el anterior documento. 2. Conozco al otorgante y tiene capacidad para el otorgamiento 3. ha suscrito el documento en su carácter de Asesor General Asesor de Patentes de Pfizer Inc, con sede en Nueva York, NY, 4. compañía legalmente constituida, con existencia legal actual y que el acto para el cual se otorgó el poder está dentro del objeto social. 5. El otorgante tiene la representación que ostenta y es legítima. 6. Baso las certificaciones 3, 4 y 5 en estos documentos.

Sello:

Certificado, verificado y autenticado en la localidad de Egham en Surrey el 8 enero, 2001, Kenneth Greenwood - Notario Público Horne Engall & Freedam- sello notarial en alto relieve.

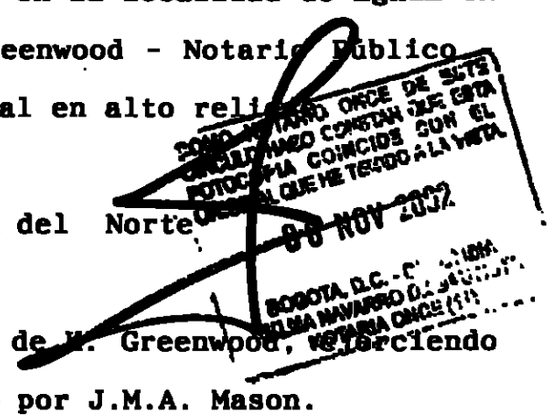
Al reverso:

Reino Unido de Gran Bretaña e Irlanda del Norte

No. D653465 - Fecha: 8 enero, 2001

Este documento lleva la firma/sello de K. Greenwood, ejerciendo funciones de Notario Público, firmado por J.M.A. Mason.

Por el Secretario de Estado Principal de su Majestad para Asuntos exteriores y de la Mancomunidad. Sello redondo: Londres.



Marlen Neira C

MARLEN NEIRA CASTRO
Traductor e Intérprete Oficial
Inglés - Español - Inglés
Resolución No. 2117 del 7 Oct 1987

5

PODER DE REPRESENTACIÓN

Poder de representación otorgado por PFIZER INC.,compañía domiciliada en el número 235 Este Calle 42 Nueva York, Estado de Nueva York, Estados Unidos, a Carlos Reinaldo Olarte.

Dado y firmado en Nueva York, el 2 de enero de 2001 por:

Trevor Lumb

Asesor General Consejero Patentes.

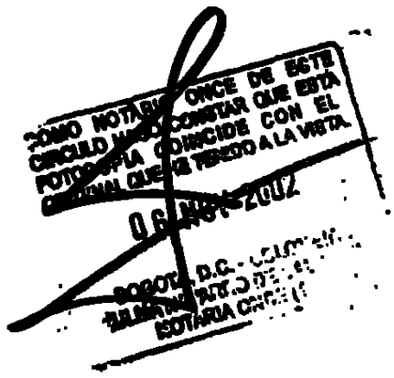
Sello en español:

Ministerio de Relaciones Exteriores No.407609_ certifica las funciones del J. Berrio a la fecha. Bogotá, febrero 2, 2001. Firma el Jefe de Legalizaciones.

--

Traducción certificada, con copia en archivo para confrontación, fechada el 6- 2-2001.

MARLEN NEIRA CASTRO
Traductor e Intérprete Oficial
Inglés - Español - Inglés
Resolución No. 2117 del 7 Oct 1987



MINISTERIO DE RELACIONES EXTERIORES

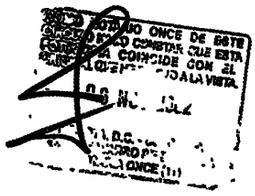
2 1 7 0 1 1

CUYA FIRMA APARECE EN EL DOCUMENTO DESEMPEÑA LAS FUNCIONES INDICADAS

EL SE ASUME RESPONSABILIDAD DEL TEXTO

1994 FEB 10 P 1:04

JEFE DE LEGALIZACIONES SANTAFE DE BOGOTA D.C.



CONSULADO GENERAL DE COLOMBIA

3RD FLOOR 15-19 GREAT TITCHFIELD STREET LONDON W1W 8HZ
TELEPHONE: 020 7637 9893 TELEFAX: 020 7637 5604
E-MAIL:CONSULCO@CONSULCO.DEMON.CO.UK

6

El suscrito Cónsul General de Colombia en Londres, vistos los documentos presentados ante este despacho,

CERTIFICA:

Que el Señor **KENNETH GREENWOOD**, quien autenticó el documento precedente, ejercía en la fecha las funciones de Notario Público de Egham, Surrey, Inglaterra, y que su firma fue debidamente reconocida por **J. M. A. MASON**, Funcionario del Departamento de Legalizaciones del Ministerio de Relaciones de la Gran Bretaña, cuya firma se encuentra registrada en este Consulado General.

Que **PFIZER INC.** es una Sociedad debidamente constituída, organizada y existente en New York, N.Y., Estados Unidos de América, y que cumple con su objeto social de acuerdo con las leyes de ese país.

Que el Señor **J. TREVOR LUMB**, en su calidad de Asesor de Patentes Generales de la mencionada Sociedad, otorga el Poder precedente y su firma ha sido autenticada por el Notario **KENNETH GREENWOOD**.

El Cónsul General no asume responsabilidad por el mencionado documento.

Dado en Londres, Reino Unido de la Gran Bretaña, a los veintidos (22) días del mes de enero del año dos mil uno (2001).

Certificación No. 01/0181
Derechos: US$106.00, CANCELADOS.

JAIRO E. BERRIO VILLARREAL
Cónsul General

NOTARIO ONCE DE ESTE CIRCULO HACE CONSTAR QUE ESTA FOTOCOPIA COINCIDE CON EL QUE HE TENIDO A LA VISTA
08 NOV 2002
BOGOTA, D.C.
NOTARIA ONCE (11)

MINISTERIO DE RELACIONES EXTERIORES

407609

CUYA FIRMA APARECE EN EL DOCUMENTO DESEMPEÑA LAS FUNCIONES JURIDICAS

NO SE ASUME RESPONSABILIDAD DEL TEXTO

2001 FEB -2 P 3:11

JEFE DE LEGALIZACIONES SANTAFE DE BOGOTA D.C



RAISBECK, LARA, RODRIGUEZ & RUEDA
Members of the Firm
BAKER & McKENZIE
ATTORNEYS AT LAW
APARTADO AEREO 3748
TELEFONO: 288-1488
SANTAFE DE BOGOTA, D.C.
COLOMBIA

7

NOTARIAL CERTIFICATION

On this 8th day of January 19. 2001, the undersigned Notary

CERTIFIES

1. That J. Trevor Lumb

of legal age and domiciled in U.S.A.

set his hand on the foregoing document.

2. That the grantor is to me personally known and that he/she has legal capacity to execute the instrument.

3. That the grantor has executed the foregoing document as

Assistant General Patent Counsel

of the juridical person

Pfizer Inc

4. That the juridical person

Pfizer Inc

having its home office in

New York, NY.

is duly organized, has present legal existence and that the purposes for which the power of attorney is granted are within the scope of its corporate purposes.

5. That the grantor has in fact he referred to authority and that his/her representation is legal.

6. That I have based certifications 3, 4 and 5 above on the following authentic documents for such purpose exhibited to me and which I mention specifically, giving their dates and their origin or antecedents:

Certified Verified And Attested
In The Town Of Egham In Surrey
This 8th Day Of January 2001
KENNETH GREENWOOD
NOTARY PUBLIC
HORNE ENGLAND FREEMAN

Notary Public

CERTIFICACION NOTARIAL

A los días del mes de de 19 ,el suscrito Notario

DA FE

1. Que

mayor y domiciliado en

suscribio de su puño y letra el documento que antecede.

2. Que conozco al otorgante y que éste tiene capacidad legal para el otorgamiento.

3. Que el otorgante ha suscrito el referido documento en su carácter de

de la persona jurídica

4. Que la persona jurídica

con sede en

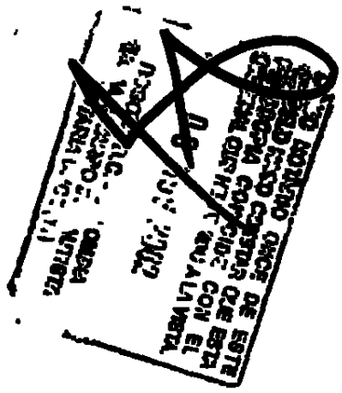
está legalmente constituida según la existencia legal actual y que el acto para el cual se ha otorgado el poder está comprendido entre los que constituyen su objeto social.

NOV 2002

5. Que el otorgante tiene efectivamente la representación que ostenta y que esta es legítima.

6. Que he basado las certificaciones 3, 4 y 5 anteriores en los siguientes documentos auténticos que al efecto se me exhibieron y que menciono especificamente con expresión de sus fechas y de su origen o procedencia:

El Notario Público



UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

No **D653465**

Date **8 January, 2001**

This document bears
the signature / seal of **K Greenwood**

Acting in the capacity of **Notary Public**

Signed by **J. M A. Mason**



For Her Majesty's Principal Secretary of State
For Foreign and Commonwealth Affairs

RAISBECK, LARA, RODRIGUEZ & RUEDA
Members of the Firm
BAKER & McKENZIE
ATTORNEYS AT LAW
APARTADO AEREO 3746
TELEFONO: 286-1466
SANTAFE DE BOGOTA, D.C.
COLOMBIA

8

POWER OF ATTORNEY

The undersigned.

PFIZER INC.

located in
235 East 42nd Street, New York
Estado de New York, Estados Unidos de América
declares that it hereby grants to

CARLOS R. OLARTE

a full and sufficient power of attorney to apply to the proper national officers and authorities for the issue of:

Letters Patent, mergers, changes of name, assignments, filing and prosecution of oppositions, changes of address

to which end it empowers them to take all steps necessary before said authorities for the objects stated, to file petitions, prepare descriptions, make protests, declarations, appeals and objections, pay imposts, apply for certifications, receive documents and securities, abandon applications and collect refunds. To the said mandatories sufficient power is granted hereby to receive notifications for and on behalf of the Company, to confer powers on those who are to represent the company judicially and extrajudicially, to answer in court in the matter of all claims and demands that may be presented in connection with the patents, and to do everything that may be required, before administrative or judicial officers of any class giving them likewise power to substitute these presents and revoke such substitutions if necessary.

Done and subscribed in New York, New York, United States of America.

On the 2nd day of January 2001

J. Trevor Lumb
Assistant General Patent Counsel

PODER

La abajo firmada.

PFIZER INC.

domiciliada en
235 East 42nd Street, New York
Estado de New York, Estados Unidos de América
declara por el presente otorgar a

CARLOS R. OLARTE

poder especial amplio y bastante para recabar de las oficinas y autoridades nacionales que correspondan, la obtención de :

Solicitudes de Patentes de Invención, cambios de nombre, cesiones, presentación y tramitación de oposiciones, cambios de nombre

a cuyo efecto los faculta para dar ante dichas autoridades, todos los pasos necesarios al objeto indicado, elevar solicitudes, formular descripciones , protestas, declaraciones, apelaciones y reclamos, oblar impuestos, solicitar testimonios, recibir documentos y valores, desistir y percibir. Se concede a los expresados mandatarios poder bastante para recibir notificaciones a nombre de la compañía, otorgar poderes a quienes vayan a representar a la compañía judicial y extrajudicialmente, responder en juicio a todas las reclamaciones o demandas que por motivo de las patentes se presentaren y hacer cuanto fuere necesario, ante las autoridades administrativas o judiciales de cualquier orden, dándoles asimismo facultad para sustituir el presente y en caso necesario revocar dichas sustituciones.

Dado y firmado en

A los días del

NOTARIO ONCE DE ESTE CIRCULO HACE CONSTAR QUE ESTA FOTOCOPIA COINCIDE CON EL QUE HA TENIDO A LA VISTA.
06 NOV 2002
BOGOTA, D.C. - COLOMBIA
NOTARIA ONCE (11)

4203

9

TRADUCCIÓN OFICIAL No. 440/02 de un documento escrito en inglés, el cual para su identificación se sella con el sello del Traductor. Esta es una Traducción Oficial efectuada el día 12 de noviembre de 2002 por la Sra. María Teresa Lara, c.c. 41.568.055 de Bogotá, Traductora Oficial según Resolución No. 01016 del 2 de octubre de 1998, expedida por el Ministerio de Justicia.

= =

Traspaso de invento a la compañía PFIZER LIMITED. Certificación notarial suscrita por Kenneth Greenwood, Notario Público de Egham, Surrey, Reino Unido, y legalizaciones subsiguientes.

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINJUSTICIA

PCS10910ARCS

CO-1

TRASPASO

Por contraprestación valiosa pagada a nosotros por **PFIZER LIMITED**, sociedad constituida en virtud de las leyes de **Inglaterra**, cuya dirección postal completa es **Ramsgate Road, Sandwich, Kent, CT13 9NJ, Reino Unido**, por el presente vendemos y traspasamos a la citada **PFIZER LIMITED** todo nuestro derecho, título e interés en y sobre nuestro invento de una nueva y útil Mejora en **DERIVADOS DE LA GLUTARAMIDA CON CICLOPENTIL SUBSTITUID UTILIZADOS COMO INHIBIDORES DE LA ENDOPEPTIDASA NEUTRA**, en cuanto hace a la República de Colombia, tal como se indica y describe plenamente en la especificación, y por el presente autorizamos y solicitamos al Comisionado de Patentes de Colombia expedir dicha patente a nombre de la citada **PFIZER LIMITED** de conformidad con este traspaso.

En fe de lo cual, hemos firmado el presente.

10

(Fdo.) Christopher Gordon BARBER
Sandwich, Kent, Inglaterra Mayo 2001 – Christopher Gordon BARBER

(Fdo.) Andrew Simon COOK
Sandwich, Kent, Inglaterra Mayo 2001 – Andrew Simon COOK

(Fdo.) Graham Nigel MAW
Sandwich, Kent, Inglaterra Mayo 2001 – Graham Nigel MAW

(Fdo.) David Cameron PRYDE
Sandwich, Kent, Inglaterra Mayo 2001 – David Cameron PRYDE

(Fdo.) Alan STOBIE
Sandwich, Kent, Inglaterra Mayo 2001 – Alan STOBIE

"RIA TERESA LAIA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01618
DE MINJUSTICIA

Certificado, verificado y autenticado
en la ciudad de Egham en Surrey

A los 31 días del mes de mayo de 2001

(Fdo.) Kenneth Greenwood

KENNETH GREENWOOD
NOTARIO PÚBLICO
HORNE ENGALL AND FREEMAN

(Adherido, sello notarial)

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. País: Reino Unido de la Gran Bretaña e Irlanda del Norte

   Este documento público

2. ha sido firmado por K Greenwood

3. actuando en calidad de Notario Público

4. lleva el sello/timbre de el antedicho Notario Público

11

Certificado

5. en Londres

6. el 31 de mayo de 2001

7. por el Secretario de Estado Principal de Su Majestad para Asuntos Exteriores y de la Commonwealth

8. No. **G899963**

9. Sello/timbre

(Sello de la Oficina de Asuntos Exteriores y de la Commonwealth)

10. Firma

(Fdo.) **Y.P. Precieux**

Por el Secretario de Estado

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINJUSTICIA

= =

La anterior es traducción fiel y completa del documento en referencia, de la cual se deja copia en este archivo para su confrontación.

María Teresa Lara R.
c.c. 41.568.055 de Bogotá
Traductora Oficial según
Resolución No. 01016 del
2 de octubre de 1998, del
Ministerio de Justicia

ASSIGNMENT

For valuable consideration paid to me (us) by **PFIZER LIMITED** a corporation organized under the laws of **England** whose full post office address is **Ramsgate Road, Sandwich, Kent, CT13 9NJ, United Kingdom,** I (we) do hereby sell and assign to the said **PFIZER LIMITED,** all my (our) right, title and interest in and to my (our) invention for a new and useful Improvement in **CYCLOPENTYL-SUBSTITUTED GLUTARAMIDE DERIVATIVES AS INHIBITORS OF NEUTRAL ENDOPEPTIDASE** so far as the Republic of Colombia is concerned, as fully set forth and described in the specification, and I (we) do hereby authorize and request the Commissioner of Patents of Colombia to issue said patent to the said **PFIZER LIMITED** in accordance with this assignment.

In witness whereof I (we) have hereunto set my (our) hand(s) this

Christopher Gordon Barber

Sandwich, Kent, England 29 May 2001 - Christopher Gordon BARBER

Andrew Simon Cook

Sandwich, Kent, England 29 May 2001 - Andrew Simon COOK

Graham Nigel Maw

Sandwich, Kent, England 29 May 2001 - Graham Nigel MAW

David Cameron Pryde

Sandwich, Kent, England 29 May 2001 - David Cameron PRYDE

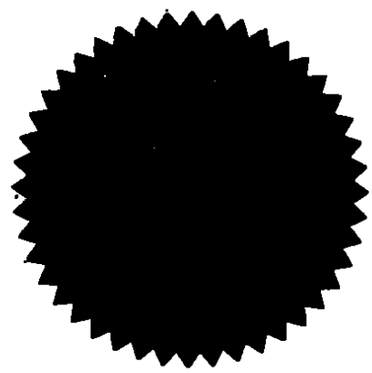
Alan Stobie

Sandwich, Kent, England 29 May 2001 - Alan STOBIE

Certified Verified and Authenticated
In The Town Of Egham In Surrey

This 31st Day Of May 2001

KENNETH GREENWOOD
NOTARY PUBLIC
HORNE ENGALL AND FREEMAN



13

APOSTILLE

(Hague Convention of 5 October 1961 / Convention de La Haye du 5 octobre 1961)

1. Country: United Kingdom of Great Britain and Northern Ireland
Pays: Royaume-Uni de Grande-Bretagne et d'Irlande du Nord

This public document / Le présent acte public

2. Has been signed by **K Greenwood**
a été signé par

3. Acting in the capacity of **Notary Public**
agissant en qualité de

4. Bears the seal/stamp of **The Said Notary Public**
est revêtu du sceau/timbre de

Certified/Attesté

5. at London/à Londres

6. the/le **31 May 2001**

7. by Her Majesty's Principal Secretary of State for Foreign and Commonwealth Affairs /
par le Secrétaire d'Etat Principal de Sa Majesté aux Affaires Etrangères et du Commonwealth.

8. Number/sous No **G899963**

9. Stamp:
timbre:



10. Signature: **Y. P. Precleux**

For the Secretary of State / Pour le Secrétaire d'Etat

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01018
DE MINJUSTICIA

440/02

TRADUCCIÓN OFICIAL No. 441/02 de un documento escrito en inglés, el cual para su identificación se sella con el sello del Traductor. Esta es una Traducción Oficial efectuada el día 12 de noviembre de 2002 por la Sra. María Teresa Lara, c.c. 41.568.055 de Bogotá, Traductora Oficial según Resolución No. 01016 del 2 de octubre de 1998, expedida por el Ministerio de Justicia.

= =

Traspaso de invento a la compañía PFIZER INC. Certificación notarial suscrita por Kenneth Greenwood, Notario Público de Egham, Surrey, Reino Unido, y legalizaciones subsiguientes.

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINJUSTICIA

PCS10910ARCS

CO-1X

TRASPASO

Por contraprestación valiosa pagada a nosotros por **PFIZER INC., sociedad constituida en virtud de las leyes del estado de Delaware, Estados Unidos de América, de 235 East 42nd Street, Nueva York, Nueva York 10017, Estados Unidos de América**, por el presente vendemos y traspasamos a la citada **PFIZER INC.** todo nuestro derecho, título e interés en y sobre nuestro invento de una nueva y útil Mejora en **DERIVADOS DE LA GLUTARAMIDA CON CICLOPENTIL SUBSTITUIDO UTILIZADOS C M INHIBIDORES DE LA ENDOPEPTIDASA NEUTRA**, en cuanto hace a la República de Colombia, tal como se indica y describe plenamente en la especificación, y por el presente autorizamos y solicitamos al Comisionado de Patentes de Colombia expedir dicha patente a nombre de la citada **PFIZER INC.** de conformidad con este traspaso.

En fe de lo cual, hemos firmado el presente.

15

PFIZER LIMITED

(Fdo.) D. J. Wood

Sandwich, Kent, Inglaterra 29 Mayo 2001 - **DAVID J. WOOD, DIRECT R, DEPARTAMENTO DE PATENTES EUROPEAS**

Certificado, verificado y autenticado
en la ciudad de Egham en Surrey

A los 31 días del mes de mayo de 2001

(Fdo.) Kenneth Greenwood

KENNETH GREENWOOD
NOTARIO PÚBLICO
HORNE ENGALL AND FREEMAN

(Adherido, sello notarial)

MARIA TERESA LABA R.
TRADUCTORA OFICIAL
RESOLUCION No. 0101
DE MINJUSTICIA

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. País: Reino Unido de la Gran Bretaña e Irlanda del Norte

   Este documento público

2. ha sido firmado por K Greenwood

3. actuando en calidad de Notario Público

4. lleva el sello/timbre de el antedicho Notario Público

Certificado

5. en Londres 6. el 31 de mayo de 2001

16

7. por el Secretario de Estado Principal de Su Majestad para Asuntos Exteriores y de la Commonwealth

8. No. **G899961**

9. Sello/timbre

(Sello de la Oficina de Asuntos Exteriores y de la Commonwealth)

10. Firma

(Fdo.) **Y.P. Precieux**

Por el Secretario de Estado

= =

La anterior es traducción fiel y completa del documento en referencia, de la cual se deja copia en este archivo para su confrontación.

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINJUSTICIA

María Teresa Lara R.
c.c. 41.568.055 de Bogotá
Traductora Oficial según
Resolución No. 01016 del
2 de octubre de 1998, del
Ministerio de Justicia

PCS10910ARCS

CO-1X

17

ASSIGNMENT

For valuable consideration paid to me (us) by **PFIZER INC., a corporation organized under the laws of the State of Delaware, United States of America of 235 East 42nd Street, New York, New York 10017, United States of America**, I (we) do hereby sell and assign to the said **PFIZER INC.**, all my (our) right, title and interest in and to my (our) invention for a new and useful Improvement in

CYCLOPENTYL-SUBSTITUTED GLUTARAMIDE DERIVATIVES AS INHIBITORS OF NEUTRAL ENDOPEPTIDASE

so far as the Republic of Colombia is concerned, as fully set forth and described in the specification, and I (we) do hereby authorize and request the Commissioner of Patents of Colombia to issue said patent to the said **PFIZER INC.** in accordance with this assignment.

In witness whereof I (we) have hereunto set my (our) hand(s) this

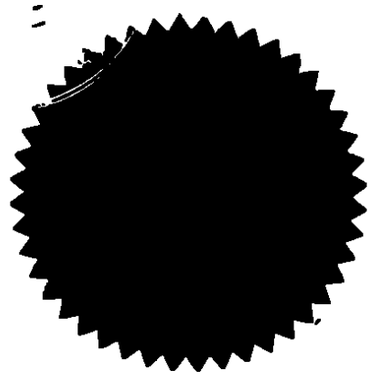
PFIZER LIMITED

D.J. Wood

Sandwich, Kent, England 29 May 2001 - **DAVID J. WOOD, DIRECTOR, EUROPEAN PATENTS DEPARTMENT**

Certified Verified and Authenticated
In The Town Of Egham In Surrey
This 31st Day Of May 2001

KENNETH GREENWOOD
NOTARY PUBLIC
HORNE ENGALL AND FREEMAN



APOSTILLE

(Hague Convention of 5 October 1961 / Convention de La Haye du 5 octobre 1961)

18

1. Country: United Kingdom of Great Britain and Northern Ireland
Pays: Royaume-Uni de Grande-Bretagne et d'Irlande du Nord

This public document / Le présent acte public

2. Has been signed by **K Greenwood**
a été signé par

3. Acting in the capacity of **Notary Public**
agissant en qualité de

4. Bears the seal/stamp of **The Said Notary Public**
est revêtu du sceau/timbre de

Certified/Attesté

5. at London/à Londres

6. the/le **31 May 2001**

7. by Her Majesty's Principal Secretary of State for Foreign and Commonwealth Affairs /
par le Secrétaire d'Etat Principal de Sa Majesté aux Affaires Etrangères et du Commonwealth.

8. Number/sous No **G899961**

9. Stamp:
timbre:

FOREIGN AND COMMONWEALTH OFFICE LONDON

10. Signature: **Y. P. Precleux**

For the Secretary of State / Pour le Secrétaire d'Etat

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01918
DE MINJUSTICIA

441/02

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 January 2002 (10.01.2002)

PCT

(10) International Publication Number
WO 02/02513 A1

(51) International Patent Classification[7]: C07C 237/22, A61K 31/19, A61P 15/00, C07C 233/58, 233/60, 235/40, 275/52, 237/24, C07D 317/58, 285/12, 207/27, 209/16, 207/14, 211/76, 213/75, 213/71, C07C 311/18, 311/13, 311/51, C07D 307/81

(21) International Application Number: PCT/IB01/01205

(22) International Filing Date: 2 July 2001 (02.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0016684.3 6 July 2000 (06.07.2000) GB
0101584.1 22 January 2001 (22.01.2001) GB

(71) Applicant *(for GB only)*: PFIZER LIMITED [GB/GB]; Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

(71) Applicant *(for all designated States except GB, US)*: PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BARBER, Christopher, Gordon [GB/GB]; Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). COOK, Andrew, Simon [GB/GB]; Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). MAW, Graham, Nigel [GB/GB]; Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). PRYDE, David, Cameron [GB/GB]; Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). STOBIE, Alan [GB/GB]; Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

(74) Agents: RUDDOCK, Keith, S. et al.; Pfizer Limited, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

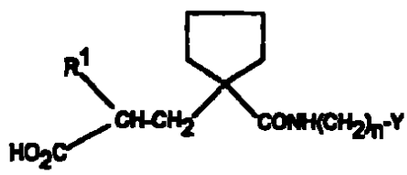
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

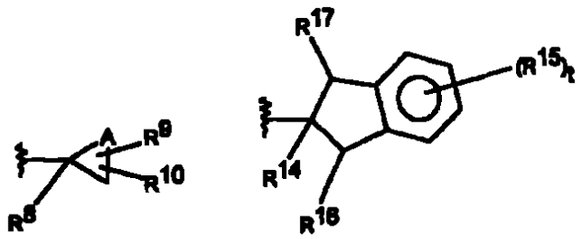
Published:
— *with international search report*

[Continued on next page]

(54) Title: CYCLOPENTYL-SUBSTITUTED GLUTARAMIDE DERIVATIVES AS INHIBITORS OF NEUTRAL ENDOPEPTIDASE



(I)



(57) Abstract: The invention provides compounds of formula I wherein R^1 is optionally substituted C_{1-6}alkyl, optionally substituted C_{3-7} cycloalkyl, optionally substituted aryl or optionally substituted heterocyclyl; n is 0, 1 or 2; and Y is $-NR^{18}S(O)_nR^{19}$ or a group shown below.

WO 02/02513 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

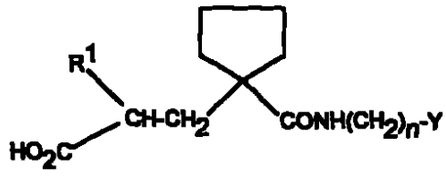
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Cyclopentyl-Substituted Glutaramide Derivatives as Inhibitors of Neutral Endopeptidase

This invention relates to inhibitors of neutral endopeptidase enzyme (NEP), uses thereof, processes for the preparation thereof, intermediates used in the preparation thereof, and compositions containing said inhibitors. These inhibitors have utility in a variety of therapeutic areas including the treatment of female sexual dysfunction (FSD) especially female sexual arousal disorder (FSAD).

NEP inhibitors are disclosed in WO 91/07386 and WO 91/10644.

According to a first aspect, the invention provides the use of a compound of formula (I), pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, in the preparation of a medicament for the treatment of female sexual dysfunction;



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6} alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino,

piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or -NR^2R^3 wherein R^2 and R^3 are as previously defined;

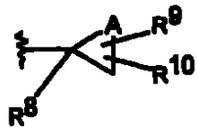
R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; p is 0, 1, 2 or 3;

n is 0, 1 or 2;

the -$(CH_2)_n$- linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

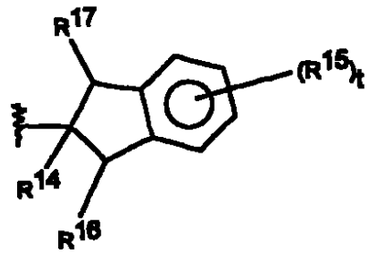
Y is the group



wherein A is -$(CH_2)_q$- where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-8}alkyl, -CH_2OH, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, -CH_2OH, -$C(O)NR^{11}R^{12}$, C_{1-8}alkyl, phenyl (optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy), or phenyl(C_{1-4}alkyl) (wherein the phenyl group is optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy), or R^9 and R^{10} together form a dioxolane; R^{11} and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

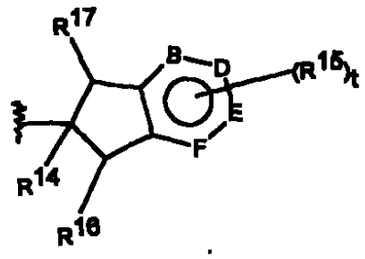
Y is the group, -C(O) NR^{11} R^{12} wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino;

C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only

contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl (preferably pyridyl); and u is 0, 1, 2 or 3.

Some of the compounds of formula I are disclosed in WO 91/10664 and WO 91/07388, but there is no teaching that they could be useful in the treatment of female sexual dysfunction. The remaining compounds of formula I are novel.

Therefore according to a second aspect, the invention provides a (novel) compound of formula (I), pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^1, n and Y are as defined in the first aspect with the proviso that Y is not the group $-C(O)NR^{11}R^{12}$ and when R^1 is propyl or phenylethyl, R^{14} is not $-CH_2OH$.

According to a third aspect, the invention provides a (novel) compound of formula (I), pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^1, n and Y are as defined in the first aspect with the proviso that Y is not the group $-C(O)NR^{11}R^{12}$ and R^{14} is not H or $-CH_2OH$.

In the above definition, unless otherwise indicated, alkyl groups having three or more carbon atoms may be straight or branched-chain. The term aryl as used herein means an aromatic hydrocarbon group such as phenyl or naphthyl which may optionally be substituted with, for example, one or more of OH, CN, CF_3, C_1-C_4 alkyl, C_1-C_4 alkoxy, halo, carbamoyl, aminosulphonyl, amino, mono or di(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups.

Halo means fluoro, chloro, bromo or iodo.

In the above definition, unless otherwise indicated the term heterocyclyl means a 5 or 6 membered nitrogen, oxygen or sulphur containing heterocyclic group which, unless otherwise stated, may be saturated, unsaturated or aromatic and which may optionally include a further oxygen or one to three nitrogen atoms in the ring and which may optionally be benzofused or substituted with for example, one or more halo, C_1-C_4 alkyl,

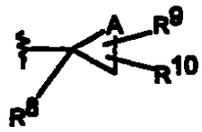
hydroxy, carbamoyl, benzyl, oxo, amino or mono or di-(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Particular examples of heterocycles include pyridyl, pyridonyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, furanyl, tetrahydrofuranyl, tetrahydropyranyl, dioxanyl, thienyl, oxazolyl, isoxazolyl, thiazolyl, oxadiazolyl, thiadiazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, quinoxalinyl, quinazolinyl and benzimidazolyl, each being optionally substituted as previously defined.

Preferred R^1 substituents are C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl, C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl or C_{1-6}alkyl substituted with aryl.

More preferred R^1 substituents are C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl (preferably methoxyethyl) or C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl (preferably methoxyethoxymethyl).

Still more preferred R^1 substituents are C_{1-4}alkyl (preferably propyl) or C_{1-6}alkoxy(C_{1-3})alkyl (preferably methoxyalkyl, more preferably methoxyethyl).

When Y is the group



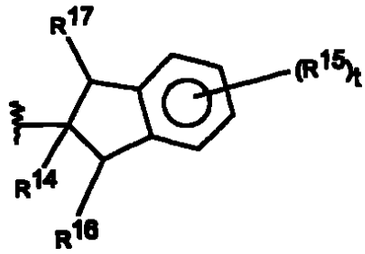
and the carbocyclic ring is fully saturated, then preferably one of R^9 or R^{10} is $-CH_2OH$; $-C(O)NR^{11}R^{12}$; C_{1-6}alkyl; phenyl optionally substituted by C_{1-4}alkyl; or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl. More, preferably the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl; phenyl optionally substituted by C_{1-4}alkyl; or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl. More preferably, R^9 and R^{10} are attached to adjacent carbon atoms in the ring. More preferably, R^8 is CH_2OH.

When Y is the group $-NR^{18}S(O)_uR^{19}$, preferably R^{18} is H. More preferably, R^{19} is benzyl or phenyl. More preferably u is 2.

Preferably Y is an optionally substituted 5-7 membered heterocyclic ring. More preferably the ring is an optionally substituted aromatic ring, particularly pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazolyl, thiazolyl, thiadiazolyl, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, pyridonyl, quinoxalinyl or quinazolinyl [especially oxadiazole (preferably 1,2,5- or 1,3,4-oxadiazole), pyridone (preferably 2-pyridone) or thiadiazole (preferably 1,3,4-thiadiazole) each of which may be substituted as defined in the first aspect. Preferably the heterocyclic ring is substituted by one or more C_{1-6}alkyl, phenyl or phenylC_{1-4}alkyl, more preferably by C_{1-4}alkyl or benzyl. Preferably Y is an *N*-substituted pyridone, preferably by benzyl or C_{1-4}alkyl.

Preferably Y is a lactam linked at the nitrogen.

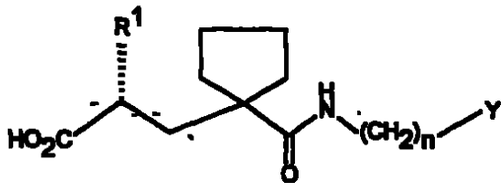
Preferably Y is



wherein R^{14} is preferably CH_2OH or $C(O)NR^{11}R^{12}$, especially $C(O)NR^{11}R^{12}$.

Preferably R^{16} and R^{17} are hydrogen. Preferably t is 0.

Preferred compounds are of formula Ie:



(Ie)

Particularly preferred compounds of the invention are:

2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]cyclopentyl)-methyl]-4-methoxybutanoic acid (Example 35);

2-[[1-([[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]-methyl]-4-phenylbutanoic acid (Example 40);

(+)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]methyl]-4-phenylbutanoic acid (Example 44);

2-[(1-[[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)methyl]-4-phenylbutanoic acid (Example 43);

cis-3-(2-methoxyethoxy)-2-[(1-[[(4-[[(phenylsulfonyl)amino]carbonyl]cyclohexyl)-amino]carbonyl]cyclopentyl)methyl]propanoic acid (Example 38);

(+)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl]pentanoic acid (Example 31);

(2*R*)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid or (-)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid (Example 29);

(2*S*)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid or (+)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid (Example 30);

2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21);

2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid (Example 22);

2-[[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 9);

trans-3-[1-({[2-(4-chlorophenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxymethyl)propanoic acid (Example 46);

trans-3-[1-({[2-(4-methoxyphenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 47);

trans-3-[1-({[2-pentylcyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 48);

3-[1-({[5-benzyl-[1,3,4]-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 49);

3-[1-({[4-butylpyridin-2-yl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 50);

3-[1-({[4-phenylpyridin-2-yl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 51);

3-[1-({[1-hydroxymethyl-3-phenylcyclopentyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 52);

2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-methoxybutanoic acid (Example 53);

trans-3-[1-({[2-phenylcyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 54);

(*R*)- 2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-methoxybutanoic acid (Example 55); and

(*S*)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-methoxybutanoic acid (Example 56).

For the avoidance of doubt, unless otherwise indicated, the term substituted means substituted by one or more defined groups. In the case where groups may be selected from a number of alternatives groups, the selected groups may be the same or different.

For the avoidance of doubt, the term independently means that where more than one substituent is selected from a number of possible substituents, those substituents may be the same or different.

The pharmaceutically or veterinarily acceptable salts of the compounds of formula I which contain a basic centre are, for example, non-toxic acid addition salts formed with inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, with carboxylic acids or with organo-sulfonic acids. Examples include the HCl, HBr, HI, sulfate or bisulfate, nitrate, phosphate or hydrogen phosphate, acetate, benzoate, succinate, saccharate, fumarate, maleate, lactate, citrate, tartrate, gluconate, camsylate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate salts. Compounds of the invention can also provide pharmaceutically or veterinarily acceptable metal salts, in particular non-toxic alkali and alkaline earth metal salts, with bases. Examples include the sodium, potassium, aluminium, calcium, magnesium, zinc, diolamine, olamine, ethylenediamine, tromethamine, choline, megulamine and diethanolamine salts. For reviews on suitable pharmaceutical salts see Berge *et al*, J. Pharm, Sci., 66, 1-19, 1977; P L Gould, International Journal of Pharmaceutics, 33 (1986), 201-217; and Bighley *et al*, Encyclopedia of Pharmaceutical Technology, Marcel Dekker Inc, New York 1996, Volume 13, page 453-497. A preferred salt is the sodium salt.

The pharmaceutically acceptable solvates of the compounds of the invention include the hydrates thereof.

Hereinafter, compounds, their pharmaceutically acceptable salts, their solvates and polymorphs, defined in any aspect of the invention (except intermediate compounds in chemical processes) are referred to as "compounds of the invention".

The compounds of the invention may possess one or more chiral centres and so exist in a number of stereoisomeric forms. All stereoisomers and mixtures thereof are included in the scope of the present invention. Racemic compounds may either be separated using preparative HPLC and a column with a chiral stationary phase or resolved to yield individual enantiomers utilising methods known to those skilled in the art. In addition, chiral intermediate compounds may be resolved and used to prepare chiral compounds of the invention.

In cases where the compounds of the invention exist as the E and Z isomers, the invention includes individual isomers as well as mixtures thereof.

In cases where compounds of the invention exist as tautomeric isomers, the invention includes individual tautomers as well as mixtures thereof.

In cases where the compounds of the invention exist as optical isomers, the invention includes individual isomers as well as mixtures thereof.

In cases where the compounds of the invention exist as diastereoisomers, the invention includes individual diastereoisomers as well as mixtures thereof.

Separation of diastereoisomers or E and Z isomers may be achieved by conventional techniques, e.g. by fractional crystallisation, chromatography or H.P.L.C (see Examples 29 and 30 herein). An individual enantiomer of a compound of the invention or intermediate may be prepared from a corresponding optically pure intermediate or by resolution, such as by H.P.L.C. of the corresponding racemate using a suitable chiral support or by fractional crystallisation of the diastereoisomeric salts formed by reaction of the corresponding racemate with a suitable optically active base, as appropriate. A

preferred optically active base is pseudoephedrine (see Preparation 2 herein).

The compounds of the invention may exist in one or more tautomeric forms. All tautomers and mixtures thereof are included in the scope of the present invention. For example, a claim to 2-hydroxypyridinyl would also cover its tautomeric form, α-pyridonyl.

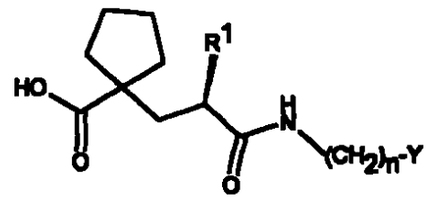
It will be appreciated by those skilled in the art that certain protected derivatives of compounds of the invention, which may be made prior to a final deprotection stage, may not possess pharmacological activity as such, but may, in certain instances, be administered orally or parenterally and thereafter metabolised in the body to form compounds of the invention which are pharmacologically active. Such derivatives may therefore be described as "prodrugs". Further, certain compounds of the invention may act as prodrugs of other compounds of the invention.

All protected derivatives and prodrugs of compounds of the invention are included within the scope of the invention. Examples of suitable pro-drugs for the compounds of the present invention are described in Drugs of Today, Volume 19, Number 9, 1983, pp 499 – 538 and in Topics in Chemistry, Chapter 31, pp 306 – 316 and in "Design of Prodrugs" by H. Bundgaard, Elsevier, 1985, Chapter 1 (the disclosures in which documents are incorporated herein by reference).

It will further be appreciated by those skilled in the art, that certain moieties, known to those skilled in the art as "pro-moieties", for example as described by H. Bundgaard in "Design of Prodrugs" (the disclosure in which document is incorporated herein by reference) may be placed on appropriate functionalities when such functionalities are present within the compounds of the invention.

Preferred prodrugs for compounds of the invention include: esters, carbonate esters, hemi-esters, phosphate esters, nitro esters, sulfate esters, sulfoxides, amides, carbamates, azo-compounds, phosphamides, glycosides, ethers, acetals and ketals.

Drug metabolism studies have shown that *in vivo*, compounds of formula I may form compounds of formula XXIII, which compounds also are inhibitors of NEP



(XXIII)

In particular, we have shown that (2*R*)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl) methyl]pentanoic acid (Example 29) *in vivo* forms (2R)-1-(2-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]pentyl)-cyclopentanecarboxylic acid.

The invention also includes all suitable isotopic variations of the compounds of the invention. An isotopic variation is defined as one in which at least one atom is replaced by an atom having the same atomic number but an atomic mass different from the atomic mass usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulphur, fluorine and chlorine such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F and ^{36}Cl, respectively. Certain isotopic variations of the invention, for example, those in which a radioactive isotope such as ^{3}H or ^{14}C is incorporated, are useful in drug and/or substrate tissue distribution studies. Tritiated, i.e. ^{3}H, and carbon-14, i.e. ^{14}C isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with isotopes such as deuterium, i.e. ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements and hence may be preferred in some circumstances. Isotopic variations of the compounds of the invention can generally be prepared by conventional procedures such as by the methods or preparations described in the Examples and Preparations hereafter using appropriate isotopic variations of suitable reagents.

The compounds of the invention are inhibitors of the zinc-dependent, neutral endopeptidase EC.3.4.24.11., and it is proposed that the compounds of the invention will treat the disease states listed below. This enzyme is involved in the breakdown of several bioactive oligopeptides, cleaving peptide bonds on the amino side of hydrophobic amino acid residues. The peptides metabolised include atrial natriuretic peptides (ANP), bombesin, bradykinin, calcitonin gene-related peptide, endothelins, enkephalins, neurotensin, substance P and vasoactive intestinal peptide. Some of these

peptides have potent vasodilatory and neurohormone functions, diuretic and natriuretic activity or mediate behaviour effects. Thus, the compounds of the invention, by inhibiting the neutral endopeptidase EC.3.4.24.11, can potentiate the biological effects of bioactive peptides.

Thus, in particular the compounds have utility in the treatment of a number of disorders, including hypertension, heart failure, angina, renal insufficiency, acute renal failure, cyclical oedema, Menières disease, hyperaldosteronism (primary and secondary) and hypercalciuria. In addition, because of their ability to potentiate the effects of ANF the compounds have utility in the treatment of glaucoma. As a further result of their ability to inhibit the neutral endopeptidase E.C.3.4.24.11 the compounds of the invention may have activity in other therapeutic areas including for example the treatment of menstrual disorders, preterm labour, pre-eclampsia, endometriosis, and reproductive disorders (especially male and female infertility, polycystic ovarian syndrome, implantation failure). Also the compounds of the invention should treat asthma, inflammation, leukemia, pain, epilepsy, affective disorders, dementia and geriatric confusion, obesity and gastrointestinal disorders (especially diarrhoea and irritable bowel syndrome), wound healing (especially diabetic and venous ulcers and pressure sores), septic shock, the modulation of gastric acid secretion, the treatment of hyperreninaemia, cystic fibrosis, restenosis, diabetic complications and atherosclerosis. In a preferred embodiment the compounds of the invention are useful in the treatment of female sexual dysfunction (FSD) preferably FSAD.

It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment.

We have found that the compounds of the invention inhibit the enzyme neutral endopeptidase. Therefore, according to a further aspect, the invention provides the use of a compound of the invention in the preparation of a medicament for the treatment or prophylaxis of a condition for which a beneficial therapeutic response can be obtained by the inhibition of neutral endopeptidase.

In accordance with the invention, FSD can be defined as the difficulty or inability of a woman to find satisfaction in sexual expression. FSD is a collective term for several diverse female sexual disorders (Leiblum, S.R. (1998). Definition and classification of

female sexual disorders. *Int. J. Impotence Res.*, 10, S104-S106; , Berman, J.R., Berman, L. & Goldstein, I. (1999). Female sexual dysfunction: Incidence, pathophysiology, evaluations and treatment options. *Urology*, 54, 385-391). The woman may have lack of desire, difficulty with arousal or orgasm, pain with intercourse or a combination of these problems. Several types of disease, medications, injuries or psychological problems can cause FSD. Treatments in development are targeted to treat specific subtypes of FSD, predominantly desire and arousal disorders.

The categories of FSD are best defined by contrasting them to the phases of normal female sexual response: desire, arousal and orgasm (Leiblum, S.R. (1998). Definition and classification of female sexual disorders, *Int. J. Impotence Res.*, 10, S104-S108). Desire or libido is the drive for sexual expression. Its manifestations often include sexual thoughts either when in the company of an interested partner or when exposed to other erotic stimuli. Arousal is the vascular response to sexual stimulation, an important component of which is genital engorgement and includes increased vaginal lubrication, elongation of the vagina and increased genital sensation/sensitivity. Orgasm is the release of sexual tension that has culminated during arousal.

Hence, FSD occurs when a woman has an inadequate or unsatisfactory response in any of these phases, usually desire, arousal or orgasm. FSD categories include hypoactive sexual desire disorder, sexual arousal disorder, orgasmic disorders and sexual pain disorders. Although the compounds of the invention will improve the genital response to sexual stimulation (as in female sexual arousal disorder), in doing so it may also improve the associated pain, distress and discomfort associated with intercourse and so treat other female sexual disorders.

Thus, in accordance with a further aspect of the invention, there is provided the use of a compound of the invention in the preparation of a medicament for the treatment or prophylaxis of hypoactive sexual desire disorder, sexual arousal disorder, orgasmic disorder and sexual pain disorder, more preferably for the treatment or propylaxis of sexual arousal disorder, orgasmic disorder, and sexual pain disorder, and most preferably in the treatment or prophylaxis of sexual arousal disorder.

Hypoactive sexual desire disorder is present if a woman has no or little desire to be sexual, and has no or few sexual thoughts or fantasies. This type of FSD can be caused

by low testosterone levels, due either to natural menopause or to surgical menopause. Other causes include illness, medications, fatigue, depression and anxiety.

Female sexual arousal disorder (FSAD) is characterised by inadequate genital response to sexual stimulation. The genitalia do not undergo the engorgement that characterises normal sexual arousal. The vaginal walls are poorly lubricated, so that intercourse is painful. Orgasms may be impeded. Arousal disorder can be caused by reduced oestrogen at menopause or after childbirth and during lactation, as well as by illnesses, with vascular components such as diabetes and atherosclerosis. Other causes result from treatment with diuretics, antihistamines, antidepressants eg SSRIs or antihypertensive agents.

Sexual pain disorders (includes dyspareunia and vaginismus) is characterised by pain resulting from penetration and may be caused by medications which reduce lubrication, endometriosis, pelvic inflammatory disease, inflammatory bowel disease or urinary tract problems.

The prevalence of FSD is difficult to gauge because the term covers several types of problem, some of which are difficult to measure, and because the interest in treating FSD is relatively recent. Many women's sexual problems are associated either directly with the female ageing process or with chronic illnesses such as diabetes and hypertension.

Because FSD consists of several subtypes that express symptoms in separate phases of the sexual response cycle, there is not a single therapy. Current treatment of FSD focuses principally on psychological or relationship issues. Treatment of FSD is gradually evolving as more clinical and basic science studies are dedicated to the investigation of this medical problem. Female sexual complaints are not all psychological in pathophysiology, especially for those individuals who may have a component of vasculogenic dysfunction (eg FSAD) contributing to the overall female sexual complaint. There are at present no drugs licensed for the treatment of FSD. Empirical drug therapy includes oestrogen administration (topically or as hormone replacement therapy), androgens or mood-altering drugs such as buspirone or trazodone. These treatment options are often unsatisfactory due to low efficacy or unacceptable side effects.

Since interest is relatively recent in treating FSD pharmacologically, therapy consists of the following:- psychological counselling, over-the-counter sexual lubricants, and investigational candidates, including drugs approved for other conditions. These medications consist of hormonal agents, either testosterone or combinations of oestrogen and testosterone and more recently vascular drugs, that have proved effective in male erectile dysfunction. None of these agents has been demonstrated to be very effective in treating FSD.

As discussed, the compounds of the invention are particularly useful for the treatment of female sexual arousal disorder (FSAD).

The Diagnostic and Statistical Manual (DSM) IV of the American Psychiatric Association defines Female Sexual Arousal Disorder (FSAD) as being:

> ..."a persistent or recurrent inability to attain or to maintain until completion of the sexual activity adequate lubrication-swelling response of sexual excitement. The disturbance must cause marked distress or interpersonal difficulty."

The arousal response consists of vasocongestion in the pelvis, vaginal lubrication and expansion and swelling of the external genitalia. The disturbance causes marked distress and/or interpersonal difficulty.

FSAD is a highly prevalent sexual disorder affecting pre-, peri- and post menopausal (± HRT) women. It is associated with concomitant disorders such as depression, cardiovascular diseases, diabetes and UG disorders.

The primary consequences of FSAD are lack of engorgement/swelling, lack of lubrication and lack of pleasurable genital sensation. The secondary consequences of FSAD are reduced sexual desire, pain during intercourse and difficulty in achieving an orgasm.

It has recently been hypothesised that there is a vascular basis for at least a proportion of patients with symptoms of FSAD (Goldstein *et al.*, Int. J. Impot. Res., 10, S84-S90,1998) with animal data supporting this view (Park *et al.*, Int. J. Impot. Res., 9, 27-37, 1997).

Drug candidates for treating FSAD, which are under investigation for efficacy, are primarily erectile dysfunction therapies that promote circulation to the male genitalia. They consist of two types of formulation, oral or sublingual medications (Apomorphine, Phentolamine, phosphodiesterase type 5 (PDE5) inhibitors e.g. Sildenafil), and prostaglandin (PGE_1) that are injected or administered transurethrally in men, and topically to the genitalia in women.

The compounds of the invention are advantageous by providing a means for restoring a normal sexual arousal response - namely increased genital blood flow leading to vaginal, clitoral and labial engorgement. This will result in increased vaginal lubrication *via* plasma transudation, increased vaginal compliance and increased genital sensitivity. Hence, the compounds of the invention provide means to restore, or potentiate, the normal sexual arousal response.

Without being bound by theory, we believe that neuropeptides such as vasoactive intestinal peptide (VIP) are major neurotransmitter candidates in the control of the female sexual arousal response, especially in the control of genital blood flow. VIP and other neuropeptides are degraded/ metabolised by NEP EC3.4.24.11. Thus, NEP inhibitors will potentiate the endogenous vasorelaxant effect of VIP released during arousal. This will lead to a treatment of FSAD, such as through enhanced genital blood flow and hence genital engorgement. We have shown that selective inhibitors of NEP EC 3.4.24.11 enhance pelvic nerve-stimulated and VIP-induced increases in vaginal and clitoral blood flow. In addition, selective NEP inhibitors enhance VIP and nerve-mediated relaxations of isolated vagina wall.

Thus the present invention is advantageous as it helps provide a means for restoring a normal sexual arousal response - namely increased genital blood flow leading to vaginal, clitoral and labial engorgement. This will result in increased vaginal lubrication *via* plasma transudation, increased vaginal compliance and increased vaginal sensitivity. Hence, the present invention provides a means to restore, or potentiate the normal sexual arousal response.

Background teachings on NEP have been presented by Victor A. McKusick *et al* on http://www3.ncbi.nlm.nih.gov/Omim/searchomim.htm. The following information concerning NEP has been extracted from that source:

"Common acute lymphocytic leukemia antigen is an important cell surface marker in the diagnosis of human acute lymphocytic leukemia (ALL). It is present on leukemic cells of pre-B phenotype, which represent 85% of cases of ALL. CALLA is not restricted to leukemic cells, however, and is found on a variety of normal tissues. CALLA is a glycoprotein that is particularly abundant in kidney, where it is present on the brush border of proximal tubules and on glomerular epithelium. Letarte et al. (1988) cloned a cDNA coding for CALLA and showed that the amino acid sequence deduced from the cDNA sequence is identical to that of human membrane-associated neutral endopeptidase (NEP; EC 3.4.24.11), also known as enkephalinase. NEP cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin. By cDNA transfection analysis, Shipp et al. (1989) confirmed that CALLA is a functional neutral endopeptidase of the type that has previously been called enkephalinase. Barker et al. (1989) demonstrated that the CALLA gene, which encodes a 100-kD type II transmembrane glycoprotein, exists in a single copy of greater than 45 kb which is not rearranged in malignancies expressing cell surface CALLA. The gene was located to human chromosome 3 by study of somatic cell hybrids and in situ hybridization regionalized the location to 3q21-q27. Tran-Paterson et al. (1989) also assigned the gene to chromosome 3 by Southern blot analysis of DNA from human-rodent somatic cell hybrids. D'Adamio et al. (1989) demonstrated that the CALLA gene spans more than 80 kb and is composed of 24 exons."

1 Barker, P. E.; Shipp, M. A.; D'Adamio, L.; Masteller, E. L.; Reinherz, E. L. The common acute lymphoblastic leukemia antigen gene maps to chromosomal region 3(q21-q27). J. Immun. 142: 283-287, 1989.

2 D'Adamio, L.; Shipp, M. A.; Masteller, E. L.; Reinherz, E. L. : Organization of the gene encoding common acute lymphoblastic leukemia antigen (neutral endopeptidase 24.11): multiple miniexons and separate 5-prime untranslated regions. Proc. Nat. Acad. Sci. 86: 7103-7107, 1989.

3 Letarte, M.; Vera, S.; Tran, R.; Addis, J. B. L.; Onizuka, R. J.; Quackenbush, E. J.; Jongeneel, C. V.; McInnes, R. R. : Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. J. Exp. Med. 168: 1247-1253, 1988.

4 Shipp, M. A.; Vijayaraghavan, J.; Schmidt, E. V.; Masteller, E. L.; D'Adamio, L.; Hersh, L. B.; Reinherz, E. L. : Common acute lymphoblastic leukemia antigen (CALLA) is active neutral endopeptidase 24.11 ('enkephalinase'): direct evidence by cDNA transfection analysis. Proc. Nat. Acad. Sci. 86: 297-301, 1989.

5 Tran-Paterson, R.; Willard, H. F.; Letarte, M. : The common acute lymphoblastic leukemia antigen (neutral endopeptidase--3.4.24.11) gene is located on human chromosome 3. Cancer Genet. Cytogenet. 42: 129-134, 1989.

"The (female) genital organs consist of an internal and external group. The internal organs are situated within the pelvis and consist of ovaries, the uterine tubes, uterus and the vagina. The external organs are superficial to the urogenital diaphragm and below the pelvic arch. They comprise the mons pubis, the labia majora and minora pudendi, the clitoris, the vestibule, the bulb of the vestibule, and the greater vestibular glands" (Gray's Anatomy, C.D. Clemente, 13th American Edition).

The compounds of the invention find application in the following sub-populations of patients with FSD: the young, the elderly, pre-menopausal, peri-menopausal, post-menopausal women with or without hormone replacement therapy.

The compounds of the invention find application in patients with FSD arising from:-

i) Vasculogenic etiologies eg cardiovascular or atherosclerotic diseases, hypercholesterolemia, cigarette smoking, diabetes, hypertension, radiation and perineal trauma, traumatic injury to the iliohypogastric pudendal vacular system.

ii) Neurogenic etiologies such as spinal cord injuries or diseases of the central nervous system including multiple sclerosis, diabetes, Parkinsonism, cerebrovascular accidents, peripheral neuropathies, trauma or radical pelvic surgery.

iii) Hormonal/endocrine etiologies such as dysfunction of the hypothalamic/pituitary/gonadal axis, or dysfunction of the ovaries, dysfunction of the pancreas, surgical or medical castration, androgen deficiency, high circulating levels of prolactin eg hyperprolactinemia, natural menopause, premature ovarian failure, hyper and hypothyroidism.

iv) Psychogenic etiologies such as depression, obsessive compulsive disorder, anxiety disorder, postnatal depression/"Baby Blues", emotional and relational

issues, performance anxiety, marital discord, dysfunctional attitudes, sexual phobias, religious inhibition or a traumatic past experiences.

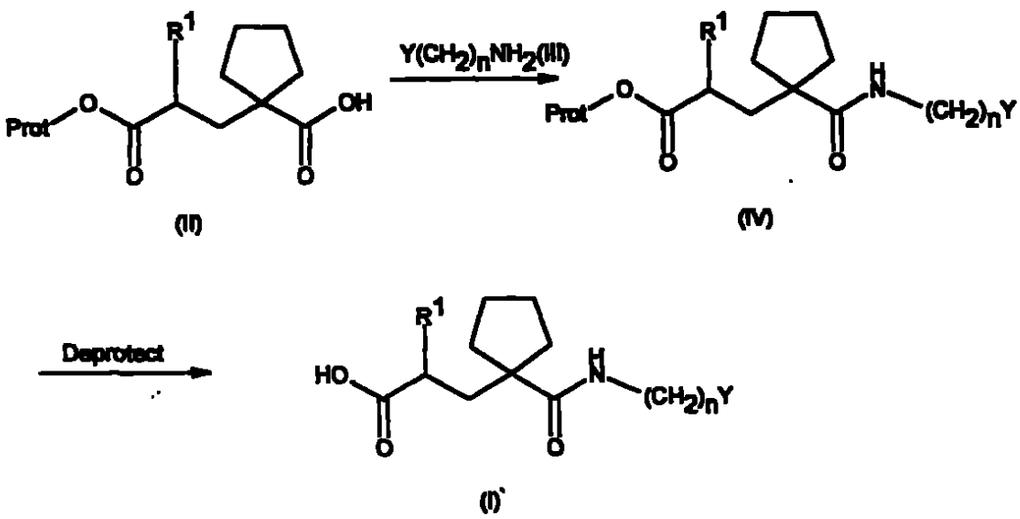
v) Drug-induced sexual dysfunction resulting from therapy with selective serotonin reuptake inhibitors (SSRIs) and other antidepressant therapies (tricyclics and major tranquillizers), anti-hypertensive therapies, sympatholytic drugs, chronic oral contraceptive pill therapy.

Compounds of the invention may be prepared, in known manner in a variety of ways. In the following reaction schemes and hereafter, unless otherwise stated R^1, n and Y are as defined in the first aspect. These processes form further aspects of the invention.

Throughout the specification, general formulae are designated by Roman numerals I, II, III, IV etc. Subsets of these general formulae are defined as Ia, Ib, Ic etc, ... IVa, IVb, IVc etc.

Compounds of general formula I may be prepared according to reaction scheme 1, by reacting a compound of formula II (where Prot is a suitable protecting group) with a primary amine of formula III to give a compound of formula IV. Deprotection gives compounds of formula I.

Scheme 1



The acid/amine coupling step can be carried out by reacting compounds of formula II with compounds of formula III (or its amine salt) in the presence of a coupling agent, optionally a catalyst, and an excess of an acid acceptor, in a suitable solvent. Typically, treatment of a mixture of compounds of formula II and compounds of formula III with a

coupling agent (for example dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSCDI), benzotriazol-1-yl diethyl phosphate, phosphorus oxychloride, titanium tetrachloride, sulfuryl chloride fluoride, Lawesson's reagent, PPACA, PYBOP or Mukaiyama's reagent) optionally in the presence of a tertiary amine base (for example triethylamine, Hunig's base, pyridine or NMM) for up to 24 hours at temperatures between –78 and 100 °C. Preferred reaction conditions comprise reacting compounds of formula II (1-1.5 equivalents) with compounds of formula III (or their salts 1-1.5 equivalents), in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCDI) or N,N'-dicyclohexylcarbodiimide (DCC) (1.1-1.3 equivalents), 1-hydroxybenzotrazole hydrate (HOBT) or dimethylaminopyridine (DMAP) (1.05-1.2 equivalents), *N*-methyl morpholine (NMM) or triethyamine (2.3-3 equivalents) in dimethylformamide or dichloromethane at between room temperature and 90°C for 16-18 hours.

Alternatively, the acid/amine coupling step may proceed via an activated intermediate (such as an acyl imidazolide, mixed anhydride or acid chloride) in the presence of an excess of acid acceptor in a suitable solvent. Typical reaction conditions comprise treatment of compounds of formula II with an activating agent (for example *N, N'*-carbonyldiimidazole, *N,N'*-carbonylbis(3-methylimidazolium) triflate, thionyl chloride or oxalyl chloride) optionally in the presence of a tertiary amine base (for example triethylamine, Hunig's base, pyridine or NMM) for up to 24 hours followed by reaction with compounds of formula III (or its salt), optionally in the presence of a catalyst (for example 4-dimethylaminopyridine) or an additive (for example hydroxybenzotriazole) in a suitable solvent (for example dichloromethane, THF, ethyl acetate, acetonitrile, DMF or toluene) optionally in the presence of an additional amine base at temperatures between –78 °C and 150 °C for up to 48 hours.

Preferred reaction conditions comprise reacting the acid chloride of compounds of formula II (1-1.1 equivalents) with compounds of formula III (or their salts, 1 to 1.5 equivalents) in the presence of triethyamine or *N*-methyl morpholine (1.4-10 equivalents) in dichloromethane solvent at room temperature for 24 hours. Alternatively, compounds of formula II can be converted to the acid chloride *in situ* by treatment with oxalyl chloride in dichloromethane in the presence of a catalytic amount of dimethylformamide for 2 hours at room temperature or by treatment of compounds of formula II with thionyl chloride in a mixture of dichloromethane and pyridine at –10 °C for 3 hours followed by

addition of triethylamine, 4-dimethylaminopyridine and the compound of formula III and allowing the mixture to react for 48 hours at 20 °C.

Compounds of formula I may be prepared from compounds of formula IV by deprotection. Methods for deprotection of an acid group depend on the protecting group. For examples of protection/deprotection methodology see "Protective groups in Organic synthesis", TW Greene and PGM Wutz.

For example, when Prot is a *tert*-butyl, deprotection conditions comprise reacting IV with trifluoroacetic acid/dichloromethane (1:1-1.5 by volume), at room temperature for 2-18 hours, optionally in the presence of a carbocation scavenger, e.g. anisole (10 equivalents). When Y contains a hydroxy group, base hydrolysis of the intermediate trifluoroacetic acid ester may be necessary. Alternative methodology for deprotection when Prot is *tert*-butyl comprises treating IV with hydrochloric acid in dichloromethane at room temperature for 3 hours. For the avoidance of doubt, Prot as *tert*-butyl is given by way of Example and is not intended to be limited to *tert*-Butyl.

Alternatively, when Prot is *tert*-butyl deprotection may be achieved by treating compounds of formula IV with a strong acid (for example gaseous or concentrated hydrochloric acid, hydrobromic acid, phosphoric acid, nitric acid or sulfuric acid, trifluoroacetic acid, chloroacetic acid, *para*-toluenesulfonic acid, trifluoromethanesulfonic acid or glacial acetic acid) in quantities ranging from catalytic to an excess, optionally in a suitable solvent (for example toluene, dichloromethane, diethyl ether, ethanol, THF or hexane) and optionally in the presence of water at temperatures between 20 °C and 150 °C for up to 48 hours.

Preferred deprotection conditions when Prot is *tert*-butyl are treatment of compounds of formula IV with a ten-fold excess of trifluoroacetic acid in dichloromethane at room temperature for 24 hours.

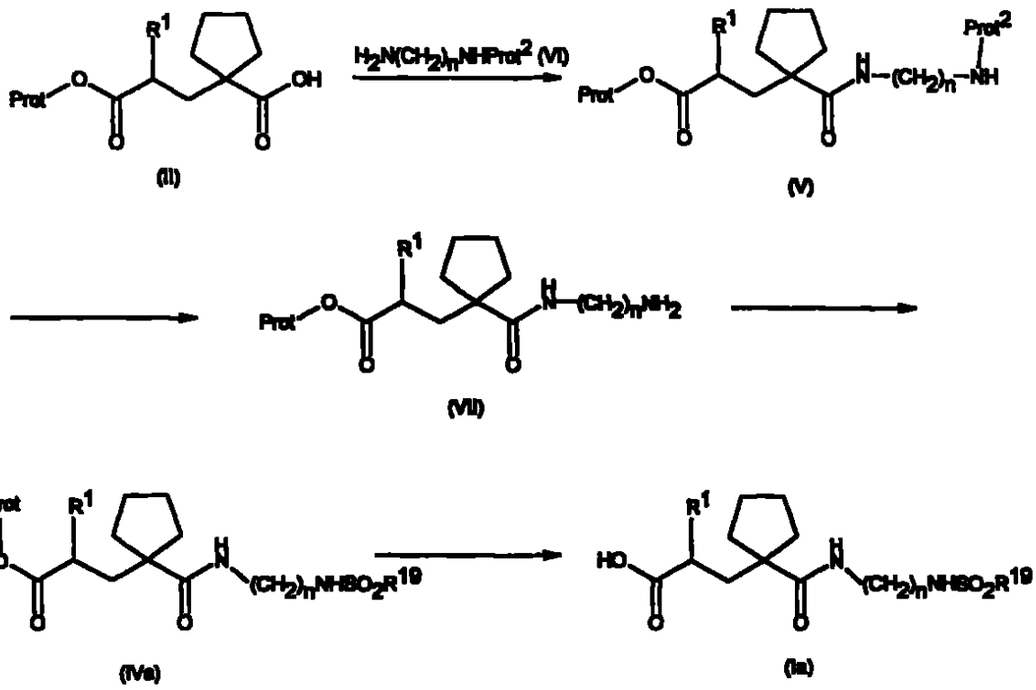
When Prot is benzyl, deprotection conditions comprise reacting IV with palladium on charcoal (5-10%) in aqueous ethanol (40-95%) at 15-60 psi at room temperature for 2hrs to 3 days.

These processes form further aspects of the invention.

Compounds of formula IV are novel and form a further aspect of the invention.

Compounds of formula Ia, i.e. compounds of general formula I where Y is $-NHSO_2R^{19}$, may be prepared according to reaction scheme 2. Compounds of formula V are first prepared by reacting compounds of formula II with compounds of formula VI where $Prot^2$ is a suitable amine protecting group. Preferred reaction conditions are analogous to those described for the acid/amine coupling step in Scheme 1 above. Selective amine deprotection of compounds of formula V gives compounds of formula VII. Compounds of formula VII are reacted with $R^{19}SO_2Cl$ in the presence of an acid acceptor in a suitable solvent to form compounds of formula IVa. Deprotection of compounds of formula IVa under analogous conditions to those described for the deprotection step of Scheme 1 gives compounds of formula Ia.

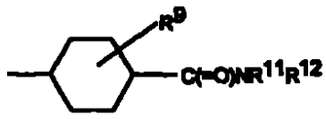
Scheme 2



Methods for deprotection of an amine group depend on the protecting group. For examples of protection/deprotection methodology see "Protective groups in Organic Synthesis", TW Greene and PGM Wutz. For example, when $Prot^2$ is benzoyloxycarbonyl, deprotection conditions comprise reacting V with palladium on charcoal (10%) in ethanol at room temperature for 18 hours.

Preferred methods for preparation of the compounds of formula IVa comprise reaction of VII with $R^{19}SO_2Cl$ (1 equivalent) in the presence of triethyamine (1.5-2.5 equivalents) in dichloromethane at room temperature for 2 to 3 days.

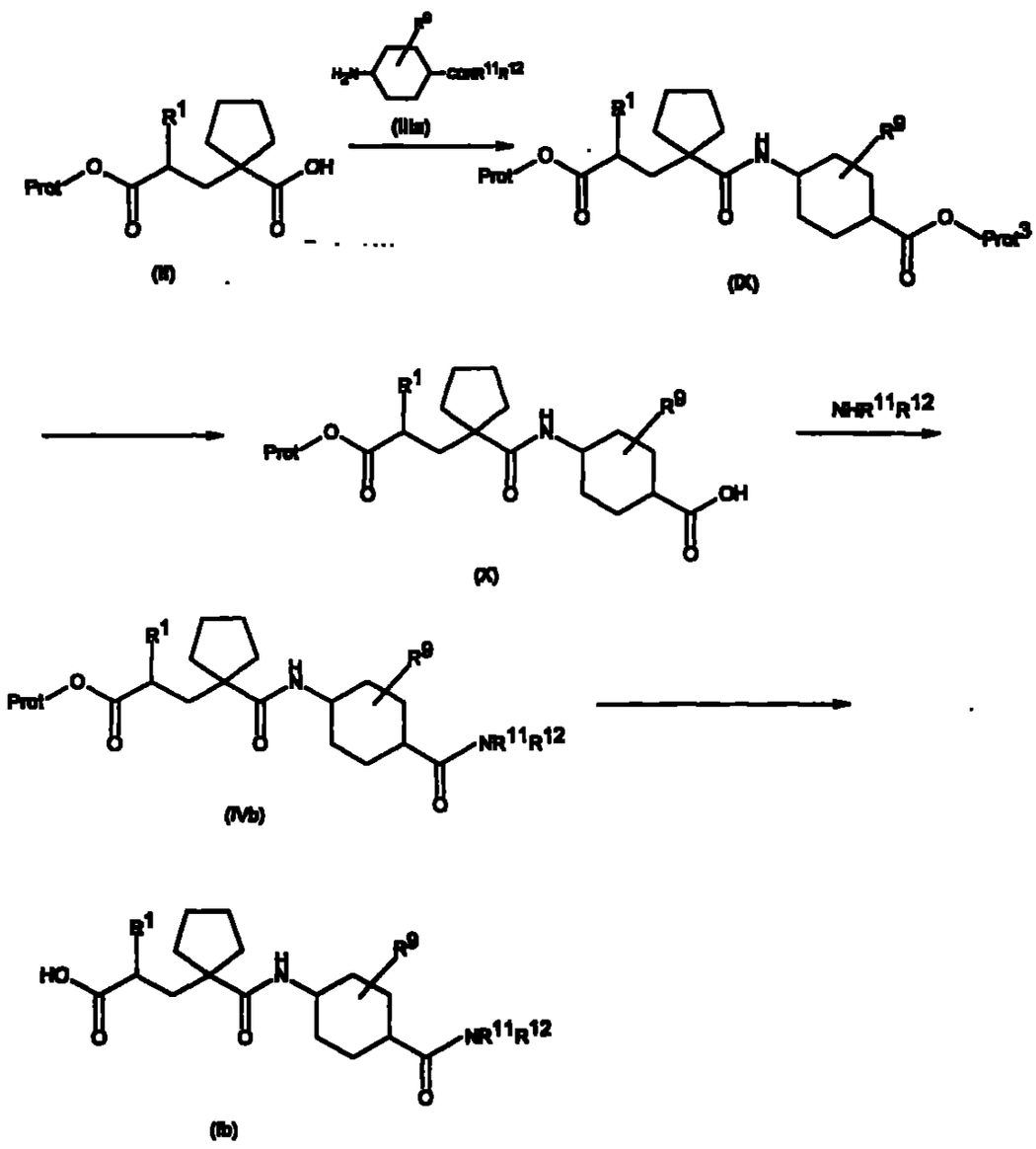
Compounds of formula Ib, i.e. compounds of formula I where n is 0 and Y is



, may be prepared according to reaction scheme 3. Compounds of formula II are reacted with compounds of formula IIIa under analogous conditions to acid/amine coupling conditions of Scheme 1 to give compounds of formula IX, where $Prot^3$ is a protecting group which can be selectively removed in the presence of protecting group Prot. A preferred protecting group $Prot^3$ is a base labile ester group. Consequently, treatment of compound of formula IX under basic conditions gives compounds of formula X. Compounds of formula X are reacted with compounds of formula $NHR^{11}R^{12}$ under analogous conditions to acid/amine coupling conditions of Scheme 1 to form compounds of formula IVb. Deprotection of compounds of formula IVb under analogous conditions to the deprotection step in Scheme 1 gives compounds of formula Ib.

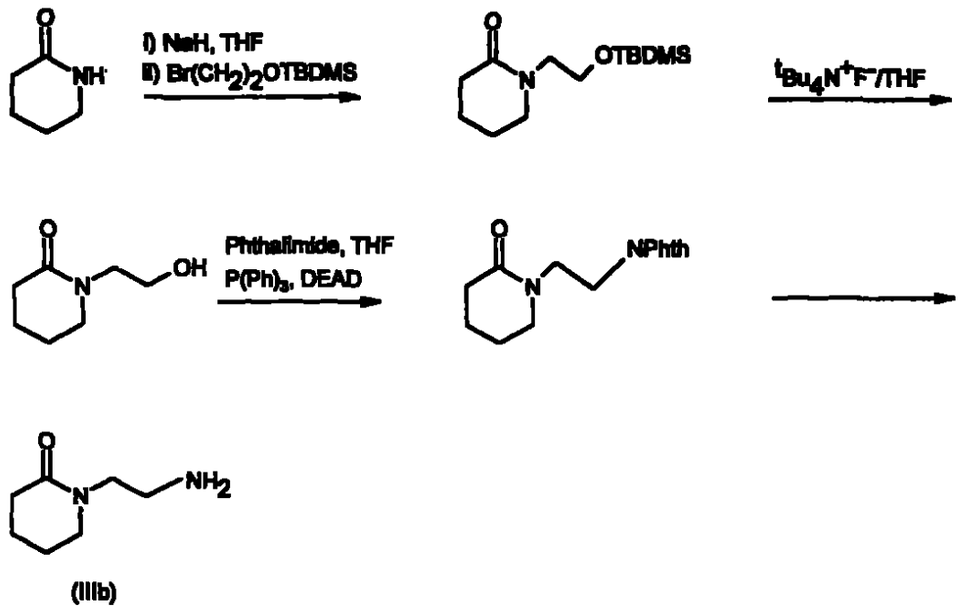
Preferred conditions for removal of protecting group $Prot^3$ from IVb comprise treatment of IVb with sodium hydroxide (1N) in methanol at room temperature for 22 hours.

Scheme 3



Compounds of formula IIIb, i.e. compounds of general formula III where n is 2 and Y is 2-oxopiperidino, may be prepared according to reaction scheme 4.

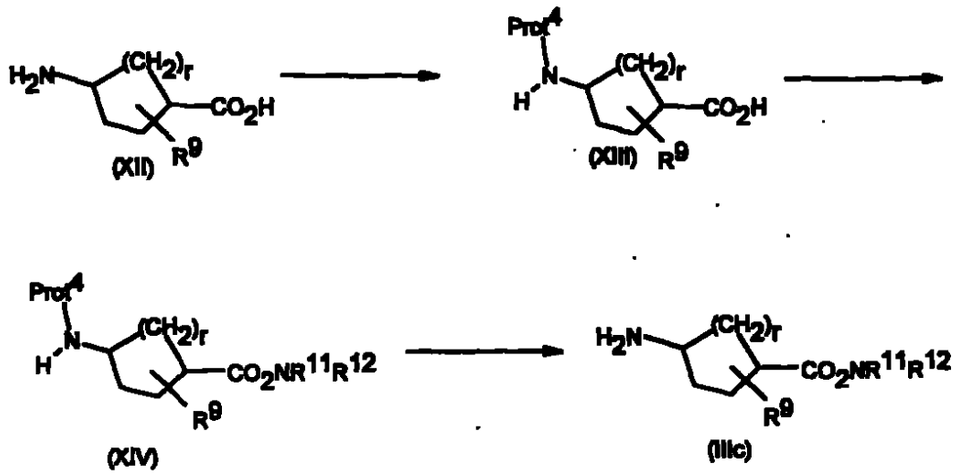
Scheme 4



(IIIb)

Compounds of formula IIIc where r is 1 or 2, may be prepared according to reaction scheme 5. Compounds of formula XII are protected at the amine moiety with a suitable protecting group $Prot^4$ to form compounds of formula XIII. A preferred protecting group is *tert*-butyloxycarbonyl. Compounds of formula XIII are reacted under typical acid/amine coupling conditions with $NHR^{11}R^{12}$ to form compounds of formula XIV, which on deprotection form compounds of formula IIIc.

Scheme 5



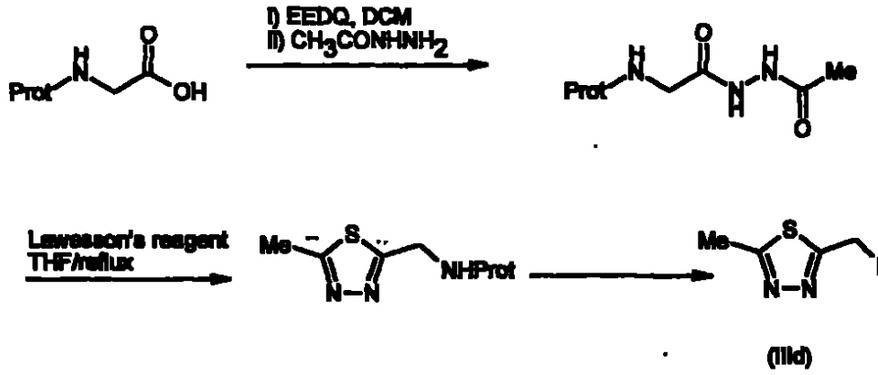
Typical reaction conditions for introducing the *tert*-butyloxycarbonyl protecting group comprise treating compounds of formula XII with (*tert*-butyloxycarbonyl)$_2$O in dioxan and 2N sodium hydroxide at room temperature for 18 hrs.

Typical acid/amine coupling conditions comprise treating compounds of formula XIII and $NHR^{11}R^{12}$ with benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PYBOP), 1-hydroxybenzotrazole hydrate (HOBT), Hünigs base, an amine (eg triethylamine), in dimethylformamide at room temperature for 2hrs. Alternatively, compounds of formula XIII and $NHR^{11}R^{12}$ may be treated with1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOBT, N-methyl morpholine (NMM), in dimethylformamide at room temperature for 18 hrs.

Typical reaction conditions for deprotection when $Prot^4$ is *tert*-butyloxycarbonyl comprise reacting XIV with hydrochloric acid or trifluoroacetic acid in dichloromethane at room temperature for 2 to 4 hrs

Compounds of formula IIId may be prepared according to reaction scheme 6. The protecting group is preferably *tert*-butyloxycarbonyl, which may be removed under standard conditions, as previously described.

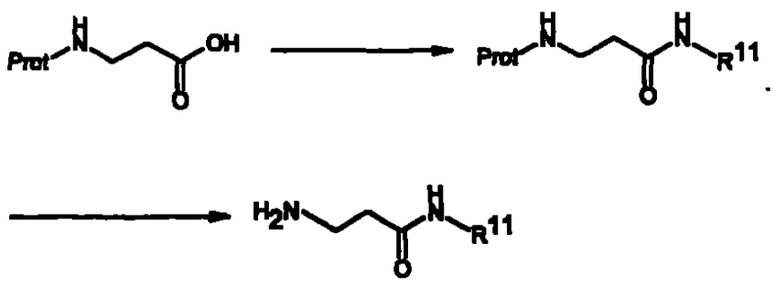
Scheme 6



(IIId)

Compounds of formula IIIe may be prepared according to reaction scheme 7 using standard acid/amine coupling reactions, as previously described. The protecting group is preferably benzyloxycarbonyl which may be removed under standard conditions, typically palladium on charcoal (5-10%) in ethanol at room temperature and 50 psi for 4 hrs.

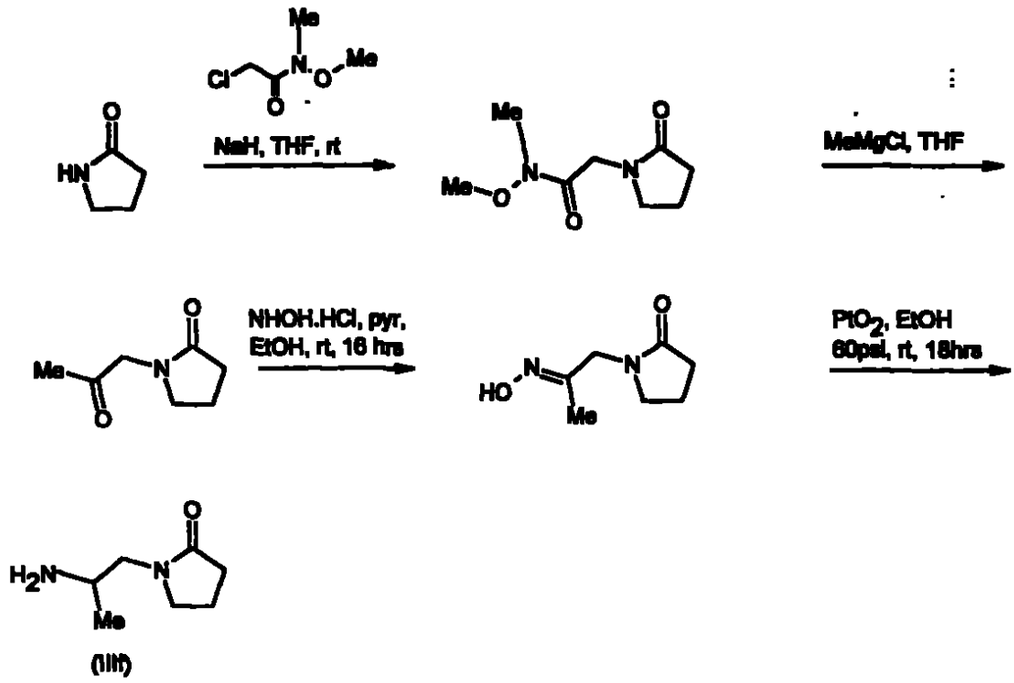
Scheme 7



(IIIe)

Compounds of formula IIIf may be prepared according to reaction scheme 8.

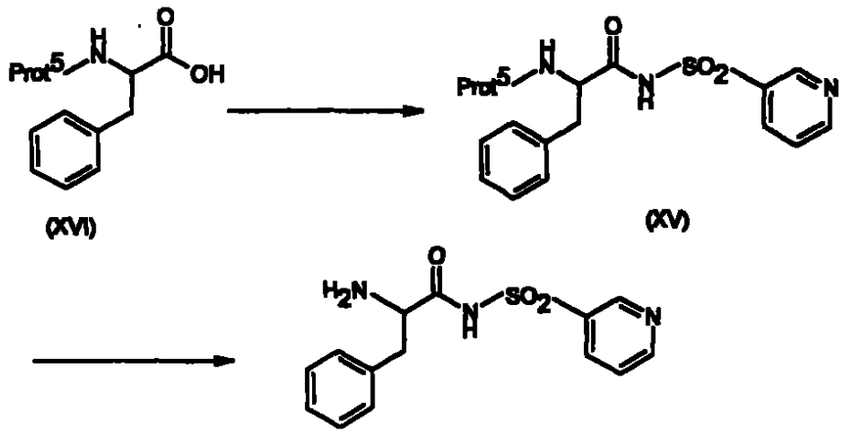
Scheme 8



(IIIf)

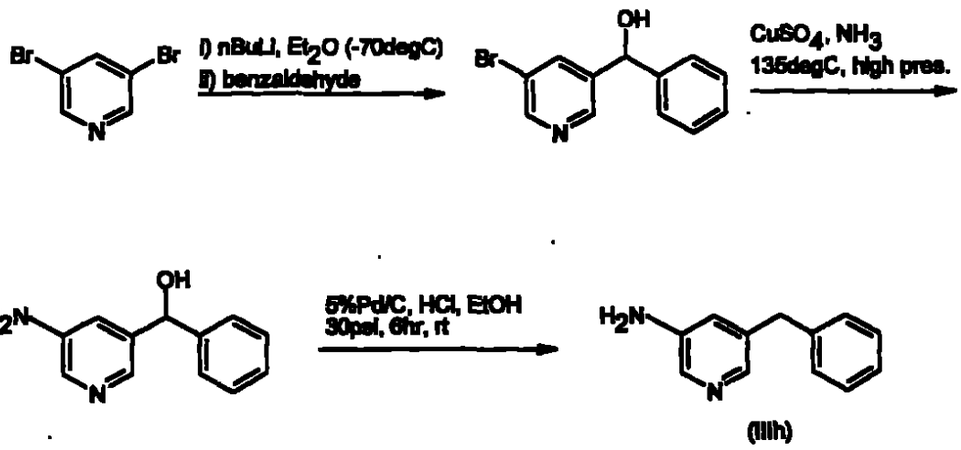
Compounds of formula IIIg may be prepared in two steps according to reaction scheme 9. As a first step, compounds of formula XV may be prepared from compounds of formula XVI using standard acid/amine coupling methodology analogous to the acid/amine coupling conditions described for reaction scheme 1. $Prot^5$ represents a suitable leaving group, preferably *tert*-butyloxycarbonyl. The second step comprises removal of $Prot^5$. When $Prot^5$ is *tert*-butyloxycarbonyl then preferred reaction conditions comprise treatment with hydrochloric acid in diethyl ether/ethyl acetate at room temperature for 18 hrs.

Scheme 9



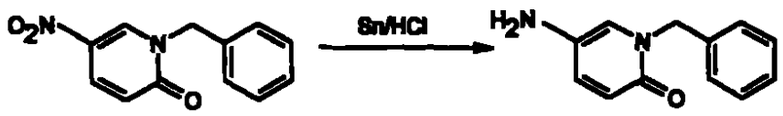
Compounds of formula IIIh may be prepared in three steps according to reaction scheme 10.

Scheme 10



Compounds of formula IIIj may be prepared by reduction of a nitro group according to reaction scheme 11.

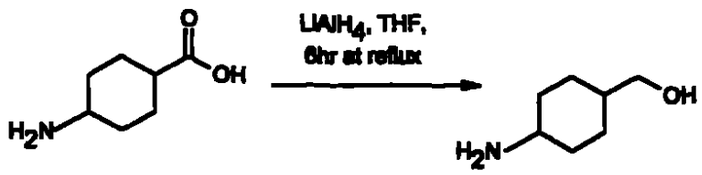
Scheme 11

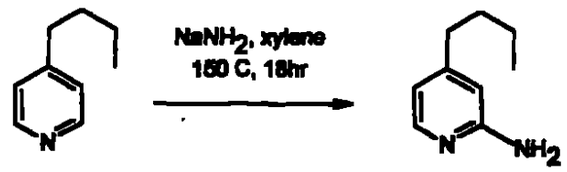


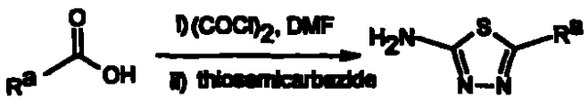
(III)

Further methods for preparing compounds of formula III are given in Scheme 12 below, where R^a is C_{1-6}alkyl or alkoxy.

Scheme 12





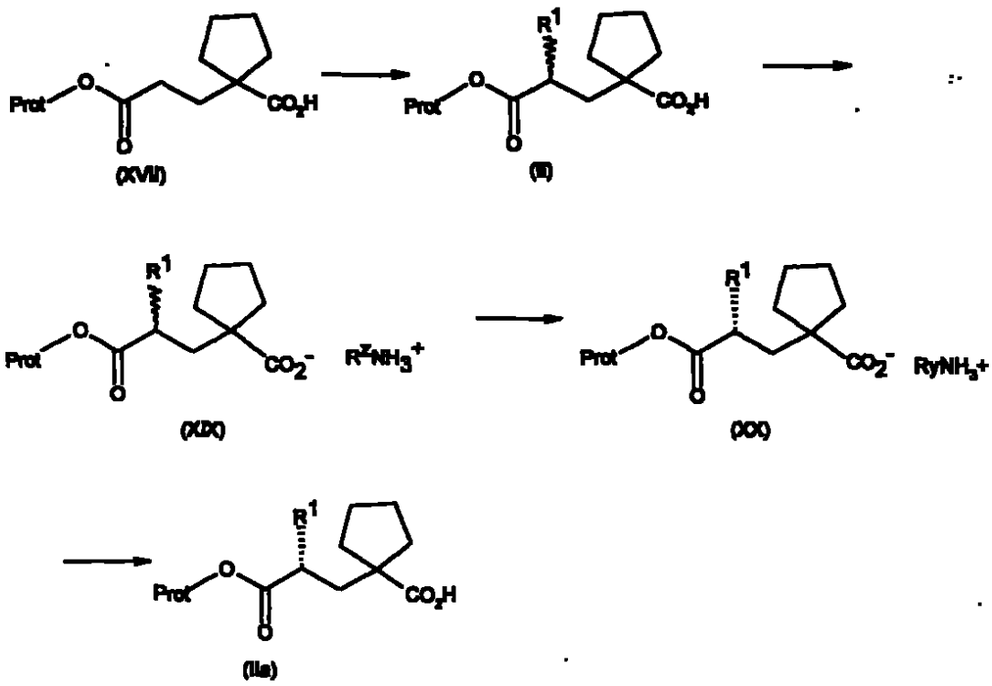


Compounds of general formula I possess a chiral centre at the carbon attached to R^1. Individual enantiomers of general formula I may be obtained by a variety of methods known to the skilled chemists, such as from a corresponding optically pure intermediate or via resolution. A preferred method of resolution is via the (+)-pseudoephedrine salt (see Preparation 2 herein). Alternatively, chiral compounds of formula I may be prepared from chiral compounds of formula II as discussed below.

A number of compounds of formula II are known in the art (see EP274234-B1 and WO9113054). Other compounds of formula II can be prepared in analogous fashion.

Chiral compounds of formula IIa may be prepared from compounds of formula XVI as shown in reaction scheme 13.

Scheme 13



Compounds of formula II may be prepared by treatment of compounds of formula XVII with R^1-X^z (where X^z is halogen) under strongly basic conditions optionally with an additive in an aprotic solvent.

Typical reaction conditions comprise firstly treating compounds of formula XVII with at least a two-fold excess of a strong base (for example lithium diisopropylamide, lithium, sodium or potassium hexamethyldisilazide, an alkyllithium, alkylmagnesium or phosphazene base) in a non-protic solvent (for example THF, diethyl ether, hexane, heptane or ethylbenzene or a mixture of these solvents), optionally with an additive (for example TMEDA, DMPU or HMPA) at temperatures between –78 °C and room temperature, and then adding R^1-X^z (for example allyl bromide, propyl bromide or methyl iodide) at –78 °C and stirring overnight whilst warming to room temperature. A suitable work-up gives the compounds of formula II.

Preferred reaction conditions for preparing compounds of formula II where R^1 is allyl, are treatment of compounds of formula XVII (1 molar equivalent) with lithium

diisopropylamide (2.3 equivalents) in a mixture of THF, n-heptane and ethylbenzene at – 10 °C for 4 hours. Allyl bromide (1.2 equivalents) is then added at – 10 °C, the reaction mixture stirred at – 10 °C for 2 hours and then warmed to 20 °C over 4 hours and stirred for a further 15 hours at 20 °C .

Resolution to compounds of formula IIb may be performed directly from compounds of formula II, however a preferred process forms an amine salt of formula XIX (i.e. R^5-NH_3^+) followed by recrystallisation to effect purification.

Typically salts are the triethylamine, isopropylamine, triethanolamine or cyclohexylamine salts. Typical reaction conditions comprise reacting II with the required amine in a suitable solvent (for example hexane, heptane or toluene) at elevated temperatures with cooling to induce crystallisation over 24 hours, followed by recrystallisation from the same or a different solvent, optionally at elevated temperatures.

A preferred salt is the cyclohexylamine salt. Preferred reaction conditions comprise treatment compounds of formula II (1 molar equivalent) with cyclohexylamine (1. equivalent) at 20 °C in heptane followed by recrystallisation from ethyl acetate at 70 °C before cooling to 50 °C over 2 hours to induce crystallisation. The mixture is then cooled to 20 °C over 2 hours and stirred for 0.5 hours.

Compounds of formula XX (where $R^7NH_3^+$ is a chiral cation) may be prepared from compounds of formula XIX by acidification in a suitable solvent system, optionally including water using a strong acid followed by classical resolution of the resulting carboxylic acid.

Typical reaction conditions comprise treatment of compounds of formula XX in a biphasic system of water and an immiscible organic solvent (for example heptane, toluene, ethyl acetate, diethyl ether or dichloromethane) with a strong acid (for example hydrochloric acid, sulfuric acid, *para*-toluenesulfonic acid, trifluoroacetic acid or phosphoric acid) at temperatures between 0 °C and 100 °C to give the free carboxylic acid, followed by treatment with a non-racemic chiral amine base (for example α-methylbenzylamine, pseudoephedrine, ephedrine, norephedrine, a cinchona alkaloid, amino acid esters, amino alcohols such as 2-pyrrolidinemethanol or quinuclidin-3-ol) optionally in a solvent (for example an ester, an alkane, an aromatic hydrocarbon, a

haloalkane, an ether or an alcohol) at temperatures between 0 °C and 150 °C to give the crude salt. This salt is then recrystallised one or more times from the same or a different solvent at temperatures between 0 °C and 150 °C, to give the chiral salt.

A preferred salt is the pseudoephedrine salt. Preferred reaction conditions comprise treatment of a suspension of a compound of formula XX in a water/n-heptane mixture with dilute hydrochloric acid at room temperature until the pH of the aqueous phase is pH 3 to give the free acid, followed by treatment of the resulting carboxylic acid with (1*S*, 2*S*)-(+)-pseudoephedrine (1 equivalent) in *n*-heptane at 80 °C followed by cooling to 45 °C over 2 hours to induce crystallisation then cooling to 20 °C over 2 hours and stirring for 4 hours. Recrystallisation from n-heptane was then carried out at 80 °C followed by cooling to 60 °C over 2 hours to induce crystallisation then cooling to 20 °C over 2 hours and stirring for 1.5 hours.

Compounds of formula IIa may be prepared from compounds of formula XX by acidification in a suitable solvent system, optionally including water using a strong acid.

Typical reaction conditions comprise treatment of compounds of formula XX in a biphasic system of water and an immiscible organic solvent (for example heptane, toluene, ethyl acetate, diethyl ether or dichloromethane) and a strong acid (for example hydrochloric acid, sulfuric acid, *para*-toluenesulfonic acid, trifluoroacetic acid or phosphoric acid) at temperatures between 0 °C and 100 °C to give compounds of formula IIa.

Preferred reaction conditions comprise treatment of a suspension of a compound of formula XX in a water/n-heptane mixture with dilute hydrochloric acid at room temperature until the pH of the aqueous phase is pH 3 to give the free acid.

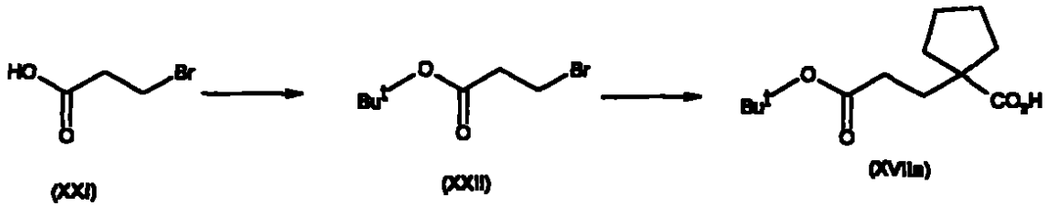
Compounds of formula IIa may be prepared by hydrogenation of the corresponding compound where R^1 is unsaturated. Typical reaction conditions comprise stirring under an atmosphere of hydrogen in a suitable solvent in the presence of a catalyst (for example palladium, platinum, nickel, iridium, rhodium or ruthenium optionally adsorbed on a suitable support such as carbon, alumina, barium sulfate, calcium carbonate or as a salt such as palladium hydroxide, or mixtures of salts such as H_2PtCl_6 and $SnCl_2.2H_2O$ or as a complex such as Wilkinson's catalyst, Crabtree's catalyst, $Co_2(CO)_8$, $RhH(PPh_3)_4$, or

$[Co(CN)_4]^{2-}$) at temperatures between room temperature and 150 °C and hydrogen pressures between 30 and 150 psi.

In a preferred process, compounds of formula IIa where R^1 is propyl, may be prepared by hydrogenation of the corresponding allyl compound. Preferred reaction conditions comprise stirring an ethanol solution of the unsaturated carboxylic acid under a hydrogen atmosphere with 9% w/w of 5% palladium on carbon at room temperature for 24 hours.

Compounds of formula XVIIa, i.e. compounds of formula XVII where Prot is *tert*-butyl, may be prepared in two steps from commercially available compounds of formula XXI according to reaction scheme 14.

Scheme 14



Compounds of formula XXII may be prepared from compounds of formula XXI by treating XXI with a source of *tert*-butyl cation or *tert*-butoxide using a suitable catalyst and/or dehydrating agent in a suitable anhydrous solvent optionally at elevated temperature, or by activation of the carboxylic acid followed by reaction with *tert*-butanol.

Typical reaction conditions comprise treating compounds of formula XXI with catalytic quantities of an acid (for example phosphoric acid, hydrochloric acid, sulfuric acid, nitric acid, *para*-toluenesulfonic acid or trifluoroacetic acid) in the presence of isobutylene, *tert*-butanol, *tert*-butyl halides or *tert*-butyl ether in a suitable solvent (for example dichloromethane, THF or toluene) between –20 and 150 °C for up to 48 hours.

Alternative reaction conditions comprise treating compounds of formula XXI with a combination of a tertiary amine base (for example triethylamine, Hunig's base, pyridine or NMM) and a dehydrating agent (for example dicyclohexylcarbodiimide, an alkyl chloroformate, phenyl dichlorophosphate, 2-chloro-1,3,5-trinitrobenzene, di-2-pyridyl carbonate, 1,1'-carbonyl diimidazole, (trimethylsilyl)ethoxyacetylene, *N,N'*-carbonylbis(3-

methylimidazolium) triflate or diethyl azodicarboxylate) and triphenyl phosphine followed by addition of *tert*-butanol, optionally with a catalyst such as 4-dimethylaminopyridine in a suitable solvent (for example dichloromethane, THF or toluene) between –20 and 150 °C for up to 48 hours.

Still further reaction conditions comprise converting compounds of formula XXI to the acid chloride using thionyl chloride, oxalyl chloride or Ghosez's reagent optionally in the presence of a tertiary amine base (for example triethylamine, Hunig's base, pyridine or NMM) followed by *treatment with tert*-butanol, optionally in the presence of a catalyst such as 4-dimethylaminopyridine in a suitable solvent (for example dichloromethane, THF or toluene) between –20 and 150 °C for up to 48 hours.

Preferred reaction conditions comprise treating compounds of formula XXI with isobutylene (5 equivalents), concentrated sulfuric acid (0.15 equivalents) and *tert*-butanol (0.16 equivalents) in dichloromethane stirring at –10 to 25 °C for 24 hours.

Compounds of formula XVIIa may be prepared from compounds of formula XXII by *treatment with cyclopentane carboxylic acid under strongly basic conditions in an aprotic* solvent, optionally in the presence of an additive.

Typical reaction conditions comprise treating cyclopentane carboxylic acid with at least a two-fold excess of a strong base (for example lithium diisopropylamide, lithium, sodium or potassium hexamethyldisilazide, an alkyllithium, alkylmagnesium or phosphazene base) in a non-protic solvent (for example THF, diethyl ether, hexane, heptane or ethylbenzene or a mixture of these), optionally in the presence of an additive (for example TMEDA, DMPU or HMPA) at temperatures ranging from –78 °C to 50 °C temperature for up to 24 hours, followed by the addition of the compound of formula XXII and reaction at –20 °C for up to 24 hours and suitable workup.

Preferred reaction conditions comprise treating of cyclopentane carboxylic acid with lithium diisopropylamide (2.15 equivalents) in a mixture of THF, n-heptane and ethylbenzene at –15 °C for 3 hours followed by treatment with *tert*-butyl-3-bromopropionate (1.06 equivalents) in THF at –15 °C for 15 hours then warming to room temperature.

Other compounds of formula II are either available from commercial sources, known in the prior art, or can be prepared from compounds known in the prior art by using methods known in the prior art or by using methods described herein (see Examples and Preparations Sections).

A pharmaceutically acceptable salt of a compound of the formula (I) may be readily prepared by mixing together solutions of a compound of the formula (I) and the desired acid or base, as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent.

The compounds of the invention may also be combined with one or more of the following for the treatment of FSD:

1) One or more naturally occurring or synthetic prostaglandins or esters thereof. Suitable prostaglandins for use herein include compounds such as alprostadil, prostaglandin E_1,prostaglandin E_0, 13, 14 - dihydroprosta glandin E_1, prostaglandin E_2, eprostinol, natural synthetic and semi-synthetic prostaglandins and derivatives thereof including those described in WO-00033825 and/or US 6,037,346 issued on 14th March 2000 all incorporated herein by reference, PGE_0, PGE_1, PGA_1, PGB_1, $PGF_1\alpha$, 19-hydroxy PGA_1, 19-hydroxy - PGB_1, PGE_2, PGB_2, 19-hydroxy-PGA_2, 19-hydroxy-PGB_2, $PGE_2\alpha$, carboprost tromethamine dinoprost, tromethamine, dinoprostone, lipo prost, gemeprost, metenoprost, sulprostune, tiaprost and moxisylate.
2) One or more α - adrenergic receptor antagonist compounds also known as α - adrenoceptors or α-receptors or α-blockers. Suitable compounds for use herein include: the α-adrenergic receptor blockerss as described in PCT application WO99/30697 published on 14th June 1998, the disclosures of which relating to α-adrenergic receptors are incorporated herein by reference and include, selective α_1-adrenoceptor or α_2-adrenoceptor blockers and non-selective adrenoceptor blockers, suitable α_1-adrenoceptor blockers include: phentolamine, phentolamine mesylate, trazodone, alfuzosin, indoramin, naftopidil, tamsulosin, dapiprazole, phenoxybenzamine, idazoxan, efaraxan, yohimbine, rauwolfa alkaloids, Recordati 15/2739, SNAP 1069, SNAP 5089, RS17053, SL 89.0591, doxazosin, terazosin, abanoquil and prazosin; α_2-blocker blockers from US 6,037,346 [14th March 2000] dibenamine, tolazoline, trimazosin and dibenamine; α-adrenergic receptors as described in US patents: 4,188,390; 4,026,894;

3,511,836; 4,315,007; 3,527,761; 3,997,666; 2,503,059; 4,703,063; 3,381,009; 4,252,721 and 2,599,000 each of which is incorporated herein by reference; α_2-Adrenoceptor blockers include: clonidine, papaverine, papaverine hydrochloride, optionally in the presence of a cariotonic agent such as pirxamine.

3) One or more NO-donor (NO-agonist) compounds. Suitable NO-donor compounds for use herein include organic nitrates, such as mono- di or tri-nitrates or organic nitrate esters including glyceryl brinitrate (also known as *nitroglycerin*), *isosorbide 5-mononitrate*, *isosorbide dinitrate*, *pentaerythritol* tetranitrate, erythrityl tetranitrate, sodium nitroprusside (SNP), 3-morpholinosydnonimine molsidomine, S-nitroso- N-acetyl penicilliamine (SNAP) S-nitroso-N-glutathione (SNO-GLU), N-hydroxy - L-arginine, amylnitrate, linsidomine, linsidomine chlorohydrate, (SIN-1) S-nitroso - N-cysteine, diazenium diolates, (NONOates), 1,5-pentanedinitrate, L-arginene, ginseng, zizphi fructus, molsidomine, Re – 2047, nitrosylated maxisylyte derivatives such as NMI-678-11 and NMI-937 as described in published PCT application WO 0012075.

4) One or more potassium channel openers or modulators. Suitable potassium channel openers/modulators for use herein include nicorandil, cromokalim, levcromakalim, lemakalim, pinacidil, diazoxide, minoxidil, charybdotoxin, glyburide, 4-amini pyridine, $BaCl_2$.

5) One or more dopaminergic agents, preferably apomorphine or a selective D2, D3 or D2/D_3agonist such as, pramipexole and ropirinol (as claimed in WO-0023056),PNU95666 (as claimed in WO-0040226).

6) One or more vasodilator agents. Suitable vasodilator agents for use herein include nimodepine, pinacidil, cyclandelate, isoxsuprine, chloroprumazine, halo peridol, Rec 15/2739, trazodone.

7) One or more thromboxane A2 agonists.

8) One or more CNS active agents.

9) One or more ergot alkoloids. Suitable ergot alkaloids are described in US patent 6,037,346 issued on 14th March 2000 and include acetergamine, brazergoline, bromerguride, clanergoline, delorgotrile, disulergine, ergonovine maleate, ergotamine tartrate, etisulergine, lergotrile, lysergide, mesulergine, metergoline, metergotamine, nicergoline, pergolide, propisergide, proterguride, terguride.

10) One or more compounds which modulate the action of natruretic factors in particular atrial naturetic factor (also known as atrial naturetic peptide), B type and C type naturetic factors such as inhibitors or neutral endopeptidase.

11) One or more compounds which inhibit angiotensin-converting enzyme such as enapril, and combined inhibitors of angiotensin-converting enzyme and neutral endopeptidase such as omapatrilat.

12) One or more angiotensin receptor antagonists such as losartan.

13) One or more substrates for NO-synthase, such as L-arginine.

14) One or more calcium channel blockers such as amlodipine.

15) One or more antagonists of endothelin receptors and inhibitors or endothelin-converting enzyme.

16) One or more cholesterol lowering agents such as statins (e.g. atorvastatin/ Lipitor- trade mark) and fibrates.

17) One or more antiplatelet and antithrombotic agents, e.g. tPA, uPA, warfarin, hirudin and other thrombin inhibitors, heparin, thromboplastin activating factor inhibitors.

18) One or more insulin sensitising agents such as rezulin and hypoglycaemic agents such as glipizide.

19) L-DOPA or carbidopa.

20) One or more acetylcholinesterase inhibitors such as donezipil.

21) One or more steroidal or non-steroidal anti-inflammatory agents.

22) One or more estrogen receptor modulators and/or estrogen agonists and/or estrogen antagonists, preferably raloxifene or lasofoxifene, (-)-cis-6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene-2-ol and pharmaceutically acceptable salts thereof the preparation of which is detailed in WO 96/21656.

23) One or more modulators of cannabinoid receptors.

24) One or more of an NPY (neuropeptide Y) inhibitor, more particularly NPY1 or NPY5 inhibitor, preferably NPY1 inhibitor, preferably said NPY inhibitors (including NPY Y1 and NPY Y5) having an IC50 of less than 100nM , more preferably less than 50nM. An assay for identifying NPY inhibitors is presented in WO-A-98/52890 (see page 96, lines 2 to 28).

25) One or more of vasoactive intestinal protein (VIP), VIP mimetic, VIP analogue, more particularly mediated by one or more of the VIP receptor subtypes VPAC1,VPAC or PACAP (pituitory adenylate cyclase activating peptide), one or more of a VIP receptor agonist or a VIP analogue (eg Ro-125-1553) or a VIP fragment, one or more of a α-adrenoceptor antagonist with VIP combination (eg Invicorp, Aviptadil).

26) One or more of a melanocortin receptor agonist or modulator or melanocortin ehancer, such as melanotan II, PT-14, PT-141 or compounds claimed in WO-09964002, WO-00074679, WO-09955679, WO-00105401, WO-00058361, WO-00114879, WO-00113112, WO-09954358.

27) One or more of a serotonin receptor agonist, antagonist or modulator, more particularly agonists, antagonists or modulators for 5HT1A (including VML 670), 5HT2A, 5HT2C, 5HT3 and/or 5HT6 receptors, including those described in WO-09902159, WO-00002550 and/or WO-00028993.

28) One or more of a testosterone replacement agent (inc dehydroandrostendione), testosternone (Tostrelle), dihydrotestosterone or a testosterone implant.

29) One or more of estrogen, estrogen and medroxyprogesterone or medroxyprogesterone acetate (MPA) (i.e. as a combination), or estrogen and methyl testosterone hormone replacement therapy agent (e.g. HRT especially Premarin, Cenestin, Oestrofeminal, Equin, Estrace, Estrofem, Elleste Solo, Estring, Eastraderm TTS, Eastraderm Matrix, Dermestril, Premphase, Preempro, Prempak, Premique, Estratest, Estratest HS, Tibolone).

30) One or more of a modulator of transporters for noradrenaline, dopamine and/or serotonin, such as bupropion, GW-320659.

31) One or more of a purinergic receptor agonist and/or modulator.

32) One or more of a neurokinin (NK) receptor antagonist, including those described in WO-09964008.

33) One or more of an opioid receptor agonist, antagonist or modulator, preferably agonists for the ORL-1 receptor.

34) One or more of an agonist or modulator for oxytocin/vasopressin receptors, preferably a selective oxytocin agonist or modulator.

35) One or more of a PDE inhibitor, more particularly a PDE 2, 3, 4, 5, 7 or 8 inhibitor, preferably PDE2 or PDE5 inhibitor and most preferably a PDE5 inhibitor (see hereinafter), said inhibitors preferably having an IC50 against the respective enzyme of less than 100nM. Suitable cGMP PDE5 inhibitors for the use according to the present invention include:

the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in EP-A-0463756; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in EP-A-0526004; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international patent application WO 93/06104; the isomeric pyrazolo [3,4-d]pyrimidin-4-ones disclosed in published international

patent application WO 93/07149; the quinazolin-4-ones disclosed in published international patent application WO 93/12095; the pyrido [3,2-d]pyrimidin-4-ones disclosed in published international patent application WO 94/05661; the purin-6-ones disclosed in published international patent application WO 94/00453; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international patent application WO 98/49166; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international patent application WO 99/54333; the pyrazolo [4,3-d]pyrimidin-4-ones disclosed in EP-A-0995751; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international patent application WO 00/24745; the pyrazolo [4,3-d]pyrimidin-4-ones disclosed in EP-A-0995750; the compounds disclosed in published international application WO95/19978; the compounds disclosed in published international application WO 99/24433 and the compounds disclosed in published international application WO 93/07124. The pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international application WO 01/27112; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international application WO 01/27113; the compounds disclosed in EP-A-1092718 and the compounds disclose din EP-A-1092719.

Further suitable PDE5 inhibitors for the use according to the present invention include: 5-[2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (sildenafil) also known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulphonyl]-4-methylpiperazine (see EP-A-0463756); 5-(2-ethoxy-5-morpholinoacetylphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see EP-A-0526004); 3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-n-propoxyphenyl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see WO98/49166); 3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see WO99/54333); (+)-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxy-1(R)-methylethoxy)pyridin-3-yl]-2-methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, also known as 3-ethyl-5-{5-[4-ethylpiperazin-1-ylsulphonyl]-2-([(1R)-2-methoxy-1-methylethyl]oxy)pyridin-3-yl}-2-methyl-2,6-dihydro-7H-pyrazolo[4,3-d] pyrimidin-7-one (see WO99/54333); 5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, also known as

1-[6-ethoxy-5-[3-ethyl-6,7-dihydro-2-(2-methoxyethyl)-7-oxo-2H-pyrazolo[4,3-d]pyrimidin-5-yl]-3-pyridylsulphonyl]-4-ethylpiperazine (see WO 01/27113, Example 8); 5-[2-*iso*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylpiperidin-4-yl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see WO 01/27113, Example 15); 5-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-phenyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see WO 01/27113, Example 66); 5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-isopropyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (seeWO 01/27112, Example 124); 5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see WO 01/27112, Example 132); (6R,12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl) -pyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione (IC-351), i.e. the compound of examples 78 and 95 of published international application WO95/19978, as well as the compound of examples 1, 3, 7 and 8; 2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one (vardenafil) also known as 1-[[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f]-as-triazin-2-yl)-4-ethoxyphenyl]sulphonyl]-4-ethylpiperazine, i.e. the compound of examples 20, 19, 337 and 336 of published international application WO99/24433; and the compound of example 11 of published international application WO93/07124 (EISAI); and compounds 3 and 14 from Rotella D P, *J. Med. Chem.*, 2000, 43, 1257.

Still other suitable PDE5 inhibitors include:4-bromo-5-(pyridylmethylamino)-6-[3-(4-chlorophenyl)-propoxy]-3(2H)pyridazinone; 1-[4-[(1,3-benzodioxol-5-ylmethyl)amiono]-6-chloro-2-quinazolinyl]-4-piperidine-carboxylic acid, monosodium salt; (+)-cis-5,6a,7,9,9,9a-hexahydro-2-[4-(trifluoromethyl)-phenylmethyl-5-methyl-cyclopent-4,5]imidazo[2,1-b]purin-4(3H)one; furazlocillin; cis-2-hexyl-5-methyl-3,4,5,6a,7,8,9,9a- octahydrocyclopent[4,5]-imidazo[2,1-b]purin-4-one; 3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6- carboxylate; 3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6-carboxylate; 4-bromo-5-(3-pyridylmethylamino)-6-(3-(4-chlorophenyl) propoxy)-3- (2H)pyridazinone; l-methyl-5(5-morpholinoacetyl-2-n-propoxyphenyl)-3-n-propyl-1,6-dihydro- 7H-pyrazolo(4,3-d)pyrimidin-7-one; 1-[4-[(1,3-benzodioxol-5-ylmethyl)amino]-6-chloro-2- quinazolinyl]-4-piperidinecarboxylic acid, monosodium salt;

Pharmaprojects No. 4516 (Glaxo Wellcome); Pharmaprojects No. 5051 (Bayer); Pharmaprojects No. 5064 (Kyowa Hakko; see WO 96/26940); Pharmaprojects No. 5069 (Schering Plough); GF-196960 (Glaxo Wellcome); E-8010 and E-4010 (Eisai); Bay-38-3045 & 38-9456 (Bayer) and Sch-51866.

If a combination of active agents are administered, then they may be administered simultaneously, separately or sequentially.

The compounds of the invention can be administered alone but, in human therapy will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

For example, the compounds of the invention, can be administered orally, buccally or sublingually in the form of tablets, capsules (including soft gel capsules), ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed-, modified-, sustained-, dual-, controlled-release or pulsatile delivery applications. The compounds of the invention may also be administered via fast dispersing or fast dissolving dosage forms.

Modified release and pulsatile release dosage forms may contain excipients such as those detailed for immediate release dosage forms together with additional excipients that act as release rate modifiers, these being coated on and/or included in the body of the device. Release rate modifiers include, but are not exclusively limited to, hydroxypropylmethyl cellulose, methyl cellulose, sodium carboxymethylcellulose, ethyl cellulose, cellulose acetate, polyethylene oxide, Xanthan gum, Carbomer, ammonio methacrylate copolymer, hydrogenated castor oil, carnauba wax, paraffin wax, cellulose acetate phthalate, hydroxypropylmethyl cellulose phthalate, methacrylic acid copolymer and mixtures thereof. Modified release and pulsatile release dosage forms may contain one or a combination of release rate modifying excipients. Release rate modifying excipients may be present both within the dosage form i.e. within the matrix, and/or on the dosage form, i.e. upon the surface or coating.

Fast dispersing or dissolving dosage formulations (FDDFs) may contain the following ingredients: aspartame, acesulfame potassium, citric acid, croscarmellose sodium,

crospovidone, diascorbic acid, ethyl acrylate, ethyl cellulose, gelatin, hydroxypropylmethyl cellulose, magnesium stearate, mannitol, methyl methacrylate, mint flavouring, polyethylene glycol, fumed silica, silicon dioxide, sodium starch glycolate, sodium stearyl fumarate, sorbitol, xylitol. The terms dispersing or dissolving as used herein to describe FDDFs are dependent upon the solubility of the drug substance used, i.e. where the drug substance is insoluble a fast dispersing dosage form can be prepared and where the drug substance is soluble a fast dissolving dosage form can be prepared.

The compositions of the invention may be administered by direct injection. The composition may be formulated for parenteral, mucosal, intramuscular, intravenous, subcutaneous, ocular, intraocular or transdermal administration. Depending upon the need, the agent may be administered at a dose of from 0.01 to 30 mg/kg body weight, such as from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight.

The term "administered" includes delivery by viral or non-viral techniques. Viral delivery mechanisms include but are not limited to adenoviral vectors, adeno-associated viral (AAV) vectos, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery mechanisms include lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by direct injection. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered topically (preferably to the genitalia). In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by inhalation. In addition or in the alternative the compositions (or component parts thereof) of the present invention may also be administered by one or more of: a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution such as by an oral route, or by a parenteral route where delivery is by an injectable form, such as, for example, by a rectal, ophthalmic (including intravitreal or intracameral), nasal, topical (including buccal and sublingual), intrauterine, vaginal or parenteral (including subcutaneous, intraperitoneal, intramuscular, intravenous, intradermal,

intracranial, intratracheal, and epidural) transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, or parenteral (e.g., intravenous, intraspinal, subcutaneous, transdermal or intramuscular) route.

By way of example, the pharmaceutical compositions of the invention may be administered in accordance with a regimen of 1 to 10 times per day, such as once or twice per day. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy.

Hence, the term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular or subcutaneous route.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethyl cellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

The compounds of the invention can also be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly,

intraurethrally intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. In addition, they may be administered in the form of an implant. For such parenteral administration they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art. Parenteral formulations may be formulated for immediate-, delayed-, modified-, sustained-, dual-, controlled-release or pulsatile delivery.

The following dosage levels and other dosage levels herein are for the average human subject having a weight range of about 65 to 70 kg. The skilled person will readily be able to determine the dosage levels required for a subject whose weight falls outside this range, such as children and the elderly.

For oral and parenteral administration to human patients, the daily dosage level of the compounds of the invention or salts or solvates thereof will usually be from 10 to 1000 mg (in single or divided doses).

Thus, for example, tablets or capsules of the compounds of the invention or salts or solvates thereof may contain from 5 to 1000mg, such as 5 mg to 500 mg of active compound for administration singly or two or more at a time, as appropriate. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention. The skilled person will also appreciate that, in the treatment of certain conditions (including FSD), compounds of the invention may be taken as a single dose on an "as required" basis (i.e. as needed or desired).

The compounds of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane,

dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A [trade mark] or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA [trade mark]), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 1 to 50 mg of a compound of the invention for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 1 to 50 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, compounds of the invention can be administered in the form of a suppository or pessary, or they may be applied topically (preferably to the genitalia) in the form of a gel, hydrogel, lotion, solution, cream, ointment or dusting powder. The compounds of the invention may also be dermally administered. The compounds of the invention may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the ocular, pulmonary or rectal routes.

For ophthalmic use, compounds can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin (preferably to the genitalia), compounds of the invention can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a

mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The compounds of the invention may also be used in combination with a cyclodextrin. Cyclodextrins are known to form inclusion and non-inclusion complexes with drug molecules. Formation of a drug-cyclodextrin complex may modify the solubility, dissolution rate, bioavailability and/or stability property of a drug molecule. Drug-cyclodextrin complexes are generally useful for most dosage forms and administration routes. As an alternative to direct complexation with the drug the cyclodextrin may be used as an auxiliary additive, e.g. as a carrier, diluent or solubiliser. Alpha-, beta- and gamma-cyclodextrins are most commonly used and suitable examples are described in WO-A-91/11172, WO-A-94/02518 and WO-A-98/55148.

In a preferred embodiment, the compounds of the invention are delivered systemically (such as orally, buccally and sublingually), more preferably orally. Preferably such systemic (most preferably oral) administration is used to treat female sexual dysfunction, preferably FSAD.

Thus in a particularly preferred embodiment, there is provided the use of the compounds of the invention in the manufacture of a systemically delivered (preferably orally delivered) medicament for the treatment or prophylaxis of FSD, more preferably FSAD.

A preferred oral formulation uses immediate release tablets; or fast dispersing or dissolving dosage formulations (FDDFs).

In a further preferred embodiment, the compounds of the invention are administered topically, preferably directly to the female genitalia, especially the vagina.

Since NEP is present throughout the body, it is very unexpected that the compounds of the invention can be administered systemically and achieve a therapeutic response in the female genitalia without provoking intolerable (adverse) side effects. Thus in the *in vivo* (rabbit) results hereafter, the compounds of the invention administered systemically increased genital blood flow, upon sexual arousal (mimicked by pelvic nerve stimulation) without adversely affecting cardiovascular parameters, such as causing a significant

hypotensive or hypertensive.

Preferably the compounds of the invention are administered for the treatment of FSD in the sexually stimulated patient (by sexual stimulation we mean to include visual, auditory or tactile stimulation).The stimulation can be before, after or during said administration.

Thus the compounds of the invention enhance the pathways/mechanisms that underlie sexual arousal in the female genitalia restoring or improving the sexual arousal response to sexual stimulation.

Thus a preferred embodiment provides the use of a compound of the invention in the preparation of a medicament for the treatment or prophyaxis of FSD in the stimulated patient.

For veterinary use, a compound of the invention, is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention . "Active ingredient" means a compound of the invention.

Formulation 1: A tablet is prepared using the following ingredients:

	weight/mg
Active ingredient	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	5
Total	665

the components are blended and compressed to form tablets.

Formulation 2: An intravenous formulation may be prepared as follows:

Active ingredient	100mg
Isotonic saline	1,000ml

Typical formulations useful for administering the compounds of the invention topically to the genitalia are as follows:

Formualtion 3: A spray

Active ingredient (1.0%) in isopropanol (30%) and water.

Formulation 4: A foam

Active ingredient, acetic acid glacial, benzoic acid, cetyl alcohol, methyl parahydroxybenzoate, phosphoric acid, polyvinyl alcohol, propylene glycol, sodium carboxymethylcellulose, stearic acid, diethyl stearamide, van Dyke perfume No. 6301, purified water and isobutane.

Formulation 5: A gel

Active ingredient, docusate sodium BP, isopropyl alcohol BP, propylene glycol, sodium hydroxide, carbomer 934P, benzoic acid and purified water.

Formulation 6: A Cream

Active ingredient, benzoic acid, cetyl alcohol, lavender, compound 13091, methylparaben, propylparaben, propylene glycol, sodium carboxymethylcellulose, sodium lauryl sulfate, stearic acid, triethanolmine, acetic acid glacial, castor oil, potassium hydroxide, sorbic acid and purified water.

Formulation 7: A pessary

Active ingredient, cetomacrogol 1000 BP, citric acid, PEG 1500 and 1000 and purified water.

The invention additionally includes:

(i) A pharmaceutical composition including a compound of the invention, together with a pharmaceutically acceptable excipient, diluent or carrier.

(ii) A compound of the invention for use as a medicament.

(iii) A method of treating FSD in a mammal including treating said mammal with an effective amount of a compound of the invention.

(iv) An FSD treating pharmaceutical composition comprising a compound of the invention together with a pharmaceutically acceptable excipient, diluent or carrier.

(v) A compound of the invention for treating FSD.

The invention is illustrated by the following non-limiting examples in which the following abbreviations and definitions are used herein below and also throughout the specification:

Arbacel®	filter agent
br	broad
Boc	*tert*-butoxycarbonyl
CDI	carbonyldiimidazole
δ	chemical shift
d	doublet
Δ	heat
DCCI	dicyclohexylcarbodiimide
DCM	dichloromethane
DMF	*N,N*-dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	dimethylsulfoxide
ES^+	electrospray ionisation positive scan
ES^-	electrospray ionisation negative scan
Ex	Example
h	hours
HMPA	hexamethylphosphoramide
HOBt	1-hydroxybenzotriazole
HPLC	high pressure liquid chromatography
m/z	mass spectrum peak
min	minutes
MS	mass spectrum
NMR	nuclear magnetic resonance
Prec	precursor
Prep	preparation
q	quartet
s	singlet
t	triplet
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid

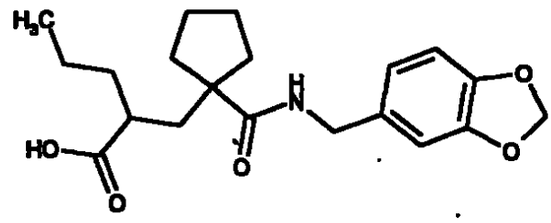
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	*N,N,N',N'*-tetramethylethylenediamine
TS^+	thermospray ionisation positive scan
WSCDI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

^1H Nuclear magnetic resonance (NMR) spectra were in all cases consistent with the proposed structures. Characteristic chemical shifts (δ) are given in parts-per-million downfield from tetramethylsilane using conventional abbreviations for designation of major peaks: e.g. s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The following abbreviations have been used for common solvents: $CDCl_3$, deuterochloroform; DMSO, dimethylsulphoxide. The abbreviation psi means pounds per square inch and LRMS means low resolution mass spectrometry. Where thin layer chromatography (TLC) has been used it refers to silica gel TLC using silica gel 60 F_{254} plates, R_f is the distance travelled by a compound divided by the distance travelled by the solvent front on a TLC plate.

The powder X-ray diffraction (PXRD) patterns were determined using a Siemens D5000 powder X-ray diffractometer fitted with a theta-theta goniometer, automatic beam divergence slits, a secondary monochromator and a scintillation counter. The specimen was rotated whilst being irradiated with copper K-alpha1 X-rays (Wavelength = 1.5046 Angstroms) filtered with a graphite monochromator (λ = 0.15405nm) with the X-ray tube operated at 40 kV/40mA. The main peaks (in degrees 2θ) of the PXRD patterns for the various solid forms are illustrated.

Example 1

2-({1-[(1,3-Benzodioxol-5-ylamino)carbonyl]cyclopentyl}methyl)pentanoic acid

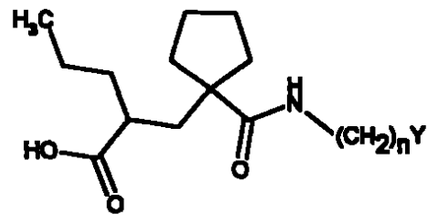


Trifluoroacetic acid (5ml) was added to a solution of the *tert*-butyl ester from preparation 34 (130mg, 0.31mmol) in dichloromethane (5ml), and the solution stirred at room temperature for 4 hours. The reaction mixture was concentrated under reduced pressure

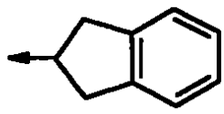
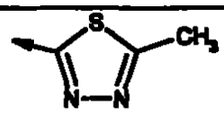
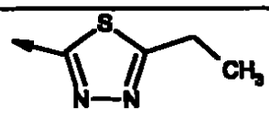
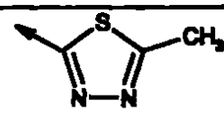
and the residue azeotroped with toluene and dichloromethane to give the title compound as a clear oil, 112 mg, ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.83 (t, 3H), 1.22-1.40 (m, 3H), 1.50-1.72 (m, 8H), 1.95 (m, 1H), 2.10 (m, 2H), 2.19 (m, 1H), 4.30 (m, 2H), 5.93 (s, 2H), 5.99 (bs, 1H), 6.74 (m, 3H); LRMS: m/z 380 (MH^-).

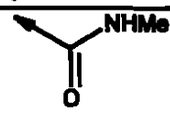
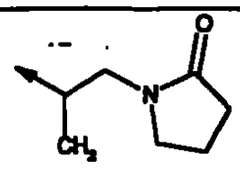
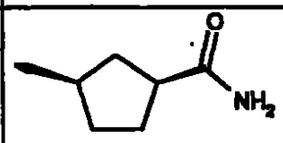
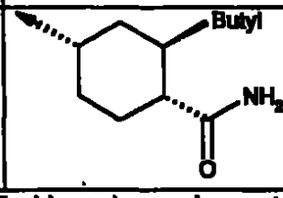
Examples 2 to 9

Compounds of formula Ic, i.e. compounds of general formula I where R^1 is propyl, were prepared from the corresponding *tert*-butyl ester following a similar procedure to that described in Example 1 from the precursor indicated.



(Ic)

Ex	Prec	n	Y	Data
2[1]	Prep 35	0		^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (s, 3H), 1.17-2.04 (m, 14H), 2.27-2.38 (m, 1H), 2.64-2.80 (m, 2H), 3.20-3.31 (m, 2H), 4.60-4.72 (m, 1H), 5.97 (d, 1H), 7.03-7.18 (m, 4H). LRMS : m/z 343.8 (M^+).
3[2,3]	Prep 36	0		^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.30-1.42 (m, 4H), 1.59-1.81 (m, 7H), 2.18 (m, 1H), 2.30 (m, 1H), 2.42 (m, 1H), 2.55 (m, 1H), 2.61 (s, 3H). LRMS : m/z 324 (MH^+). Mp 184-186° C; Anal. Found: C, 55.50; H, 7.22; N, 12.61. $C_{16}H_{25}N_3O_3S$ requires C, 55.36; H, 7.14; N, 12.91%.
4[3]	Prep 37	0		^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.92 (t, 3H), 1.35 (t, 3H), 1.25-1.80 (m, 11H), 2.20-2.50 (m, 4H), 2.95 (q, 2H), 12.10 (bs, 1H); LRMS : m/z 339.8 (MH^+) Anal. Found: C, 56.46; H, 7.46; N, 12.36. $C_{16}H_{25}N_3O_3S$ requires C, 56.62; H, 7.44; N, 12.37%.
5[2]	Prep 38	1		^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 1.20-1.70 (m, 11H), 1.90-2.20 (m, 3H), 2.25 (m, 1H), 2.70 (s, 3H), 4.75 (m, 2H), 7.10 (bs, 1H). LRMS : m/z 340.6 (MH^+)

Ex	Prec	n	Y	Data
6[2]	Prep 39	2		^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.25-1.40 (m, 3H), 1.41-1.70 (m, 8H), 1.92 (m, 1H), 2.00-2.18 (m, 2H), 2.38 (m, 1H), 2.42 (t, 2H), 2.80 (d, 3H), 3.40-3.60 (m, 2H), 6.50 (bs, 1H), 6.74 (bs, 1H). LRMS : m/z 313.2 (MH^+)
7	Prep 40	0		^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.19 (d, 3H), 1.21-1.69 (m, 11H), 1.89-2.10 (m, 5H), 2.30 (m, 1H), 2.41 (m, 2H), 2.95 (m, 1H), 3.35 (m, 1H), 3.63 (m, 2H), 4.20 (m, 1H), 6.58-6.70 (m, 1H). LRMS : m/z 353.1 (MH^+)
8	Prep 41	0		^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.39 (m, 3H), 1.41-2.10 (m, 1H), 2.80 (m, 1H), 4.35 (m, 17H), 5.81 (d, 1H), 6.30 (bs, 0.5H), 6.43 (bs, 0.5H), 7.40 (bd, 0.5H), 7.81 (bd, 0.5H). LRMS : m/z 339.8 (MH^+)
9	Prep 32	0		^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (m, 6H), 1.08-2.08 (m, 29H), 4.29 (m, 1H), 5.95 (d, 1H), 6.43 (s, 1H), 7.80 (d, 1H). LRMS : m/z 409.5 (MH^+)

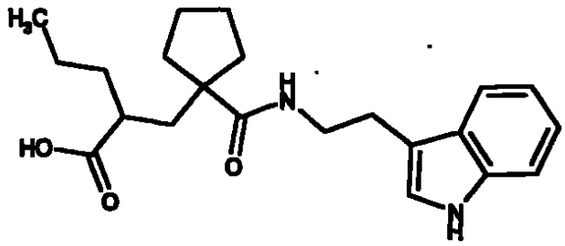
1 = additionally purified by column chromatography on silica gel using ethyl acetate:pentane as eluant.

2 = additionally purified by column chromatography on silica gel using dichloromethane:methanol as eluant.

3 = recrystallised from ether

Example 10

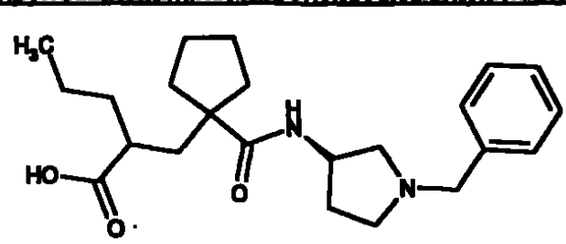
2-[[1-({[2-(1*H*-Indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]methyl]pentanoic acid



Trifluoroacetic acid (2.61ml, 33.9mmol) was added to a solution of the *tert*-butyl ester from preparation 44 (482mg, 1.13mmol) and anisole (1.23ml, 11.3mmol) in dichloromethane (4ml), and the reaction stirred at room temperature for 4 hours. The mixture was washed with water, then brine, dried ($MgSO_4$), concentrated under reduced pressure and the residue azeotroped with toluene. The residual brown oil was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant, and re-columned using an elution gradient of ethyl acetate:pentane (30:70 to 50:50) to afford the title compound as a clear foam, 136mg, 32%; ^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.82 (s, 3H), 1.16-1.77 (m, 12H), 1.78-2.03 (m, 2H), 2.36 (m, 1H), 2.97 (m, 2H), 3.61 (m, 2H), 5.83 (m, 1H), 7.04 (s, 1H), 7.09-7.23 (m, 2H), 7.39 (d, 1H), 7.61 (d, 1H), 8.15 (m, 1H); LRMS : m/z 371.8 (MH^+).

Example 11

2-[[1-({[(3*S*)-1-Benzylpyrrolidinyl]amino}carbonyl)cyclopentyl]methyl]pentanoic acid

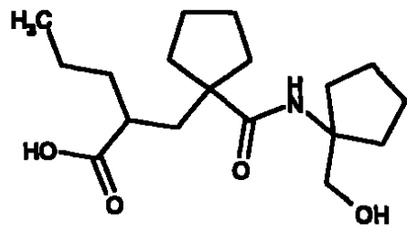


A solution of the *tert*-butyl ester from preparation 45 (70mg, 0.16mmol) in trifluoroacetic acid (1ml) and dichloromethane (1ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with dichloromethane. The residue was partitioned between water (1ml) and ethyl acetate (5ml), and the pH of the aqueous layer adjusted to 8 using sodium bicarbonate solution. The layers were separated, the organic phase dried (Na_2SO_4), evaporated under reduced pressure and the residue azeotroped with dichloromethane to give the title compound as a beige foam, 45mg, 73%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-

2.95 (m, 19H), 3.52 (m, 1H), 3.75 (m, 1H), 3.95 (m, 1H), 4.25 (m, 1H), 4.45 (m, 1H), 6.96 (bs, 1H), 7.39 (m, 5H); LRMS : m/z 387 (MH^+); Anal. Found: C, 61.11; H, 7.69; N, 6.00. $C_{23}H_{34}N_2O_3;CH_2Cl_2$ requires C, 61.14; H, 7.70; N, 5.94%.

Example 12

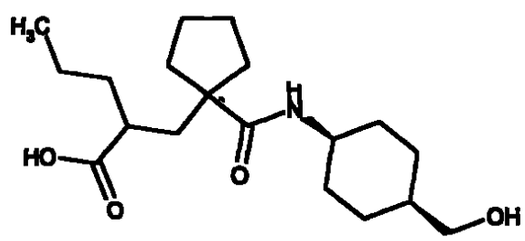
2-[[1-([1-(Hydroxymethyl)cyclopentyl]amino)carbonyl)cyclopentyl]-methyl]pentanoic acid



A solution of the *tert*-butyl ester from preparation 33 (38mg, 0.1mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with toluene and then dichloromethane to give a colourless gum. This was suspended in a solution of potassium carbonate (50mg, 0.3mmol) in methanol, and the mixture stirred for 2 hours at room temperature. The methanol was removed under reduced pressure, the residual aqueous mixture diluted with water (20ml), and acidifed to pH 2 using 2N hydrochloric acid. This solution was extracted with ethyl acetate (2x20ml), and the combined organic solutions dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil, 32mg, 97%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.20-1.40 (m, 3H), 1.41-1.90 (m, 17H), 2.01-2.20 (m, 2H), 2.40 (m, 1H), 3.71 (dd, 2H), 5.80 (bs, 1H); LRMS : m/z 326.1 (MH^+).

Example 13

Cis-2-[[1-([[4-(Hydroxymethyl)cyclohexyl]amino]carbonyl)cyclopentyl]methyl] pentanoic acid

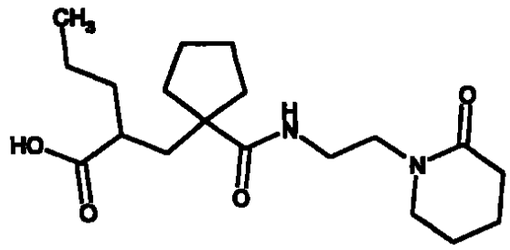


The title compound was obtained as a colourless gum in 68%, from the *tert*-butyl ester from preparation 43, following the procedure described in example 12, except the

product was additionally purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.21-1.40 (m, 6H), 1.52-1.70 (m, 15H), 1.92-2.11 (m, 3H), 2.39 (m, 1H), 3.55 (d, 2H), 4.01 (m, 1H), 5.90 (m, 1H); LRMS : m/z 340.3 (MH^+).

Example 14

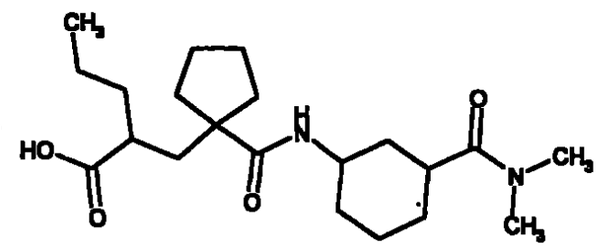
2-[[1-({[2-(2-Oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]methyl]pentanoic acid



Hydrogen chloride gas was bubbled through an ice-cold solution of the *tert*-butyl ester from preparation 47 (43mg, 0.105mmol) in dichloromethane (10ml) for 20 minutes. The solution was then stirred at room temperature for 3 hours. The mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give a glass-like solid. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound, 6mg; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.36 (m, 4H), 1.41-1.69 (m, 7H), 1.79 (m, 4H), 1.90-2.10 (m, 3H), 2.30 (m, 1H), 2.38 (t, 2H), 3.30-3.60 (m, 6H), 7.00 (bs, 1H); LRMS : m/z 351 $(M-H)^-$.

Example 15

2-({1-[({3-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl) pentanoic acid

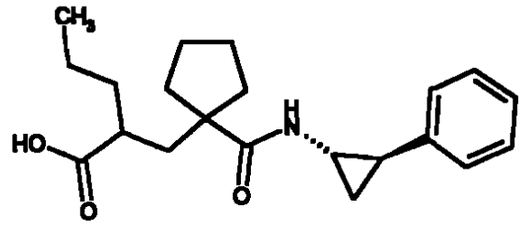


The title compound was obtained as a solid in 85% yield from the *tert*-butyl ester from preparation 42, following a similar method to that described in example 14, except that dichloromethane:methanol:acetic acid (95:3:2) was used as the chromatographic eluant; ^{1}H NMR ($CDCl_3$, 400MHz) d: 0.89 (t, 3H), 1.09-1.76 (m, 12H), 1.80-2.17 (m, 10H), 2.37

(m, 1H), 2.68 (m, 1H), 2.95 (s, 3H), 3.04 (s, 3H), 3.83 (m, 1H), 6.06 (m, 1H); LRMS : m/z 381 (MH^+); Anal. Found: C, 63.31; H, 9.17; N, 6.53. $C_{21}H_{36}N_2O_4.H_2O$ requires C, 63.29; H, 9.61; N, 7.03%.

Example 16

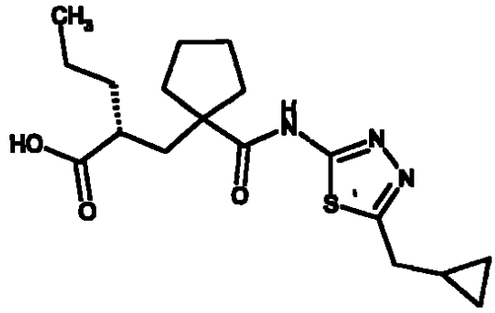
2-[[1-({[(1*R*,2*R*)-2-Phenylcyclopropyl]amino}carbonyl)cyclopentyl]-methyl]pentanoic acid



The title compound was obtained quantitatively as an orange gum from the *tert*-butyl ester from preparation 46, following a similar procedure to that described in example 14; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.12-2.14 (m, 17H), 2.38 (m, 1H), 2.67 (m, 1H), 6.10 (s, 1H), 7.13 (m, 3H), 7.25 (m, 2H); LRMS : m/z 344.3 (MH^+).

Example 17

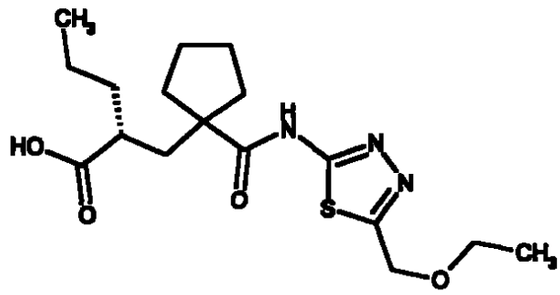
(2*R*)-2-[[1-({[5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)-cyclopentyl]methyl]pentanoic acid



A solution of the *tert*-butyl ester from preparation 50 (63mg, 0.15mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml), was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to give the title compound as a white foam, 46mg, 83%; ^1H NMR (CD_3OD, 400MHz) δ: 0.38 (m, 2H), 0.62 (m, 2H), 0.82 (t, 3H), 1.12 (m, 1H), 1.26 (m, 2H), 1.38 (m, 1H), 1.52 (m, 1H), 1.78-1.78 (m, 6H), 1.90 (m, 1H), 2.23 (m, 4H), 2.92 (d, 2H); LRMS: m/z 366.0 (MH^+); $[\alpha]_D$ = -7.75° (c = 0.08, methanol).

Example 18

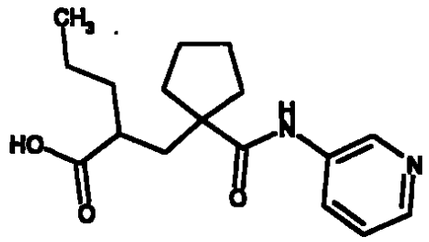
(2*R*)-2-[[1-({[5-(Ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]-methyl]pentanoic acid



The title compound was obtained as a white foam in 62% yield from the *tert*-butyl ester from preparation 51, following a similar procedure to that described in example 17; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.82 (t, 3H), 1.21-1.40 (m, 7H), 1.50 (m, 1H), 1.60-1.77 (m, 7H), 1.88 (m, 1H), 2.23 (m, 4H), 3.62 (q, 2H); $[\alpha]_D$ = -6.08° (c = 0.25, methanol).

Example 19

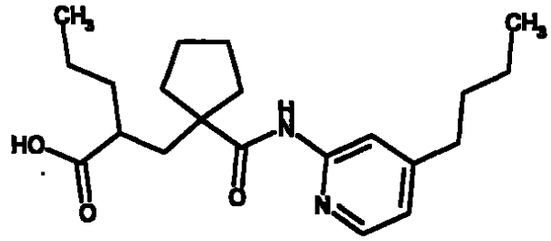
2-({1-[(3-Pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 52 (130mg, 0.33mmol) and 10% palladium on charcoal (20mg) in 95% aqueous ethanol (3ml) was hydrogenated at 15psi and room temperature for 2 hours. The reaction was filtered through Arbocel®, washing through with ethanol, and the filtrate evaporated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 103mg, 83%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 2H), 1.44 (m, 1H), 1.58-1.82 (m, 8H), 2.19 (m, 1H), 2.39 (m, 2H), 2.52 (m, 1H), 6.88 (m, 1H), 7.67 (m, 1H), 7.82 (d, 1H), 8.38 (d, 1H), 9.78 (s, 1H); LRMS : m/z 305 (MH^+).

Example 20

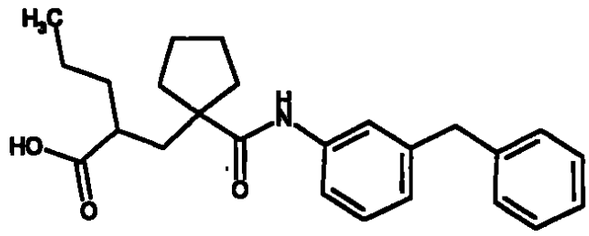
2-[(1-[[(4-Butyl-2-pyridinyl)amino]carbonyl]cyclopentyl)methyl]pentanoic acid



The title compound was obtained in 92% yield from the benzyl ester from preparation 55, following a similar procedure to that described in example 19; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (m, 6H), 1.28-1.50 (m, 5H), 1.58-1.81 (m, 10H), 2.20 (m, 1H), 2.40 (m, 2H), 2.58 (m, 3H), 6.70 (d, 1H), 7.68 (d, 1H), 8.22 (s, 1H), 9.90 (bs, 1H).

Example 21

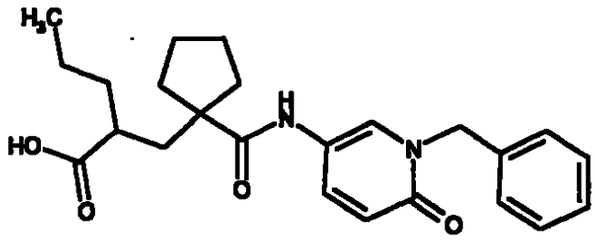
2-({1-[(3-Benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 53 (1.3mg, 2.47mmol) and 5% palladium on charcoal (130mg) in water (10ml) and ethanol (40ml) was hydrogenated at 30 psi and room temperature for 2 hours. The reaction mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure, and the residue triturated with dichloromethane. The residual gum was triturated with ether, then hexane, and dried at 50°C, to give the title compound as a solid, 0.79g, 81%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.95 (t, 3H), 1.24-1.51 (m, 3H), 1.58-1.80 (m, 7H), 1.88 (dd, 1H), 2.15 (m, 2H), 2.24 (m, 1H), 2.48 (m, 1H), 4.00 (s, 2H), 6.98 (d, 1H), 7.24 (m, 6H), 7.40 (m, 3H); Anal. Found: C, 75.48; H, 7.76; N, 3.59. $C_{25}H_{31}NO_3$;0.25H_2O requires C, 75.44; H, 7.98; N, 3.51%.

Example 22

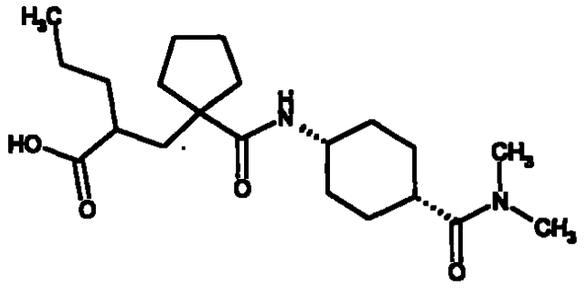
2-[(1-[[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]-cyclopentyl)-methyl]pentanoic acid.



The title compound was obtained as a white foam in 51% yield from the benzyl ester from preparation 56, following a similar procedure to that described in example 21, except the product was purified by column chromatography on silica gel, using ethyl acetate as eluant; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.28-1.80 (m, 12H), 2.01 (m, 1H), 2.30-2.52 (m, 2H), 5.02 (dd, 2H), 6.60 (d, 1H), 7.27 (m, 5H), 7.70 (s, 1H), 8.34 (s, 1H); Anal. Found: C, 69.52; H, 7.41; N, 6.51. $C_{24}H_{30}N_2O_4$;0.25H_2O requires C, 69.45; H, 7.41; N, 6.75.

Example 23

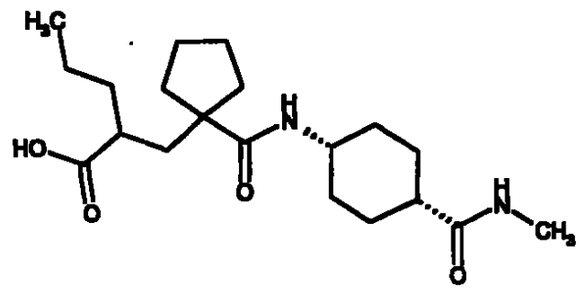
cis-2-({1-[({4-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 58 (150mg, 0.33mmol) and 10% palladium on charcoal (20mg) in water (0.3ml) and ethanol (3.5ml) was hydrogenated at 15 psi and room temperature for 3 days. The reaction mixture was filtered through Arbocel®, and the filtrate concentrated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 85mg, 65%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.29-1.96 (m, 18H), 2.01-2.23 (m, 4H), 2.37 (m, 1H), 2.62 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.96 (m, 1H), 5.98 (m, 1H); LRMS : m/z 381.8 (MH^+); Anal. Found: C, 63.81; H, 9.58; N, 6.99. $C_{21}H_{36}N_2O_4$;0.2CH_2Cl_2 requires C, 64.06; H, 9.23; N, 7.05%.

Example 24

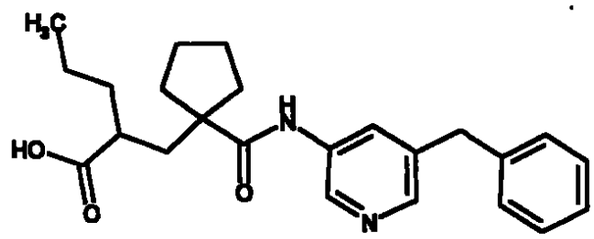
cis-2-({1-[({4-[(Methylamino)carbonyl]cyclohexyl}amino)carbonyl]cyclopentyl}-methyl)pentanoic acid



The title compound was obtained as a white solid in 34% yield from the benzyl ester from preparation 59, following the procedure described in example 23; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.90 (t, 3H), 1.26-2.02 (m, 20H), 2.19 (m, 3H), 2.39 (m, 1H), 2.82 (d, 3H), 4.00 (m, 1H), 5.69 (m, 1H), 6.00 (d, 1H); LRMS : m/z 365 ($M-H^+$).

Example 25

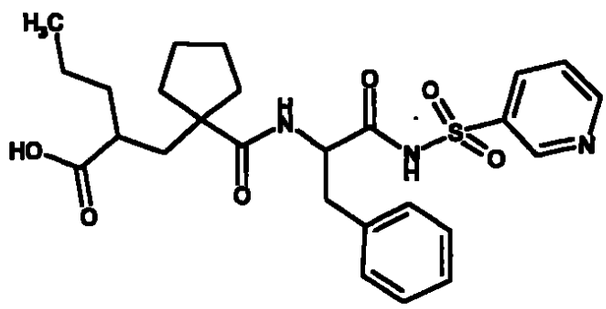
2-[(1-[[(5-Benzyl-3-pyridinyl)amino]carbonyl]cyclopentyl)methyl]pentanoic acid.



A mixture of the benzyl ester from preparation 54 (850mg, 1.76mmol) and 5% palladium on charcoal (100mg) in 20% aqueous ethanol (30ml) was hydrogenated at 30 psi and room temperature for 2 hours. The mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure, and the residue azeotroped with dichloromethane to give the title compound as a foam, 0.63g; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.92 (t, 3H), 1.30-1.83 (m, 11H), 2.07 (m, 1H), 2.42 (m, 3H), 3.82 (s, 2H), 7.15-7.38 (5H), 7.80 (s, 1H), 8.48 (s, 1H), 8.59 (s, 1H), 8.62 (s, 1H); Anal. Found: C, 72.29; H, 7.70; N, 6.90. $C_{24}H_{30}N_2O_3$;0.25H_2O requires C, 72.24; H, 7.70; N, 7.02%.

Example 26

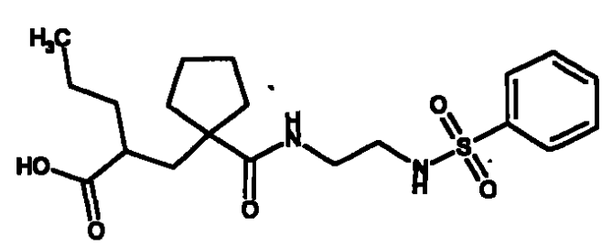
2-({1-[({1-Benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoic acid.



A mixture of the benzyl ester from preparation 57 (918mg, 1.52mmol) and 10% palladium on charcoal (90mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 4 ½ hours. Tlc analysis showed starting material remaining, so additional catalyst (70mg) was added, and the mixture hydrogenated for a further 18 hours. Tlc analysis, again showed starting material remaining, so further catalyst (70mg) was added, and hydrogenation continued for an additional 6 hours. The reaction mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:acetic acid:ethanol (99:1:0 to 79.1:0.9:20) to afford the title compound as a white foam, 271mg, 35%; ^{1}H NMR (DMSOd_6, 300MHz) δ: 0.75 (m, 3H), 0.96-1.42 (m, 11H), 1.61-1.99 (m, 4H), 2.75-3.02 (m, 2H), 4.45 (m, 1H), 7.20 (m, 6H), 7.62 (m, 1H), 8.24 (m, 1H), 8.83 (s, 1H), 9.01 (s, 1H), 11.98 (bs, 1H), 12.70 (bs, 1H); IR (KBr disc) 1185, 1195 (m), 1455, 1515, 1640, 1704, 2870, 2930, 2960 (s).

Example 27

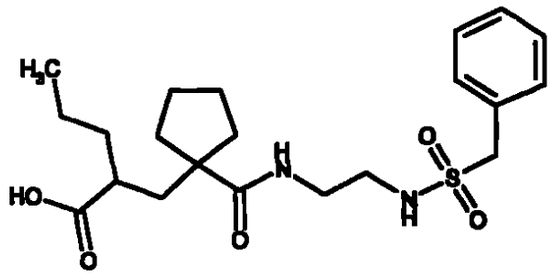
2-({1-[({2-[(Phenylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}-methyl)pentanoic acid



A mixture of the amine from preparation 61 (235mg, 0.72mmol), benzenesulphonyl chloride (127mg, 0.72mmol) and triethylamine (150µl, 1.08mmol) in dichloromethane (6ml) was stirred at room temperature for 2 days. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to give a clear oil. This was then dissolved in trifluoroacetic acid (3ml) and dichloromethane (3ml) and the solution stirred at room temperature for 6 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) to afford the title compound as a clear oil, 204mg, 69%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.22-1.43 (m, 4H), 1.43-2.18 (m, 10H), 2.36 (m, 1H), 3.11 (m, 2H), 3.20-3.31 (m, 1H), 3.42-3.53 (m, 1H), 6.13-6.24 (m, 1H), 7.42-7.59 (m, 3H), 7.84 (m, 2H); LRMS : m/z 411.8 (MH^+); Anal. Found: C, 57.26; H, 7.40; N, 6.61. $C_{20}H_{30}N_2O_5S$ requires C, 57.18; H, 7.22; N, 6.62%.

Example 28

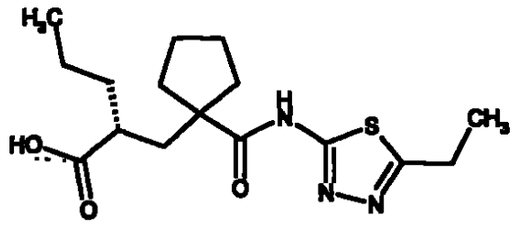
2-({1-[({2-[(Benzylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}-methyl)pentanoic acid



The title compound was obtained as a clear oil in 97% yield, from the amine from preparation 61, following the procedure described in example 27, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.87 (t, 3H), 1.19-1.72 (m, 11H), 1.80-1.96 (m, 1H), 2.00-2.16 (m, 2H), 2.27-2.38 (m, 1H), 2.92-3.21 (m, 3H), 3.23-3.39 (m, 1H), 4.25 (s, 2H), 5.80-6.06 (m, 1H), 6.38 (m, 1H), 7.29-7.43 (m, 5H); LRMS : m/z 425.8 (MH^+).

Example 29

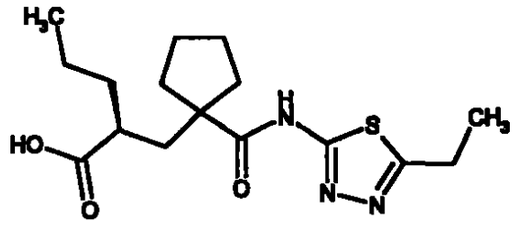
(2*R*)-2-[(1-[[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl) methyl]pentanoic acid



and

Example 30

(2*S*)-2-[(1-[[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl) methyl]pentanoic acid



The acid from Example 4 (824mg) was further purified by HPLC using an AD column and using hexane:*iso*-propanol:trifluoroacetic acid (85:15:0.2) as eluant to give the title compound of example 29 as a white foam, 400mg, 99.5% ee, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 6H), 1.50-1.80 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.30 (bs, 1H), LRMS : m/z 338 (MH^+), $[\alpha]_D$ = -9.0 ° (c = 0.1, methanol), and the title compound of example 30 as a white foam, 386mg, 99% ee, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 6H), 1.50-1.79 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.27 (bs, 1H); LRMS: m/z 338 (MH^+); and $[\alpha]_D$ = +3.8° (c = 0.1, methanol).

Alternatively, Example 29 may be prepared as follows:

To a solution of the product from Preparation 51a (574 g, 1.45 mol) in dichloromethane (2.87 L) was added trifluoroacetic acid (1.15 L) over a period of 50 minutes with cooling at 10 °C. After addition was complete, the reaction was allowed to warm to ambient temperature with stirring under a nitrogen atmosphere for 24 hours. Deionised water (2.6 L) was then added. The reaction mixture was then washed with deionised water (3 x 2.6 L). The dichloromethane layer was concentrated to a volume of approximately 1 L to

give the crude title compound (439 g, 1.29 mol, 96% yield) as a solution in dichloromethane. A purified sample of the title compound was obtained using the following procedure. To a dichloromethane solution (2.34 L) of the crude product, that had been filtered to remove any particulate contamination, was added isopropyl acetate (1.38 L). The resultant mixture was distilled at atmospheric pressure whilst being simultaneously replaced with isopropyl acetate until the solution temperature reached 87 °C. The heating was stopped and the solution was allowed to cool to ambient temperature with stirring for 14 hours to give a cloudy brown solution. The agitation rate was then increased and crystallisation commenced. The suspension was then allowed to granulate for 12 hours at ambient temperature. The resultant suspension was then cooled to 0 °C for 3.5 hours and the solid was then collected by filtration. The filter cake was then washed with isopropyl acetate (2 x 185 ml, then 2 x 90 ml) and the solid was dried under vacuum at 40-45 °C for 18 hours to give the title compound (602 g, 0.18 mol, 70% yield) as a cream coloured, crystalline solid; m.p. : 130-136 °C; LRMS (negative APCI) : m/z $[M-H]^-$ 338; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.92 (t, 3H), 1.27-1.52 (m, 7H), 1.52-1.89 (m, 8H), 2.11-2.27 (m, 1H), 2.27-2.37 (m, 1H), 2.42-2.55 (m, 1H), 2.65 (dd, 2H), 3.00 (q, 2H), 12.25 (bs, 1H).

Example 29 may be purified as follows:

The title product from Example 29 was dissolved in methanol. To this solution was added sodium methoxide (1 equivalent) in methanol (1 ml/g of Example 29) and the mixture was stirred at room temperature for 20 minutes. The solvent was removed *in vacuo* and the residue was azeotoped with ethyl acetate to give a brown residue. Ethyl acetate was added and the solution filtered to give a brown solid which was washed with *tert*-butylmethyl ether to give the crude sodium salt of Example 29. This crude product (35g) was partitioned between water (200ml) and ethyl acetate (350ml). Concentrated hydrochloric acid (~7ml) was added until the pH of the aqueous layer was pH2. The aqueous phase was washed with ethyl acetate (2 x 100ml). The combined layers were dried using magnesium sulphate. The solvent was removed *in vacuo* to give a light brown solid (31g). Ethyl acetate (64ml, 2ml/g) and diisopropyl ether (155ml, 5ml/g) were added and the mixture heated to 68°C until a clear solution was obtained (~30min). Upon cooling to room temperature, crystallisation of the free acid occurred. After 30 minutes stirring at room temperature the product was collected by filtration and washed with diisopropyl ether. The product was dried in a vacuum oven at 50°C overnight.

(20.2g, 61% recovery from the sodium salt.); m.p. 135 degC (determined using a Perkin Elmer DSC7 at a heating rate of 20°C/minute).

The main peaks (from 2 to 40 degrees 2θ) in the PXRD pattern are as follows. The PXRD was performed without reference to an internal standard (e.g. silicon powder). The peak positions are therefore subject to possible instrumental zero offset and sample height errors.

Angle ° 2-Theta	Intensity %	Angle ° 2-Theta	Intensity %	Angle ° 2-Theta	Intensity %
6.701	59.8	20.121	36.1	28.539	17.3
8.908	11.2	20.425	7.1	30.092	56.0
9.305	8.6	21.104	8.9	31.236	8.8
9.590	4.5	21.328	5.7	32.010	12.8
10.039	25.3	21.733	100.0	32.810	8.0
10.206	59.3	22.150	18.7	33.106	6.6
10.578	11.3	22.816	10.2	33.720	8.0
10.984	12.2	23.327	46.2	34.033	9.2
12.075	10.3	23.681	6.4	34.388	12.4
13.054	5.5	23.960	9.5	34.646	8.5
14.256	8.2	24.248	14.0	34.821	8.2
15.695	45.2	24.722	9.4	35.806	7.3
16.316	17.1	25.505	15.5	36.272	13.5
16.418	8.5	25.779	10.5	36.633	12.1
16.698	24.1	26.267	7.0	37.500	7.8
17.129	16.5	26.724	20.4	37.862	11.7
17.605	20.9	26.890	70.4	38.281	6.5
18.575	23.6	27.663	7.2	39.975	11.8
19.296	14.0	28.158	16.8		

Salts of Example 29

Sodium Salts

a) Lower Melting Form

The title product from Example 29 was dissolved in methanol (80mg in 2.5mls-33ml/g). Sodium hydroxide (0.024mls as 10N solution) was added. On evaporation of the methanol crystallisation of the sodium salt occurred, which was used with no further purification. The salt was dried on high vacuum for 5 hrs to give 85mg, quantitative yield; m.p. 214 degC (determined using a TA instruments DSC2910 at a heating rate of 10°C/minute).

The main peaks (from 2 to 40 degrees 2θ) in the PXRD pattern are as follows. The PXRD was performed without reference to an internal standard (e.g. silicon powder). The peak positions are therefore subject to possible instrumental zero offset and sample height errors.

Angle ° 2-Theta	Intensity %	Angle ° 2-Theta	Intensity %	Angle ° 2-Theta	Intensity %
7.748	92.8	15.814	14.0	25.995	15.4
8.380	17.8	16.496	43.9	26.753	18.4
8.950	14.3	17.349	40.9	28.085	20.4
9.515	8.9	17.687	100.0	28.883	19.9
10.043	11.7	18.229	35.3	29.737	19.7
11.000	13.2	18.736	40.9	30.227	21.6
11.829	17.5	19.754	37.5	31.204	28.1
12.258	17.3	20.258	50.3	31.729	20.6
13.087	19.0	20.498	93.1	34.825	19.7
13.464	22.3	21.719	47.4	35.394	24.2
13.808	13.4	22.499	35.5	36.018	22.9
14.840	14.3	23.310	29.9	37.722	24.5
15.197	27.1	24.111	19.5	39.304	20.6
15.489	21.4	25.165	19.1		

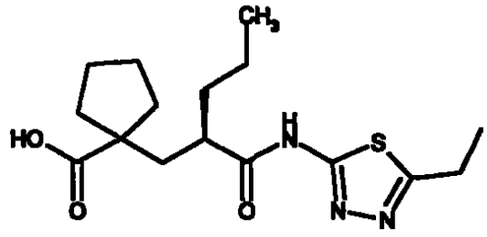
b) Higher Melting Form

The title product from Example 29 was dissolved in methanol (450mg in 15ml - 33ml/g). Sodium hydroxide (1.33mls as 1N solution) was added. The methanol was stripped using a rotavap *in vacuo* to give a gum. The gum was azeotroped once with isopropyl alcohol (8mls) to remove residual methanol and water before a further portion of isopropyl alcohol (15mls) was added. The mixture was heated until a homogenous mixture was obtained. On cooling crystallisation of the sodium salt occurred. The mixture was held at room temperature for 10 minutes. The salt was filtered off, washed with isopropyl alcohol and dried in a vacuum oven at 60°C for 30 min; 200mg of sodium salt was recovered; m.p. 252 degC (determined using a TA Instruments DSC2910 at a heating rate of 10°C/minute).

The main peaks (from 2 to 40 degrees 2θ) in the PXRD pattern are as follows. The PXRD was performed without reference to an internal standard (e.g. silicon powder). The peak positions are therefore subject to possible instrumental zero offset and sample height errors.

Angle ° 2-Theta	Intensity %	Angle ° 2-Theta	Intensity %	Angle ° 2-Theta	Intensity %
3.419	0.8	20.682	1.5	30.090	1.1
6.674	100.0	20.758	1.7	30.735	0.9
7.394	0.4	20.841	1.4	31.360	1.4
8.200	1.7	21.058	1.0	32.025	3.5
11.485	0.4	21.462	0.5	32.742	0.7
13.010	1.5	21.935	2.0	33.072	0.7
13.337	0.8	22.965	0.6	33.723	1.2
16.414	0.7	23.585	1.1	34.261	0.7
17.288	2.8	24.868	1.4	35.946	1.8
18.185	0.9	25.387	1.1	36.839	0.7
18.987	1.4	25.733	1.8	37.484	0.7
19.330	0.5	26.124	0.9	38.519	1.4
19.687	0.8	26.826	0.9	38.801	1.3
20.069	1.8	28.719	1.8		

The title compound of Example 29 metabolysed to form (2*R*)-1-(2-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}pentyl)cyclopentanecarboxylic acid.



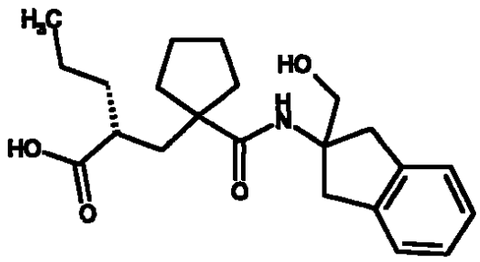
This compound was prepared as follows:

The product from Preparation 102 (430mg, 1mmol) was taken up in ethanol (5mls) and methanol (1ml) and hydrogenated at 30psi hydrogen pressure at room temperature for 2h. The mixture was then filtered through a plug of Arbocel® and evaporated to a yellow oil. This oil was purified by column chromatography using firstly 19:1, then 9:1 DCM:MeOH as eluant to provide the product as a clear oil (120mg, 35%); ^{1}HNMR (400MHz, $CDCl_3$) 0.88 (t, 3H), 1.20-1.88 (m, 13H), 1.90-2.03 (m, 1H), 2.24-2.38 (m, 1H), 2.43-2.72 (m, 2H), 2.95 (q, 2H); LRMS m/z 340.2 (M+H).

Example 31

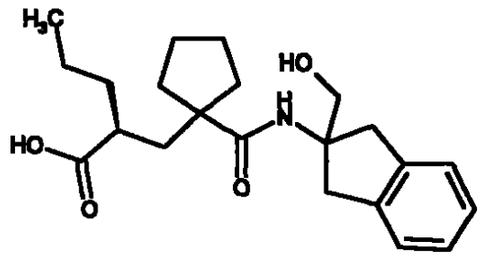
(*R*)-2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]pentanoic acid

and



Example 32

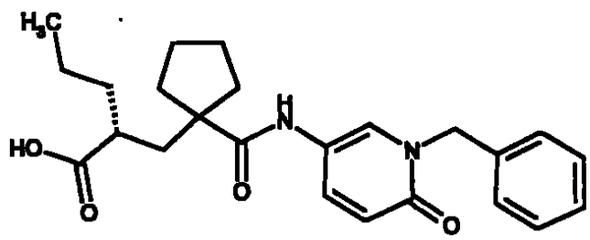
(*S*)-2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1H-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]pentanoic acid



2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]pentanoic acid (WO 9110644, Example 8) was further purified by HPLC using an AD column and hexane:isopropanol:trifluoroacetic acid (90:10:0.1) as eluant, to give the title compound of Example 31, 99% ee, $[\alpha]_D$ = +10.4° (c = 0.067, ethanol) and the title compound of Example 32, 99% ee, $[\alpha]_D$ = -10.9° (c = 0.046, ethanol).

Example 33

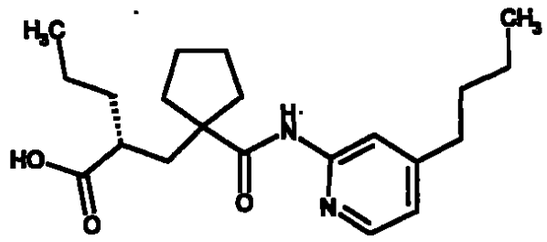
(2*R*)-2-[(1-[[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]-cyclopentyl)methyl]-pentanoic acid



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (191mg, 1.0mmol), 1-hydroxybenzotriazole hydrate (135mg, 01.0mmol), N-methylmorpholine (165µl, 1.5mmol) and finally the amine from preparation 28 (150mg, 0.69mmol) were added to a solution of the acid from preparation 2 (284mg, 1.0mmol) in N,N-dimethylformamide (8ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (4x50ml) and brine (50ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using ethyl acetate:pentane (30:70) to give a yellow oil, 191mg. This intermediate was dissolved in dichloromethane (3ml) and trifluoroacetic acid (3ml) and the solution stirred at room temperature for 5 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to give the title compound as a foam, 77mg, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (t, 3H), 1.20-1.76 (m, 12H), 1.93-2.02 (m, 1H), 2.20-2.46 (m, 3H), 4.95 (d, 1H), 5.04 (d, 1H), 6.61 (d, 1H), 7.21 (m, 1H), 7.50 (s, 1H), 8.23 (s, 1H); LRMS : m/z 411.6 (MH)+; $[\alpha]_D$ = -3.8° (c = 0.052, ethanol).

Example 34

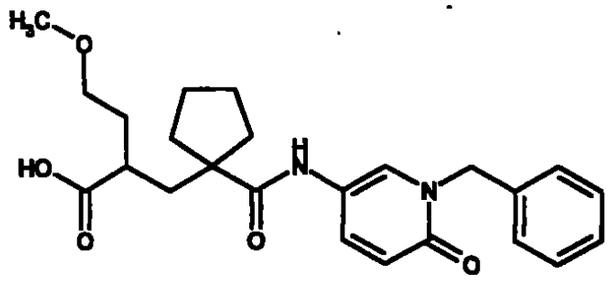
(2*R*)-2-[(1-[[(4-Butyl-2-pyridinyl)amino]carbonyl]cyclopentyl)methyl]pentanoic acid



The title compound was obtained in 43% yield from the acid from preparation 2 and the amine from preparation 30, following a similar procedure to that described in Example 33, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80-1.00 (m, 6H), 1.22-1.84 (m, 18H), 2.03-2.56 (m, 3H), 2.77 (m, 1H), 7.14 (d, 1H), 8.08 (d, 1H), 8.23 (s, 1H), 11.71 (brs, 1H); LRMS : m/z 361.7 $(MH)^+$, $[\alpha]_D$ = -1.4° (c = 0.14, ethanol).

Example 35

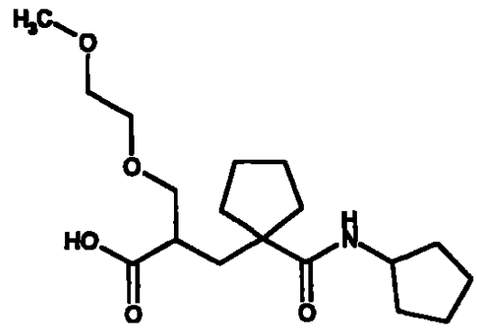
2-[(1-[[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]cyclopentyl)methyl]-4-methoxybutanoic acid



A mixture of the benzyl ester from preparation 62 (850mg, 1.64mmol), and 5% palladium on charcoal (250mg) in 40% aqueous ethanol (21ml), was hydrogenated at 30 psi and room temperature for 30 minutes. The reaction mixture was filtered through Hyflo®, and the filtrate evaporated under reduced pressure. The residual foam was purified by column chromatography on silica gel using dichloromethane:methanol (97:3) as eluant to give the title compound as a white foam, 550mg, 79%; ^{1}H NMR (DMSO-d_6, 300MHz) δ: 1.24-2.17 (m, 12H), 2.18-2.31 (m, 1H), 3.07 (s, 3H), 3.21 (t, 2H), 5.08 (s, 2H), 6.63 (d, 1H), 7.23-7.41 (m, 5H), 7.72 (d, 1H), 8.24 (s, 1H); Anal. Found: C, 67.46; H, 7.18; N, 6.24. $C_{24}H_{30}N_2O_5$ requires C, 67.58; H, 7.09; N, 6.57%.

Example 36

3-[1-[(Cyclopentylamino)carbonyl]cyclopentyl]-2-[(2-methoxyethoxy)methyl]-propanoic acid

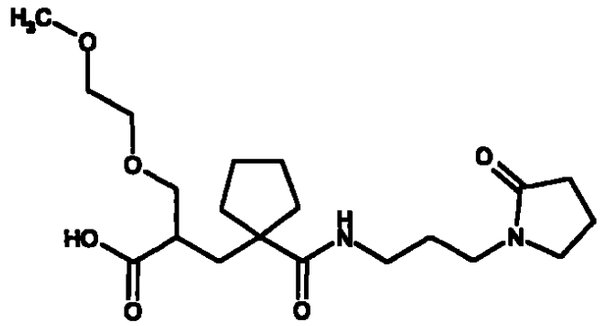


A solution of the *tert*-butyl ester from preparation 64 (320mg, 0.80mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room temperature for 8 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) to give the title compound as a clear oil, 171mg, 62%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.29-1.40 (m, 2H), 1.42-1.69 (m, 10H), 1.75 (dd, 1H),

1.87-2.03 (m, 5H), 2.64 (m, 1H), 3.34 (s, 3H), 3.43-3.52 (m, 3H), 3.57 (m, 2H), 3.61 (m, 1H), 4.08-4.20 (m, 1H), 5.89 (d, 1H); LRMS : m/z 340 (MH^+).

Example 37

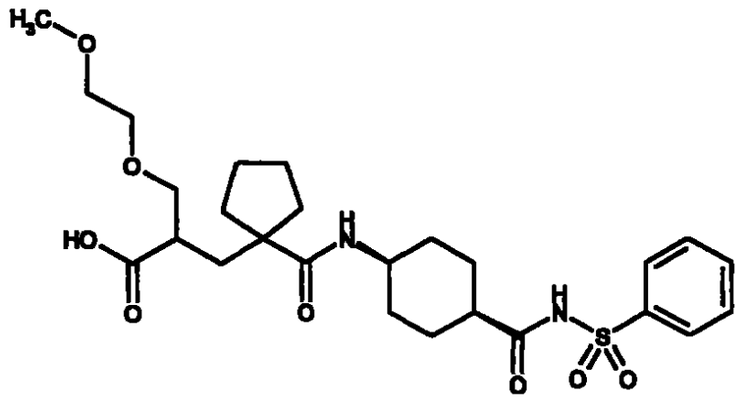
3-(2-Methoxyethoxy)-2-[[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)-cyclopentyl]methyl]propanoic acid



The title compound was obtained as a clear oil in 57% yield from the *tert*-butyl ester of preparation 65, following the procedure described in example 36, ^1H NMR ($CDCl_3$, 300MHz) δ: 1.56-1.78 (m, 8H), 1.94-2.17 (m, 6H), 2.44 (m, 2H), 2.68-2.76 (m, 1H), 3.10-3.21 (m, 1H), 3.22-3.31 (m, 1H), 3.37 (s, 3H), 3.40 (m, 2H), 3.44-3.56 (m, 5H), 3.60 (m, 2H), 3.68 (m, 1H), 6.91-7.01 (m, 1H); LRMS : m/z 398.7 (M^+).

Example 38

cis-3-(2-Methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}cyclohexyl)-amino]carbonyl}cyclopentyl)methyl]propanoic acid

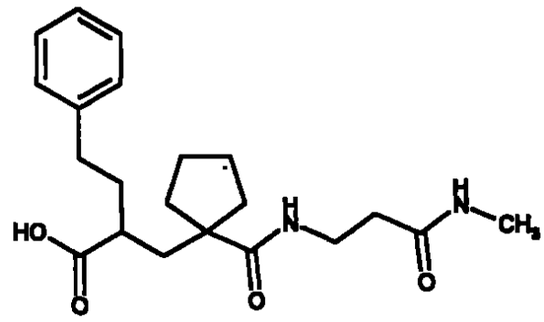


A solution of the *tert*-butyl ester from preparation 66 (446mg, 0.75mmol) in dichloromethane (5ml) and trifluoroacetic acid (5ml) was stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and the residue azeotroped with dichloromethane, then toluene, and finally ether, to afford the title compound as a white foam, 385mg, 95%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.48-2.17

(m, 18H), 2.40 (s, 1H), 2.66 (s, 1H), 3.37 (s, 3H), 3.50-3.70 (m, 6H), 3.94 (s, 1H), 6.10 (d, 1H), 6.59 (s, 1H), 7.55 (t, 2H), 7.61 (m, 1H), 8.02 (d, 2H), 9.11 (s, 1H); Anal. Found: C, 54.88; H, 6.90; N, 5.04. $C_{28}H_{38}N_2O_5S;1.7H_2O$ requires C, 57.97; H, 7.11; N, 5.20%.

Example 39

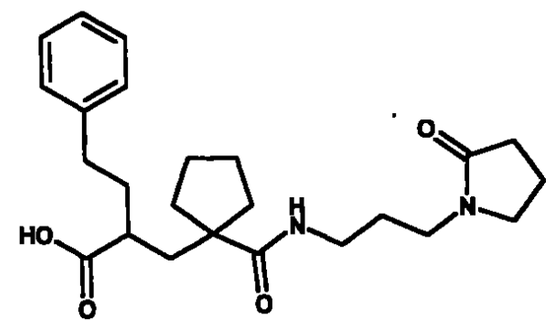
2-[[1-({[3-(Methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl]-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 68 (160mg, 0.34mmol) and 10% palladium on charcoal (100mg) in ethanol (30ml) was hydrogenated at room temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel® and the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:acetic acid (95:5:0 to 95:5:0.5) to afford the title compound as a white foam, 100mg, 79%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.40-1.70 (m, 8H), 1.95 (m, 3H), 2.10 (m, 1H); 2.35 (d, 3H), 2.59 (m, 2H), 2.75 (t, 3H), 3.42 (m, 2H), 6.25 (bs, 1H), 6.70 (bs, 1H), 7.13-7.25 (m, 5H); and LRMS: m/z 375.0 (MH^+).

Example 40

2-[[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]-methyl]-4-phenylbutanoic acid.

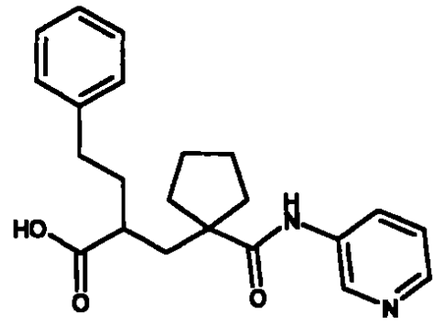


A mixture of the benzyl ester from preparation 67 (780mg, 1.55mmol) and 10%

palladium on charcoal (100mg) in ethanol:water (90:10 by volume; 30ml) was hydrogenated at room temperature under 60psi H_2 pressure for 1.5 hours. The catalyst was filtered off, and the filtrate evaporated under reduced pressure to provide the title compound as a white foam, 473mg, 74%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.26-1.77 (m, 10H), 1.78-2.46 (m, 11H), 2.49-2.70 (m, 2H), 2.95-3.36 (m, 4H), 6.92-7.38 (m, 5H); Anal. Found: C, 64.05; H, 7.73; N, 6.22. $C_{24}H_{34}N_2O_4$.0.75H_2O requires C, 65.88; H, 7.83; N, 6.40%.

Example 41

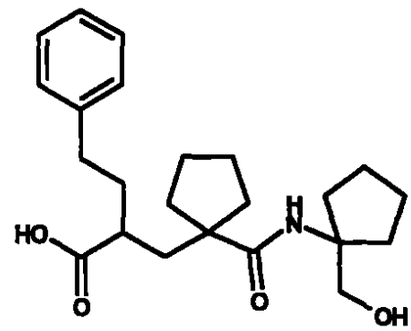
4-Phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoic acid



A mixture of the benzyl ester from preparation 71 (700mg, 1.53mmol) and 5% palladium on charcoal (70mg) in ethanol:water (90:10 by volume, 50ml) was hydrogenated at room temperature under 30 psi H_2 pressure for 5 hours. The catalyst was filtered through Arbocel®, washing well with ethanol, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant to provide the title compound as a white foam, 510mg, 91%; m.p. 80-85°C (collapses to a gum); ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.40-2.78 (m, 15H), 6.93-7.39 (m, 5H), 7.93 (m, 1H), 8.59 (d, 1H), 9.17 (d, 1H), 9.41 (s, 1H); Anal. Found: C, 70.83; H, 7.10; N, 7.64. $C_{22}H_{26}N_2O_3$.0.3H_2O requires C, 70.94; H, 7.22; N, 7.52%.

Example 42

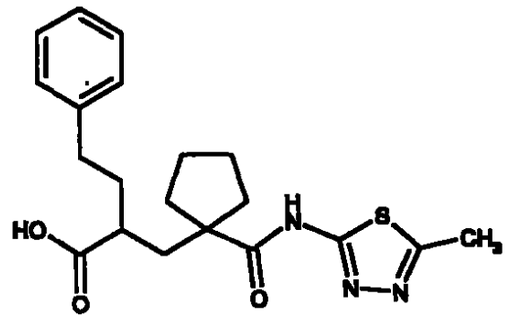
2-[[1-({[1-(Hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl]-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 69 (118mg, 0.25mmol) and 10% palladium on charcoal (100mg) in ethanol (20ml) was hydrogenated at room temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane to give the title compound as a colourless gum, 95mg, 98%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.41-1.80 (m, 17H), 1.90 (m, 1H), 1.92-2.20 (m, 3H), 2.40 (m, 1H), 2.60 (m, 2H), 3.60 (d, 1H), 3.71 (d, 1H), 5.80 (bs, 1H), 7.15-7.30 (m, 5H); LRMS : m/z 388.1 (MH^+).

Example 43

2-[(1-{[(5-Methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid

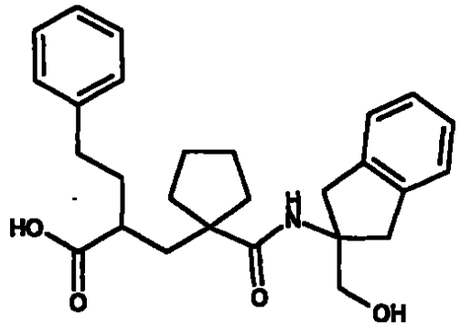


A mixture of the benzyl ester from preparation 70 (187mg, 0.39mmol) and 10% palladium on charcoal (80mg) in ethanol (20ml) was hydrogenated at 60 psi for 18 hours. Tlc analysis showed starting material remaining, so additional 10% palladium on charcoal (100mg) was added, and the reaction continued for a further 5 hours. Tlc analysis again showed starting material remaining, so additional catalyst (100mg) was added, and hydrogenation continued for 18 hours. The mixture was filtered through Arbocel , and the filtrate concentrated under reduced pressure, and azeotroped with

dichloromethane. The crude product was purified by chromatography on silica gel using a Biotage® column, and dichloromethane:methanol (95:5) as eluant to afford the title compound as a clear oil, 80mg, 53%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.51-1.89 (m, 9H), 2.03 (m, 1H), 2.20 (m, 1H), 2.40 (m, 2H), 2.60 (m, 5H), 7.15-7.30 (m, 5H); LRMS : m/z 387.8 (MH^+).

Example 44

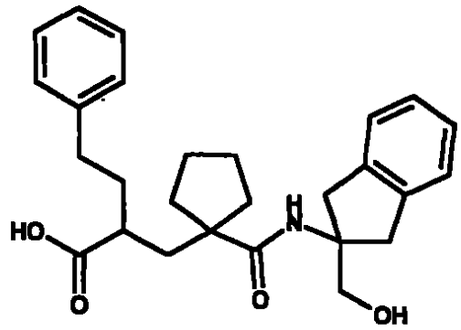
(R)-2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]-methyl]-4-phenylbutanoic acid



and

Example 45

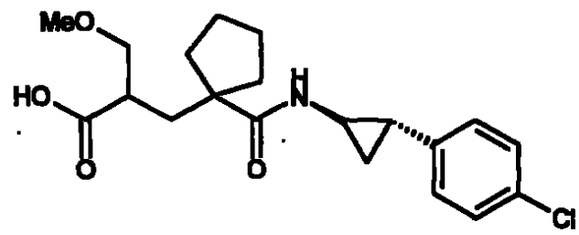
(S)-2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-phenylbutanoic acid



2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-phenylbutanoic acid (WO 9110644, Example 9) was purified by standard HPLC procedures using an AD column and hexane:isopropanol: trifluoroacetic acid (70:30:0.2) as eluant, to give the title compound of Example 44, 99.5% ee; $[\alpha]_D$ = +9.1° (c = 1.76 in ethanol); and the title compound of Example 45, 99.5% ee; $[\alpha]_D$ = -10.5 ° (c = 2.2 in ethanol).

Example 46

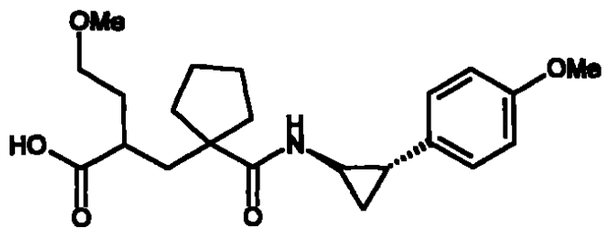
trans-3-[1-({[2-(4-Chlorophenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxymethyl)propanoic acid



The product from preparation 72 (160mg, 0.39mmol) was taken up in 50ml DCM and cooled to 0°C. Hydrogen chloride gas was then bubbled through the solution for 15mins and then allowed to stir at room temperature for 16h. The reaction mixture was concentrated *in vacuo* and then purified by column chromatography using 5:95 MeOH:DCM as eluant to provide the title product (18mg, 12%); R_f 5:95 (DCM:MeOH) 0.2; ^{1}HNMR (400MHz, $CDCl_3$) 1.04-1.18 (m, 2H), 1.20-1.36 (m, 2H), 1.36-1.79 (m, 7H), 1.83-2.08 (m, 4H), 2.57-2.66 (m, 1H), 2.73-2.83 (m, 1H), 3.27 (s, 3H, OMe), 3.32-3.41 (m, 1H), 3.48 (app. dd, 1H, C<u>H</u>OMe), 6.21 (s, N<u>H</u>), 7.03 (d, 2H, Ar), 7.18 (d, 2H, Ar); LRMS : m/z, M-H 378; HRMS Found MH+ 380.1622. Calculated MH+ 380.1623.

Example 47

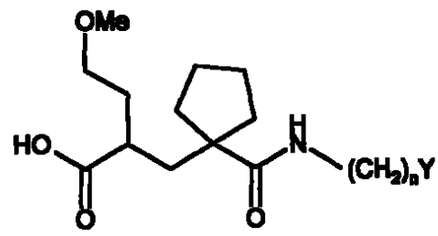
trans-3-[1-({[2-(4-Methoxyphenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid



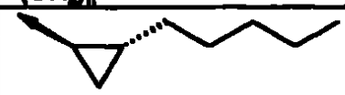
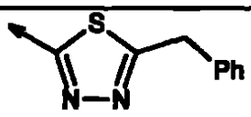
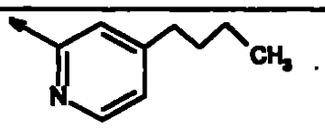
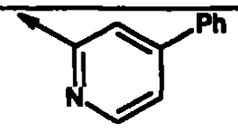
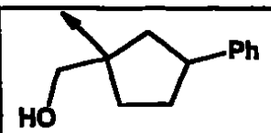
The product from preparation 81 (113mg, 0.25mmol) was taken up in a 4M solution of hydrogen chloride in dioxane (10mls) and stirred for 3h. The mixture was concentrated *in vacuo* and purified by column chromatography using 5:95 (MeOH:DCM) as eluant to provide the acid as a colourless film (45mg, 44%); R_f 95:5 (DCM:MeOH) 0.2; LRMS : m/z, M-H, 388; ^{1}HNMR (400MHz, $CDCl_3$) 1.01-1.22 (m, 2H), 1.40-2.22 (m, 15H), 2.42-

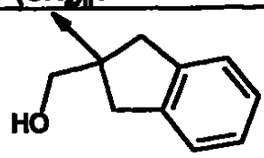
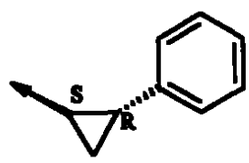
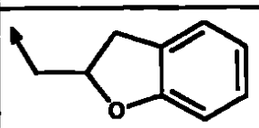
2.57 (m, 1H), 2.73-2.82 (m, 1H), 3.23 (s, 3H, OMe), 3.27-3.44 (m, 2H), 3.72 (s, 3H, OMe), 6.12 (s, 1H, NH), 6.78 (d, 2H, Ar), 7.06 (d, 2H, Ar).

Compounds of formula Id, i.e. compounds of formula I where R^1 is methoxyethyl were prepared either from a) the indicated tert-butyl ester following a similar procedure to that described in Example 47, or b) the indicated benzyl ester following a similar procedure to that described in Example 42.



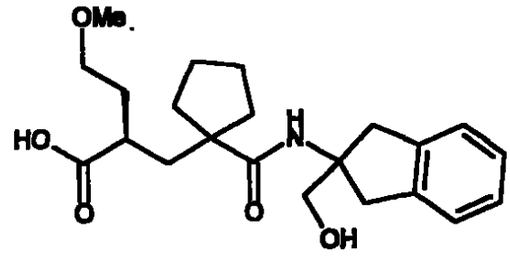
(Id)

Ex	Prec	-$(CH_2)_nY$	Data
48	Prep 89		^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.50-0.63 (m, 3H), 0.82 (t, 3H, Me), 0.77-0.84 (m, 1H), 1.01-1.18 (m, 1H), 1.20-1.78 (m,), 1.82-2.08 (m, 2H), 3.27 (s, 3H, OMe), 3.33-3.41 (m, 2H), 5.92 (s, 1H, NH). LRMS : m/z 352 (M-H)
49	Prep 95		^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.62-2.57 (m, 13H), 3.07 (s, 3H, OMe), 2.96-3.44 (m, 2H), 4.09 (s, 2H), 7.20 (brs, 5H). HRMS : Found m/z 418.1796. $C_{21}H_{27}N_3O_4S$ requires m/z 418.1795.
50	Prep 91		^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.92 (t, 3H, Me), 1.24-2.60 (m, 19H), 3.25 (s, 3H, OMe), 3.39 (t, 2H, CH_2OMe), 6.68-6.71 (m, 1H, Ar), 7.63-7.70 (m, 1H, Ar), 8.21 (s, 1H, Ar), 9.77 (brs, NH). LRMS : m/z 378 (M^+).
51	Prep 92		^{1}HNMR ($CDCl_3$, 400MHz) δ: 1.24-2.34 (m, 10H), 2.37-2.54 (m, 1H), 2.54-2.73 (m, 2H), 3.33 (s, 3H, OMe), 3.38-4.49 (m, 2H), 7.00 (d, 1H. Ar), 7.38-7.56 (m, 3H), 7.59-7.69 (m, 2H), 7.80 (t, 1H, Ar), 8.66 (s, 1H, Ar), 9.77-9.93 (m, NH). LRMS : m/z 395 (M-H).
52	Prep 88		^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.84 (br.t, 3H), 1.20-2.20 (m, 19H), 2.24-2.58 (m, 2H), 3.07-3.33 (m, 1H), 3.60-3.96 (m, 2H), 5.82-5.98 (m, 1H), 7.14-7.36 (m, 5H). LRMS m/z M-H 400

Ex	Prec	-$(CH_2)_nY$	Data
53	Prep 93		^{1}HNMR ($CDCl_3$, 400MHz) δ: 1.43-1.76 (m, 7H), 1.80-2.24 (m, 4H), 2.57-2.68 (m, 2H), 3.06 (d, 1H), 3.12 (d, 1H), 3.27 (d, 1H), 3.32 (s, 3H), 3.36-3.48 (m, 2H), 3.80 (d, 1H), 3.87 (d, 1H), 6.04 (s, 1H), 7.16-7.22 (m, 4H).
54	Prep 81a		^{1}HNMR ($CDCl_3$, 400MHz) δ : 1.02-1.28 (m, 2H), 1.37-1.84 (m, 7H), 1.85-2.16 (m, 4H), 2.62 (br.s, 1H), 2.80-2.93 (m, 1H), 3.29 (s, 3H, Me), 3.22-3.58 (m, 2H), 6.21 (br.s, 1H), 7.03-7.34 (m, 5H). LRMS m/z M+H 346 HRMS m/z M+H Found 346.2011. $C_{20}H_{27}NO_4$ requires 346.2013.
55	Prep 96		^{1}HNMR ($CDCl_3$, 400MHz) δ : 1.45-1.67 (m, 8H), 1.85-2.00 (m, 4H), 2.05 (m, 1H), 2.46 (bs, 1H), 2.90 (dd, 1H), 3.30 (s, 3H), 3.35 (m, 2H), 3.40 (m, 2H), 3.70 (bm, 1H), 4.90 (bs, 1H), 6.25 (bs, 1H), 6.75 (d, 1H), 6.81 (t, 1H), 7.10 (d, 1H), 7.15 (t, 1H); LRMS m/z M-H 374; HRMS m/z M+H Found 376.2118. $C_{20}H_{27}NO_4$ requires 376.2123.

Example 56

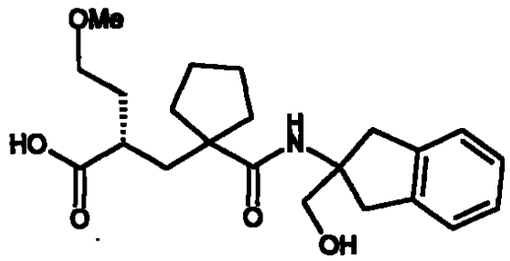
(*R*)- 2-[[1-([[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino)carbonyl)-cyclopentyl]methyl]-4-methoxybutanoic acid



and

Example 57

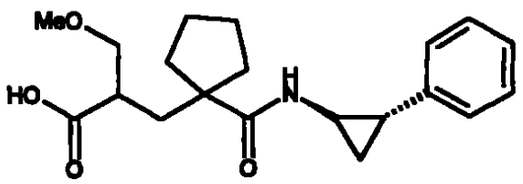
(*S*)-2-[[1-([[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino)carbonyl)-cyclopentyl]methyl]-4-methoxybutanoic acid



The product from Example 53 was purified by HPLC using a Chiralcel OD column (250*20mm) at ambient temperature using a mixture of 70% hexane containing 0.3% TFA and 0.2% DEA and 30% IPA containing 0.3% TFA and 0.2% DEA at a flow rate of 10ml/min. Example 55 is the R enantiomer which eluted first after 6mins (α_D 11.00 c1mg/ml in EtOH). Example 56 is the S enantiomer which eluted second after 7mins (α_D –8.62 c1.07mg/ml in EtOH).

Example 58

3-Methoxy-2-[[1-({[(*trans*)-2-phenylcyclopropyl]amino}carbonyl)cyclopentyl]-methyl]propanoic acid

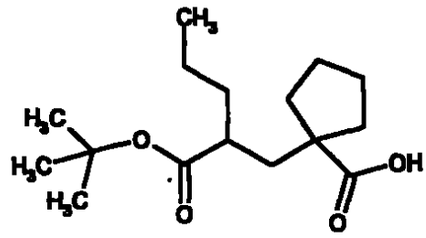


The title compound was prepared according to the procedure of Example 47 from the title product from preparation 82; ^{1}HNMR ($CDCl_3$, 400MHz) δ: 1.2 (m, 2H), 1.5 (m, 3H), 1.6 (bs, 3H), 1.8 (d, 1H), 2.0 (m, 4H), 2.6 (bs, 1H), 2.9 (bs, 1H), 3.3 (s, 3H), 3.4 (t, 1H), 3.5 (t, 1H), 6.4 (s, 1H), 7.1 (m, 3H), 7.3 (t, 2H); LRMS 344 (M-H).

The following Preparations describe the preparation of certain intermediates used in the preceding Examples.

Preparation 1

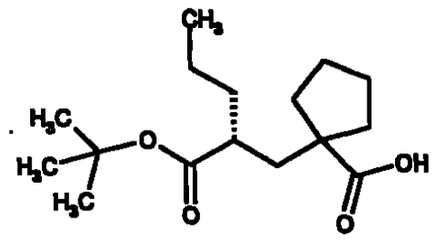
1-[2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid



A mixture of 1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (EP 274234, Example 44) (23g, 81.5mmol) and 10% palladium on charcoal (2g) in dry ethanol (200ml) was hydrogenated at 30psi and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel, using ethyl acetate:pentane (40:60) as the eluant, to provide the title product as a clear oil, 21g, 91%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); LRMS : m/z 283 $(M-H)^-$.

Preparation 2

1-[(2*R*)-2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid



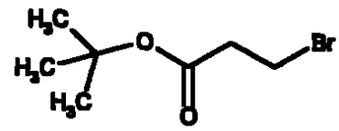
A mixture of (*R*)-1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (WO 9113054, Example 10) (10g, 35.4mmol) and 10% palladium on charcoal (600mg) in dry ethanol (25ml) was hydrogenated at 1 atm. and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give the title compound as a yellow oil, 9.6g, 95%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); $[\alpha]_D$ = -3.3° (c = 0.09, ethanol).

Alternatively the title product from Preparation 2 may be prepared as follows:

A solution of the product from stage f) below (1.12 kg, 4.0 mol) in ethanol (6.5 L) was split into two equal portions which were both subjected to the following reaction conditions. To a solution of the product from stage f) below (561 g, 2.0 mol) in ethanol (3.25 L) was added the hydrogenation catalyst (50.5 g of 5% Pd on carbon - 50% wet) and the reaction vessel was pressurised with hydrogen gas (30 psi). The reaction was stirred for 18 hours at room temperature before recombining the two batches and removing the catalyst by filtration. The filter cake was washed with ethanol (2 x 450 ml) and the combined filtrates were then concentrated under vacuum. The resultant suspension was then filtered and n-heptane (1 L) was added. The solvent was removed by distillation under vacuum and n-heptane (1 L) was added. The solvent was removed by distillation under vacuum to give the title compound (1.1 kg, 3.86 mol, 97% yield) as a yellow oil that was used directly in the next step; LRMS (negative APCI) : m/z $[M-H]^-$ 283; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.79-0.98 (t, 3H), 1.24-1.39 (m, 3H), 1.42-1.50 (s, 9H), 1.50-1.61, (m, 3H), 1.61-1.74 (m, 4H), 1.74-1.84 (m, 1H), 2.02-2.23 (m, 3H), 2.23-2.35 (m, 1H).

Preparation of Starting Materials

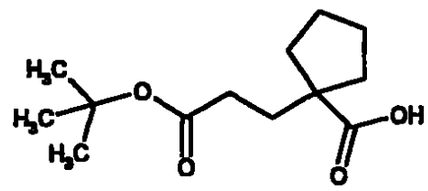
a) *tert*-Butyl-3-bromopropionate



To a solution of 3-bromopropionic acid (6.0 kg, 39.2 mol) in dichloromethane (60 L) at 0 °C was added *tert*-butanol (0.6 L) and conc. sulfuric acid (0.33 L). The resultant solution was cooled to -15 °C and isobutylene was bubbled through (11 kg, 196 mol). The reaction was then stirred for 3 hours at –5 °C before warming to 20 °C over 4 hours and was then stirred at this temperature for 15 hours. The reaction was quenched by cautious addition into saturated aqueous sodium bicarbonate solution (0.6 M, 72 L, 43.2 mol). The layers were then separated and the organic layer was washed with saturated aqueous sodium bicarbonate solution (2 x 48 L) followed by deionised water (48 L). This washing cycle was repeated and the pH of the aqueous layer was measured and was shown to be above pH 7. Potassium carbonate (90 g, 1.5% w/w) was added to the organic layer before concentrating the solution to a volume of 9 L by distillation at

atmospheric pressure. Tetrahydrofuran (40 L) was added and the remainder of the dichloromethane was removed by distillation at atmospheric pressure to give a solution (12 L) of the title compound (5.27 kg, 25.2 mol, 64% yield) in tetrahydrofuran that was used directly in the next step; ^{1}H-NMR ($CDCl_3$, 300 MHz,) δ: 1.45 (s, 9H), 2.60 (t, 2H), 3.53 (t, 2H); LRMS (EI) : m/z [MH$^+$] 209 (^{79}Br).

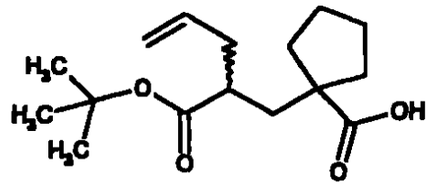
b) 1-(3-*tert*-Butoxy-3-oxopropyl)cyclopentane carboxylic acid



To a solution of commercially supplied lithium diisopropylamide (20.2 kg of a 2M solution in tetrahydrofuran/n-heptane/ethylbenzene, 51.0 mol) at -15 °C was added a solution of cyclopentane carboxylic acid (2.7 kg, 23.7 mol) in anhydrous tetrahydrofuran (8.1 L) cautiously over a period of 1 hour with stirring under a nitrogen atmosphere. The resultant solution was stirred at 0 °C for 3 hours during which time a precipitate formed. This suspension was then added to a solution of product from stage a) above (5.24 kg, 25.1 mol) in tetrahydrofuran (52 L) at –15°C over a period of 1.25 hours. After the addition was complete, the reaction was stirred at 0 °C for 1 hour and then warmed to 20 °C over 4 hours and left to stir at this temperature for 13.5 hours. The reaction mixture was then cooled to -15 °C and to this was added n-heptane (27 L) and 5M aqueous hydrochloric acid (23.7 L, 118.5 mol) cautiously with stirring. The layers were then separated and the aqueous phase was extracted with n-heptane (13.5 L). The combined organic phases were extracted with 5% aqueous sodium bicarbonate solution (3 x 30 L) and then with 10% aqueous potassium carbonate solution (3 x 30 L). The aqueous extracts were kept separate and analysed for product content. The three aqueous potassium carbonate extracts were combined and n-heptane (27 L) was added before the pH of this mixture was then adjusted to pH 7-8 using 5 M aqueous hydrochloric acid (10.5 L) with stirring. The layers were then separated and more n-heptane (40.5 L) was added. The pH of the mixture was then adjusted further to pH 3 using 5 M aqueous hydrochloric acid (19.5 L). The layers were then separated and the organic phase was washed with deionised water (2 x 27 L). The organic phase then azeotropically dried by distillation under vacuum and the solvent volume was reduced to approximately 4 L. The solution

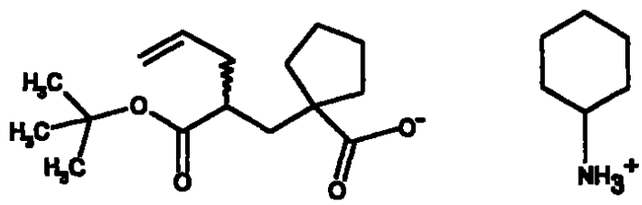
was then cooled to 0 °C with stirring to allow crystallisation to occur. Stirring was continued at 0 °C for 5 hours after which the product was collected by filtration. The resultant solid was dried under vacuum at 50 °C for 22.5 hours to give the title compound (1.17 kg, 4.8 mol, 20% yield) as a white crystalline solid; m.p. : 89-92 °C; LRMS (negative APCI) : m/z $[M-H]^-$ 241; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.47 (s, 9H), 1.50-1.60 (m, 2H), 1.60-1.82 (m, 4H), 1.69-2.0 (m, 2H), 2.08-2.23 (m, 2H), 2.23-2.34 (m, 2H).

c) 1-[2-(*tert*-Butoxycarbonyl)-4-pentenyl]cyclopentane carboxylic acid



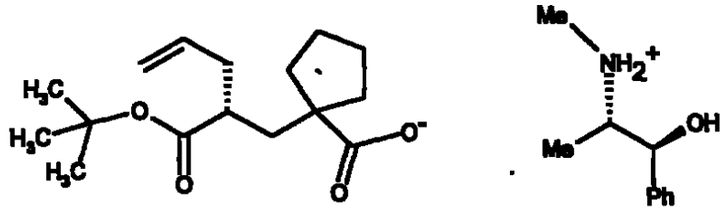
To a solution of commercially supplied lithium diisopropylamide (7.63 kg of a 2M solution in tetrahydrofuran/n-heptane/ethylbenzene, 19.3 mol) in anhydrous tetrahydrofuran (18.3 L) at -10 °C was added a solution of the product from stage b) above (2.0 kg, 8.25 mol) in anhydrous tetrahydrofuran (10 L) with stirring over a period of 4 hours whilst maintaining the reaction temperature at -10 °C. To the resultant solution was added a solution of allyl bromide (1.2 kg, 9.9 mol) in tetrahydrofuran (10 L) over a period of 2 hours before warming the reaction to 20 °C over a period of 4 hours. After stirring at this temperature for 9.5 hours the reaction was quenched by the addition of water (40 L) and the two phases were separated. The organic phase was then extracted successively with water (20 L) and 0.3 M aqueous potassium hydroxide solution (12 L). To the combined aqueous phases was then added n-heptane (20 L), and 5 M aqueous hydrochloric acid (7.5 L) until the pH of the aqueous layer was pH 2. The layers were then separated and the aqueous phases were then extracted with n-heptane (20 L). The combined organic phases were then washed with saturated brine (2 x 8.0 L) and concentrated under vacuum to give the crude product (2.22 kg, 7.86 mol, 95% yield) as a solution in n-heptane (12.5 kg total solution weight) that was used directly in the next step; LRMS (EI) : $[M^+ - C_4H_8]$ 226, $[M^+ - HO^tBu]$ 208, [208-CO] 180; ^{1}H-NMR ($CDCl_3$, 300 MHz), δ: 1.45 (s, 9H), 1.48-1.60 (m, 2H), 1.60-1.76 (m, 4H), 1.80 (dd, 1H), 2.03-2.27 (m, 4H), 2.27-2.45 (m, 2H), 5.06 (dd, 2H), 5.75 (ddt, 1H).

d) Cyclohexanaminium 1-[2-(*tert*-butoxycarbonyl)-4-pentenyl] cyclopentane carboxylate



To a solution of crude product from stage c) above (3.83 kg, 13.6 mol) in n-heptane (23 L) was added cyclohexylamine (1.35 kg, 13.8 mol) in n-heptane (7.0 L) over a period of 0.5 hours. The delivery lines were washed with n-heptane (0.7 L, 0.2 ml/g) and this was added to the reaction mixture. The resultant slurry was granulated at 20 °C for 2 hours and the solid was then collected by filtration. The filter cake was washed with n-heptane (2 x 1.9 L) and was dried under vacuum at 50 °C for 23 hours. The resultant white solid (4.42 kg, 11.6 mol, 85% yield) was dissolved in ethyl acetate (24 L, 5.4 ml/g) and was heated to 70 °C to form a clear solution. The resultant solution was then cooled to 50 °C and was seeded with authentic compound (1 g). The suspension was then cooled from 50 °C to 20 °C over a period of 4 hours. The suspension was granulated at 20 °C for 0.5 hours and the solid was collected by filtration. The filter cake was washed with ethyl acetate (2 x 1.8 L), and the solid was dried under vacuum at 45 °C for 14.5 hours to give the title compound (3.76 kg, 9.85 mol, 85% recovery) as a white crystalline solid; m.p. : 129.5-131.0 °C; Anal. Found: C, 69.28; H, 10.31; N, 3.60. $C_{22}H_{39}NO_4$ requires C, 69.25; H, 10.30; N, 3.67%; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.05-1.35 (m, 6H), 1.35-1.53 (m, 10H), 1.53-1.69 (m, 5H), 1.69-1.89 (m, 3H), 1.89-2.02 (m, 3H), 2.02-2.18 (m, 2H), 2.18-2.31 (m, 2H), 2.31-2.44 (m, 1H), 2.71-2.94 (m, 1H), 5.03 (dd, 2H), 5.73 (ddt, 1H), 6.4 (bs, 3H).

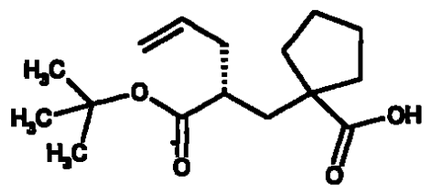
e) (1*S*, 2*S*)-1-Hydroxy-*N*-methyl-1-phenyl-2-propanaminium 1-[(2*R*)-2-(*tert*-butoxycarbonyl)-4-pentenyl]cyclopentane carboxylate



To a mixture of water (22.6 L) and n-heptane (22.6 L) was added the product from stage d) above (3.76 kg, 9.85 mol) with stirring. 5 M aqueous hydrochloric

acid (2.2 L, 11.0 mol) was added until the pH of the aqueous phase was pH 3. The layers were separated and the aqueous phase was extracted further with n-heptane (2 x 22.6 L). The combined organic extracts were then washed with saturated brine (3.8 L, 10 ml/g) and were then concentrated under vacuum to a volume of 20.3 L total solution volume). To this solution was added (1*S*, 2*S*)-(+)-pseudoephedrine (1.63 kg, 9.86 mol, 1.0 eq) with stirring and the suspension was heated to 80 °C, whereupon complete dissolution occurred. The resultant solution was held at this temperature for 20 minutes before cooling to 45 °C. A sample of seed crystals (0.2 g) was then added and the suspension was cooled to 20 °C over a period of 2 hours. The resultant slurry was granulated for a period of 4 hours and the solid was then collected by filtration. The filter cake was then washed with n-heptane (3 x 0.5 L) and was dried under vacuum at 40-45 °C for 23 hours to give a white solid (2.15 kg, 4.8 mol, 49% yield). A suspension of this material (2.15 kg, 4.8 mol) in n-heptane (10.8 L) was heated to 80 °C to give a clear solution. After holding this temperature for 10 minutes the solution was cooled to 60 °C and a sample of seed crystals (1 g) was added. The resultant suspension was then cooled to 20 °C over a period of 2 hours and was then granulated for 1.5 hours at this temperature. The solid was then collected by filtration, washed with n-heptane (2 x 0.59 L) and was dried under vacuum at 50 °C for 17.5 hours to give the title compound (1.80 kg, 4.0 mol, 84% recovery) as a white crystalline solid; *m.p.* : 109-110 °C; Anal. Found: C, 69.48; H, 9.25; N, 3.17. $C_{29}H_{41}NO_5$ requires C, 69.77; H, 9.23; N, 3.17%; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.05 (d, 3H), 1.34-1.55 (m, 2H), 1.44 (s, 9H), 1.55-1.73 (m, 4H), 1.82-2.03 (m, 2H), 2.03-2.21 (m, 2H), 2.21-2.35 (m, 2H), 2.35-2.41 (m, 1H), 2.60 (s, 3H), 3.03 (pent, 1H), 4.52 (d, 1H), 5.03 (dd, 2H), 5.76 (ddt, 1H), 7.21-7.45 (m, 5H).

f) 1-[(2*R*)-2-(*tert*-Butoxycarbonyl)-4-pentenyl]cyclopentane carboxylic acid

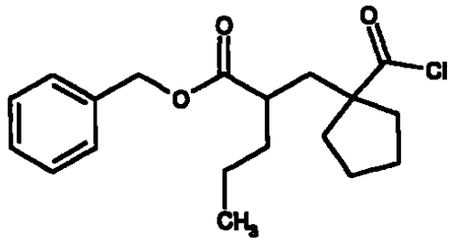


To a mixture of deionised water (10.8 L) and n-heptane (10.8 L) was added the product from stage e) above (1.80 kg, 4.0 mol) and 5 M aqueous hydrochloric acid (1.3 L, 6.5 mol) until the pH of the aqueous layer was pH 3. The layers were then separated and the aqueous layer was extracted with n-heptane (2 x 10.8 L).

The combined organic layers were washed with brine (1.8 L) and were then concentrated by distillation at atmospheric pressure to a volume of 6.4 L. Ethanol (18.0 L) was then added and the solution was again concentrated by distillation at atmospheric pressure to give the title compound (1.14 kg, 4.0 mol, 100% recovery) as a solution in ethanol (6.4 L total solution volume) that was used directly in the next step (see above); LRMS (EI) : $[M^+-C_4H_8]$ 226, $[M^+-HO^tBu]$ 208, [208-CO] 180; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.45 (s, 9H), 1.48-1.60 (m, 2H), 1.60-1.76 (m, 4H), 1.80 (dd, 1H), 2.03-2.27 (m, 4H), 2.27-2.45 (m, 2H), 5.06 (dd, 2H), 5.75 (ddt, 1H).

Preparation 3

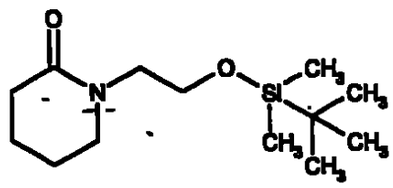
Benzyl 2-[(1-(chlorocarbonyl)cyclopentyl]methyl]pentanoate



Oxalyl chloride (1.15ml, 13.2mmol) was added to an ice-cooled solution of 1-[2-[(benzyloxy)carbonyl]pentyl]cyclopentanecarboxylic acid (EP 274234, Example 16) (2.0g, 6.3mmol) in dry dichloromethane (20ml), and the solution stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give the title compound as a golden oil, 2.1g; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.28 (m, 2H), 1.43 (m, 2H), 1.63 (m, 6H), 2.00 (m, 1H), 2.08-2.35 (m, 3H), 2.44 (m, 1H), 5.15 (s, 2H), 7.28 (m, 5H).

Preparation 4

1-(2-[(*tert*-Butyl(dimethyl)silyl]oxy)ethyl)-2-piperidinone

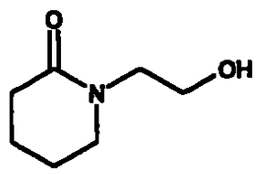


Sodium hydride (807mg, 60% dispersion in mineral oil, 20.18mmol) was added portionwise to a solution of *d*-valerolactam (2.0g, 20.2mmol) in tetrahydrofuran (100ml) under nitrogen. (2-Bromoethoxy)(*tert*-butyl)dimethylsilane (ex Aldrich Chemical Co.)

(4.33ml, 20.2mmol) was added portionwise, and the reaction heated at 70°C for 18 hours. Water (50ml) was added to the cooled reaction, the mixture concentrated *in vacuo*, to remove the tetrahydrofuran, and extracted with ethyl acetate (200ml). The organic solution was dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 97:3) to give the title compound, 3.25g; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.00 (s, 6H), 0.83 (s, 9H), 1.75 (m, 4H), 2.35 (m, 2H), 3.39 (m, 4H), 3.75 (t, 2H); LRMS : m/z 257.9 (M^+).

Preparation 5

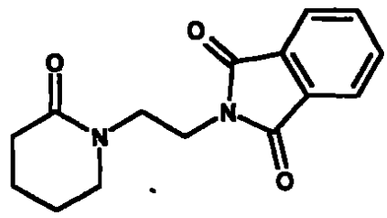
1-(2-Hydroxyethyl)-2-piperidinone



Tetra-n-butylammonium fluoride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added to a solution of the lactam from preparation 4 (3.3g, 12.8mmol) in tetrahydrofuran (50ml), and the reaction stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure, the residue azeotroped with dichloromethane, and purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 95:5) to give the title compound as an oil; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.80 (m, 4H), 2.40 (t, 2H), 3.38 (t, 2H), 3.42 (t, 1H), 3.56 (t, 2H), 3.80 (t, 2H).

Preparation 6

2-[2-(2-Oxo-1-piperidinyl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione



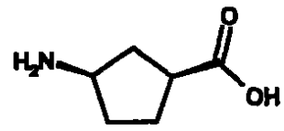
Pthalimide (952mg, 6.47mmol) was added to a solution of the product from preparation 5 (842mg, 5.88mmol) in tetrahydrofuran (30ml), and the mixture sonicated until a solution was obtained. Polymer supported triphenyl phosphine (2.5g, 7.5mmol) and diethyl azodicarboxylate (1.15ml, 7.31mmol) were added, and the reaction stirred at room

64

temperature for 18 hours. The mixture was filtered through Arbocel , the filtrate concentrated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 100:0), to give the title compound as a white foam, 1.6g (containing some impurities); ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.60-1.80 (m, 4H), 2.17 (m, 2H), 3.30 (m, 2H), 3.60 (m, 2H), 3.83 (m, 2H), 7.62 (m, 2H), 7.79 (m, 2H); LRMS : m/z 273.2 (MH^+).

Preparation 7

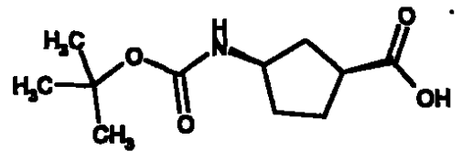
(1*S*,3*R*)-3-Aminocyclopentanecarboxylic acid



Platinum oxide (1g) was added to a solution of (1*R*,4*S*)-4-amino-cyclopent-2-ene carboxylic acid (Taylor *et al.*, J. Chem. Soc., Chem. Commun. (1990), (16), 1120-1) (5.3g, 41.7mmol) in water (70ml), and the mixture was hydrogenated at 45 psi and room temperature for 18 hours. The mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure, and the residue azeotroped with toluene, to afford the title compound as an off-white solid; ^{1}H NMR (D_2O, 400MHz) δ: 1.70-1.92 (m, 3H), 2.00 (m, 2H), 2.18 (m, 1H), 2.77 (m, 1H), 3.68 (m, 1H); LRMS : m/z 129.8 (MH^+).

Preparation 8

(1*S*,3*R*)-3-[(*tert*-Butoxycarbonyl)amino]cyclopentanecarboxylic acid

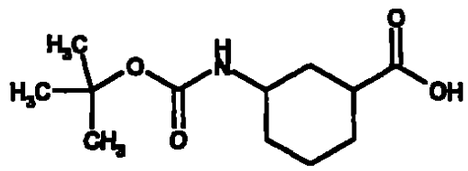


Di-*tert*-butyl dicarbonate (10g, 45.8mmol) was added to an ice-cooled solution of the product from preparation 7 (5.4g, 41.8mmol) in dioxan (42.5ml) and sodium hydroxide solution (42.5ml, 1N, 42.5mmol), and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure to remove the dioxan, then acidified to pH 2 using 2N hydrochloric acid. The aqueous solution was extracted with ethyl acetate (5x100ml), the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure to give a white solid. This was triturated with hexane, to give the title product, 8.0g, 83%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H),

1.58-2.06 (m, 5H), 2.21 (m, 1H), 2.84 (m, 1H), 4.01 (m, 1H), 4.84 (m, 1H); LRMS: m/z 228 $(M-H)^-$.

Preparation 9

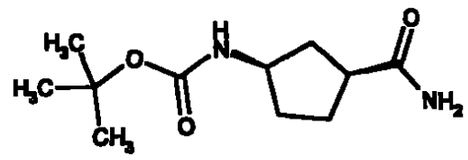
3-[(*tert*-Butoxycarbonyl)amino]cyclohexanecarboxylic acid



The title compound was obtained as a white solid in 81% yield, from 3-aminocyclohexanecarboxylic acid, following the procedure described in preparation 8; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.04 (m, 1H), 1.19-1.50 (m, 13H), 1.83 (m, 1H), 1.97 (m, 2H), 2.24 (m, 1H), 2.40 (m, 1H), 3.44 (bs, 1H), 4.42 (bs, 1H).

Preparation 10

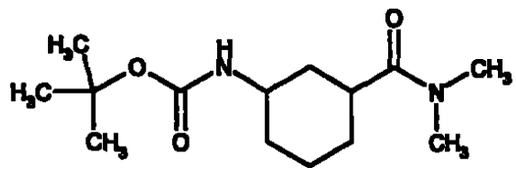
tert-Butyl (1*R*,3*S*)-3-(aminocarbonyl)cyclopentylcarbamate



Benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (3.4g, 6.54mmol), 1-hydroxybenzotriazole hydrate (883mg, 6.54mmol), ammonium chloride (467mg, 8.72mmol) and N-ethyldiisopropylamine (3.04ml, 17.5mmol) were added sequentially to a solution of the acid from preparation 8 (1.0g, 4.37mmol) in N,N-dimethylformamide (16ml), and the reaction stirred at room temperature for 2 hours. The mixture was diluted with ethyl acetate (100ml), washed with water (3x), and brine, then dried ($MgSO_4$) and evaporated under reduced pressure. The residual gum was purified by chromatography on silica gel using a Biotage® column, and an elution gradient of dichloromethane:methanol (98:2 to 95:5). The product was triturated with ether to afford the title compound as a white solid, 438mg, 44%; ^1H NMR ($DMSOd_6$, 400MHz) δ: 1.34 (s, 9H), 1.40 (m, 2H), 1.64 (m, 3H), 1.90 (m, 1H), 2.55 (m, 1H), 3.70 (m, 1H), 6.70 (bs, 1H), 6.80 (d, 1H), 7.22 (bs, 1H).

Preparation 11

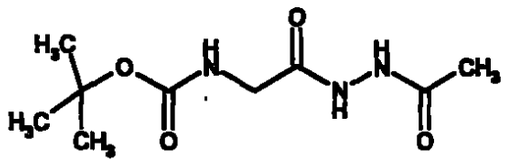
tert-Butyl 3-[(dimethylamino)carbonyl]cyclohexylcarbamate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.19g, 6.19mmol), 1-hydroxybenzotriazole hydrate (840mg, 6.19mmol), N-methylmorpholine (1.1ml, 10.1mmol) and finally 33% ethanolic dimethylamine (1.5ml) were added to a solution of the acid from preparation 9 (1.37g, 5.6mmol) in N,N-dimethylformamide (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure, the residue diluted with ethyl acetate and washed with water (2x). The mixture was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of methanol:dichloromethane (5:95 to 10:90), to give the title compound, 998mg, 66%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.12 (m, 1H), 1.40 (m, 11H), 1.70 (m, 2H), 1.85 (m, 1H), 2.00 (m, 2H), 2.62 (m, 1H), 2.96 (s, 3H), 3.05 (s, 3H), 3.50 (m, 1H), 4.50 (m, 1H).

Preparation 12

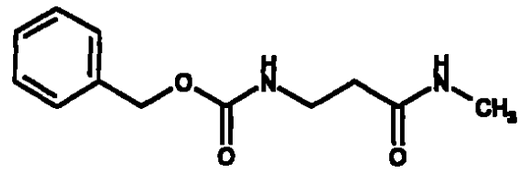
tert-Butyl 2-(2-acetylhydrazino)-2-oxoethylcarbamate



2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (7.06g, 28.5mmol) was added to a solution of N-*tert*-butoxycarbonylglycine (5.0g, 28.6mmol) in dichloromethane (75ml), and the solution stirred for 15 minutes. Acetic hydrazide (2.6g, 35.1mmol) was added, and the reaction stirred at room temperature for 18 hours. The resulting precipitate was filtered, and dried *in vacuo*, to afford a white crystalline solid, 2.42g. The filtrate was concentrated under reduced pressure, diluted with ether, and the resulting precipitate filtered and dried *in vacuo*, to afford additional product as a white solid, 4.4g, 67% in total; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 2.02 (s, 3H), 3.87 (d, 2H), 5.22 (bs, 1H), 8.27 (bs, 1H), 8.84 (bs, 1H); LRMS : m/z 249.2 (MNH_4^+); Anal. Found: C, 46.41; H, 7.36; N, 17.98, $C_9H_{17}N_3O_4$ requires C, 46.66; H, 7.41; N, 18.13%.

Preparation 13

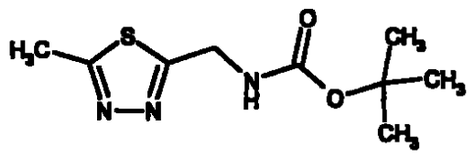
Benzyl 3-(methylamino)-3-oxopropylcarbamate



A mixture of N-[(benzyloxy)carbonyl]-β-alanine (10g, 44.8mmol), methylamine hydrochloride (3.33g, 49.28mmol), 1-hydroxybenzotriazole hydrate (6.05g, 44.8mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.3g, 53.76mmol) and N-methylmorpholine (11.33ml, 103mmol) in dichloromethane (200ml) was stirred at room temperature for 18 hours. The resulting precipitate was filtered off to give the desired product as a colourless foam, and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:hexane (90:10 to 100:0) to give additional product, 7.98g, 75% in total; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 2.42 (t, 2H), 2.80 (s, 3H), 3.50 (m, 2H), 5.21 (s, 2H), 5.49 (bs, 1H), 5.63 (bs, 1H), 7.36 (m, 5H); Anal. Found: C, 60.68; H, 7.00; N, 11.95. $C_{12}H_{16}N_2O_3$ requires C, 61.00; H, 6.83; N, 11.86%.

Preparation 14

tert-Butyl (5-methyl-1,3,4-thiadiazol-2-yl)methylcarbamate

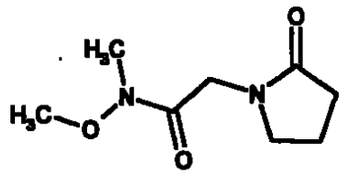


Lawesson's reagent (960mg, 2.38mmol) was added to a solution of the hydrazide from preparation 12 (500mg, 2.16mmol) in tetrahydrofuran (40ml) and the reaction heated under reflux for 3 hours, then stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 80:20) to give an oil. Ethyl acetate (100ml) and charcoal (2g) were added and the mixture was stirred for 10 minutes then filtered. The filtrate was concentrated under reduced pressure, and the residue azeotroped with dichloromethane to afford the title compound as a crystalline solid, 441mg, 89%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.45 (s, 9H), 2.77 (s, 3H), 4.66 (d, 2H), 5.22 (bs, 1H); LRMS : m/z 230.1 (MH^+).

66

Preparation 15

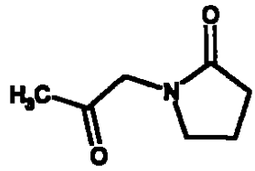
N-Methoxy-*N*-methyl-2-(2-oxo-1-pyrrolidinyl)acetamide



2-Chloro-N-methoxy-N-methylacetamide (ex Aldrich Chemical Co.) (3.2g, 23.3mmol) was added to a suspension of 2-pyrrolidinone (2.0g, 23.5mmol) and sodium hydride (940mg, 60% dispersion in mineral oil, 23.5mmol) in tetrahydrofuran (60ml), and the reaction stirred at room temperature for 48 hours. The mixture was quenched with water (150ml), and extracted with ethyl acetate (200ml) and dichloromethane (200ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was triturated with hexane, then ether to afford the title compound as white crystals, 1.8g, 41%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.02 (m, 2H), 2.40 (t, 2H), 3.17 (s, 3H), 3.48 (t, 2H), 3.72 (s, 3H), 4.19 (s, 2H); LRMS : m/z 186.9 (MH^+).

Preparation 16

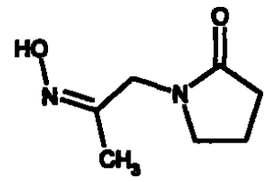
1-(2-Oxopropyl)-2-pyrrolidinone



Methylmagnesium chloride (2.7ml, 3M in tetrahydrofuran, 8.1mmol) was added to a cooled (-20°C) solution of the amide from preparation 15 (1.5g, 8.1mmol) in tetrahydrofuran (50ml), and the reaction allowed to warm to room temperature, then stirred for an hour. The mixture was quenched by the addition of aqueous ammonium chloride solution, then extracted with ethyl acetate (3x50ml). The combined organic solutions were dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as an oil, 645mg, 56%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.07 (m, 2H), 2.17 (s, 3H), 2.42 (t, 2H), 3.42 (t, 2H), 4.10 (s, 2H).

Preparation 17

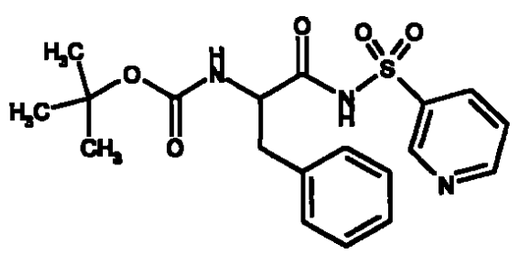
1-[2-(Hydroxyimino)propyl]-2-pyrrolidinone



Hydroxylamine hydrochloride (316mg, 4.55mmol) and then pyridine (370µl, 4.58mmol) were added to a solution of the amide from preparation 16 (643mg, 4.55mmol) in ethanol (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 90:10). The product was triturated with ether to give the title compound as a white solid, 375mg, 53%; ^{1}H NMR (DMSOd_6, 400MHz) δ: 1.60 (s, 3H), 1.87 (m, 2H), 2.20 (t, 2H), 3.19 (t, 2H), 3.78 (s, 2H), 10.77 (s, 1H); LRMS : m/z 157.4 (MH^+).

Preparation 18

tert-Butyl 1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethylcarbamate

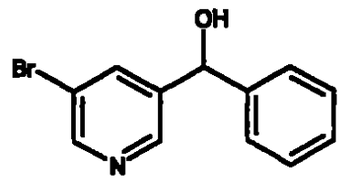


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (939mg, 4.9mmol), 1-hydroxybenzotriazole hydrate (562mg, 4.15mmol), and N-methylmorpholine (952mg, 9.42mmol) were added to an ice-cold solution of N-*tert*-butoxycarbonyl-L-phenylalanine (1.0g, 3.77mmol) in dichloromethane (20ml), and the mixture stirred for 15 minutes. 3-Pyridinesulphonamide (Mon. für Chemie; 72; 77; 1938) (596mg, 3.77mmol) was added, and the reaction stirred at room temperature for 24 hours. The mixture was evaporated under reduced pressure and the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The aqueous layer was extracted with ethyl acetate, then dichloromethane, the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified twice by column chromatography on silica gel, using an elution gradient of ethyl acetate:ethanol (100:0 to 90:10) to give the desired product as a white foam, 1.01g, 66%; ^{1}H NMR (DMSOd_6,

300MHz) δ: 1.30 (s, 9H), 2.77 (m, 1H), 2.97 (m, 1H), 3.84 (m, 1H), 5.95 (bs, 1H), 6.96 (m, 2H), 7.08 (m, 3H), 7.42 (m, 1H), 8.05 (d, 1H), 8.60 (d, 1H), 8.84 (m, 1H); $[\alpha]_D$ = -10° (0.1% solution in methanol).

Preparation 19

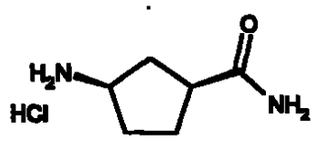
(5-Bromo-3-pyridinyl)(phenyl)methanol



n-Butyl lithium (17ml, 2.5M in hexanes, 42.5mmol) was added dropwise to cooled (-78° C) solution of 3,5-dibromopyridine (10g, 42.2mmol) in ether (200ml), so as to maintain an internal temperature <-70°C. The mixture was then stirred for 15 minutes and a solution of benzaldehyde (4.5g, 42.5mmol) in ether (20ml) was added dropwise, again maintaining the temperature <-70°C. The mixture was stirred for 15 minutes, then allowed to warm to room temperature over an hour. The reaction was quenched by the addition of 0.9M ammonium chloride solution (200ml), the layers separated, and the aqueous phase extracted with ether. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:ether (95:5 to 80:20) to give the title compound as a yellow oil, 7.6g, 68%; ^1H NMR (D_2O, 300MHz) δ: 5.80 (s, 1H), 7.37 (m, 5H), 7.90 (s, 1H), 8.40 (s, 1H), 8.44 (s, 1H).

Preparation 20

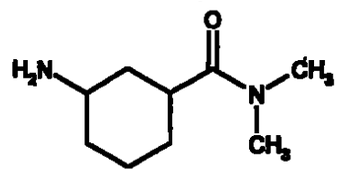
(1S,3R)-3-Aminocyclopentanecarboxamide hydrochloride



Hydrogen chloride gas was bubbled through an ice-cooled solution of the amide from preparation 10 (438mg, 1.92mmol) in dichloromethane (50ml) for 10 minutes, and the resulting suspension stirred at room temperature for 2 hours. The mixture was purged with nitrogen, then evaporated under reduced pressure. The residue was triturated with ether, to afford the title compound as a solid; ^1H NMR (D_2O, 400MHz) δ: 1.63-1.82 (m, 3H), 1.92-2.07 (m, 2H), 2.19 (m, 1H), 2.82 (m, 1H), 3.62 (m, 1H).

Preparation 21

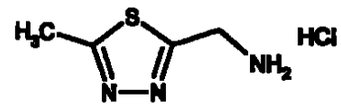
3-Amino-*N,N*-dimethylcyclohexanecarboxamide



A solution of the amide from preparation 11 (997mg, 3.69mmol) in trifluoroacetic acid (8ml) and dichloromethane (8ml) was stirred at room temperature for 4 hours. The mixture was concentrated under reduced pressure and the residue partitioned between dichloromethane (25ml) and sodium bicarbonate solution (25ml). The pH was adjusted to 9 using sodium hydroxide solution, the layers separated, and the aqueous phase evaporated under reduced pressure. The resulting solid was triturated with hot ethyl acetate, the suspension filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (84:14:2) to afford the title compound as a colourless oil, 346mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.08 (m, 1H), 1.25-1.54 (m, 6H), 1.72 (m, 1H), 1.86 (m, 2H), 2.53-2.75 (m, 2H), 2.96 (s, 3H), 3.03 (s, 3H).

Preparation 22

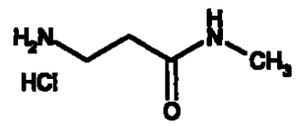
(5-Methyl-1,3,4-thiadiazol-2-yl)methylamine hydrochloride



Hydrogen chloride gas was bubbled through an ice-cooled solution of the thiadiazole from preparation 14 (425mg, 1.85mmol) in dichloromethane (50ml) for 15 minutes, and the reaction stirred at room temperature for 1 hour. The mixture was purged with nitrogen, then evaporated under reduced pressure to afford the title compound as a white solid; ^{1}H NMR ($DMSOd_6$, 400MHz) δ: 2.75 (s, 3H), 4.48 (m, 2H), 8.80 (bs, 3H).

Preparation 23

3-Amino-N-methylpropanamide hydrochloride

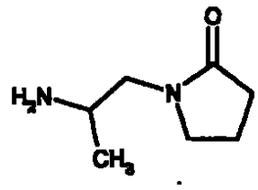


A mixture of the benzyl carbamate from preparation 13 (7.92g, 33.5mmol) and 5% palladium on charcoal (800mg) in ethanol (300ml) was hydrogenated at 50 psi and room

temperature for 4 hours. The reaction mixture was filtered through Arbocel washing through with ethanol, and 1N hydrochloric acid (36.9ml, 36.9mmol) was added to the combined filtrate. This solution was evaporated under reduced pressure and the residue azeotroped with dichloromethane to afford the title compound, 4.66g, ^{1}H NMR ($DMSOd_6$, 300MHz) δ: 2.46 (t, 2H), 2.60 (s, 3H), 2.95 (m, 2H), 7.98-8.16 (m, 2H).

Preparation 24

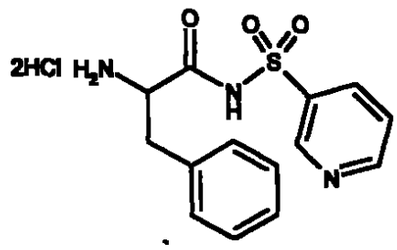
1-(2-Aminopropyl)-2-pyrrolidinone



A mixture of the oxime from preparation 17 (375mg, 2.40mmol) and platinum oxide (300mg) in ethanol (20ml) was hydrogenated at 60psi and room temperature for 18 hours. Tlc analysis showed starting material remaining, so additional platinum oxide (100mg) was added and the reaction continued for a further 4 hours. The mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:0.88 ammonia (95:5:0.5 to 90:10:1) to give the title compound as a clear oil, 170mg, 50%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.02 (d, 3H), 1.36 (bs, 2H), 2.00 (m, 2H), 2.38 (t, 2H), 3.00-3.16 (m, 2H), 3.21 (m, 1H), 3.35-3.45 (m, 2H); LRMS : m/z 143 (MH^+).

Preparation 25

N-(2-Amino-3-phenylpropanoyl)-3-pyridinesulphonamide dihydrochloride

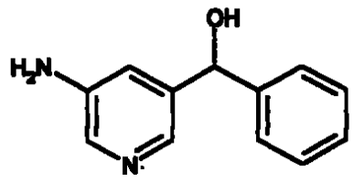


Saturated ethereal hydrochloric acid (40ml) was added to an ice-cold solution of the sulphonamide from preparation 18 (959mg, 2.37mmol) in ethyl acetate (30ml) and ether (10ml), and the solution stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with

dichloromethane (3x) to afford the title compound as a white solid, 959mg; ^{1}H NMR (DMSOd_6, 300MHz) δ: 3.23-3.50 (m, 1H), 3.70-3.98 (m, 1H), 4.13 (m, 1H), 7.05 (m, 2H), 7.20 (m, 3H), 7.78 (m, 1H), 8.36 (d, 1H), 8.44 (bs, 2H), 8.95 (d, 1H), 9.02 (s, 1H);$[\alpha]_D$ = +138° (0.5% solution in methanol).

Preparation 26

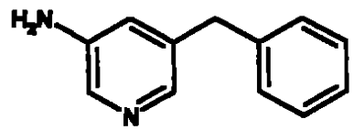
(5-Amino-3-pyridinyl)(phenyl)methanol



A mixture of the bromide from preparation 19 (2.0g, 7.60mmol) and copper (II) sulphate pentahydrate (350mg, 1.40mmol) in 0.88 ammonia (18ml) was heated at 135°C in a sealed vessel for 24 hours. Sodium hydroxide solution (1N, 10ml) was added to the cooled solution, and the mixture was then extracted with ether (6x). The combined organic extracts were dried ($MgSO_4$), and concentrated under reduced pressure. The resulting precipitate was filtered, washed with ether and dried to give the title compound as a solid, 1.25g, 83%; m.p. 92-94°C; ^{1}H NMR (DMSOd_6, 300MHz) δ: 5.22 (s, 2H), 5.59 (d, 1H), 5.86 (d, 1H), 6.83 (s, 1H), 7.20 (m, 1H), 7.34 (m, 4H), 7.78 (m, 2H).

Preparation 27

5-Benzyl-3-pyridinylamine

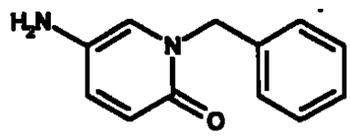


A mixture of the alcohol from preparation 26 (700mg, 3.5mmol) and 5% palladium on charcoal (70mg) in hydrochloric acid (5ml, 1N) and ethanol (20ml) was hydrogenated at 30 psi and room temperature for 6 hours. The mixture was filtered through Arbocel®, and the filtrate concentrated under reduced pressure. The residue was basified using aqueous sodium bicarbonate solution, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (92:8:0.4) as eluant, to give the title compound as a solid, 500mg, 78%; mp 107-109°C; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 3.61 (bs, 2H), 3.94 (s, 2H), 6.78 (s, 1H), 7.24 (m, 5H), 7.98 (s, 2H).

69

Preparation 28

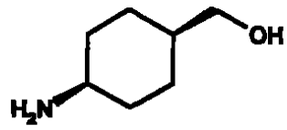
5-Amino-1-benzyl-2(1*H*)-pyridinone



A mixture of 1-benzyl-5-nitro-1H-pyridin-2-one (Justus Liebigs Ann. Chem. 484; 1930; 52) (1.0g, 4.35mmol), and granulated tin (3.5g, 29.5mmol) in concentrated hydrochloric acid (14ml) was heated at 90°C for 1.5 hours. The cooled solution was diluted with water, neutralised using sodium carbonate solution, and extracted with ethyl acetate (250ml in total). The combined organic extracts were filtered, dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as a pale green solid, (turned blue with time), 440mg, 51%; ^1H NMR ($CDCl_3$, 250MHz) δ: 4.12-4.47 (bs, 2H), 5.00 (s, 2H), 6.31 (d, 1H), 6.86 (s, 1H), 7.07 (m, 1H), 7.14-7.42 (m, 5H).

Preparation 29

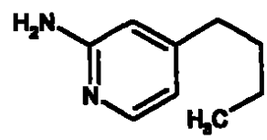
Cis-(4-Aminocyclohexyl)methanol



Lithium aluminium hydride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added dropwise to an ice-cooled solution of cis-4-aminocyclohexanecarboxylic acid (1.33g, 9.29mmol) in tetrahydrofuran (50ml), and once addition was complete, the reaction was heated under reflux for 6 hours. The resulting suspension was cooled to 5°C, and water (0.6ml), aqueous sodium hydroxide solution (1.1ml, 2M), then water (0.6ml) were added sequentially. The resulting suspension was filtered, and the filtrate evaporated under reduced pressure to give an oil, which was used without further purification; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.40-1.80 (m, 12H), 3.00 (m, 1H), 3.55 (d, 2H); LRMS : m/z 130.2 (MH^+).

Preparation 30

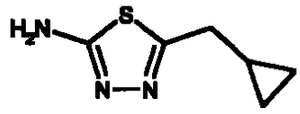
2-Amino-4-butylpyridine



A mixture of 4-butylpyridine (5.0g, 37.0mmol) and 95% sodium amide (1.7g, 40.7mmol) in xylene (10ml) was heated at 150°C for 18 hours. The cooled mixture was diluted with ether (100ml) and extracted with 2N hydrochloric acid (twice). The aqueous extracts were basified using sodium hydroxide solution, and re-extracted with ether. These combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (97:3:0.15) as eluant, to afford the title compound as a crystalline solid, 2.1g, 38%; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.38 (m, 2H), 1.60 (m, 2H), 2.52 (t, 2H), 4.38 (bs, 2H), 6.38 (s, 1H), 6.55 (d, 1H), 7.98 (d, 1H); Anal. Found: C, 72.01; H, 9.47; N, 18.53. $C_9H_{14}N_2$ requires C, 71.96; H, 9.39; N, 18.65%.

Preparation 31

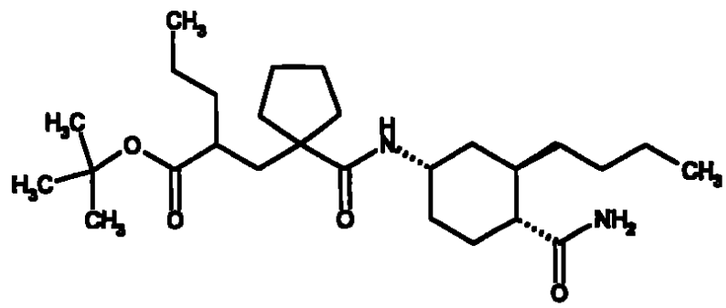
5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-amine



Oxalyl chloride (3.13ml, 35.9mmol) and N,N-dimethylformamide (1 drop) were added to a solution of cyclopropylacetic acid (3g, 29.9mmol) in dichloromethane (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure and azeotroped with dichloromethane to give a brown oil. A mixture of this intermediate acid chloride (887mg, 7.48mmol) and thiosemicarbazide (455mg, 4.99mmol) were heated at 70°C for 18 hours, then cooled. Water was added, the mixture basified to pH 9 using 50% aqueous sodium hydroxide solution, and the resulting precipitate filtered and dried, to give the title product, 410mg, 53%; ^1H NMR (CD_3OD, 400MHz) δ: 0.28 (m, 2H), 0.60 (m, 2H), 1.02 (m, 1H), 2.77 (d, 2H); LRMS : m/z 155.2 (MH^+).

Preparation 32

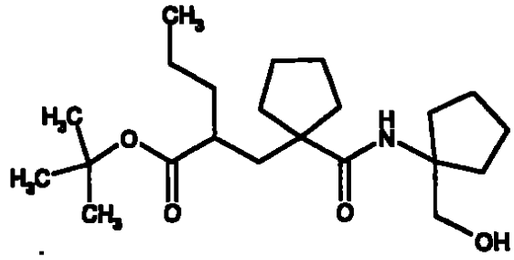
(1*R*, 2*R*,4*S*)-4-[({1-[2-(*tert*-Butoxycarbonyl)pentyl]cyclopentyl}carbonyl)amino]-2-butylcyclohexanecarboxamide



The product from Preparation 49 (65mg, 0.14mmol) was taken up in DCM (2mls) and 1,1'-carbonyldiimidazole (23mg, 0.15mmol) added in one portion. 0.88 aqueous ammonia solution (0.5mls) was added and the mixture stirred for 16h. The volatiles were removed under reduced pressure, and the resulting suspension treated with saturated aqueous $NaHCO_3$ solution (5mls). The organics were extracted with EtOAc (2x), dried ($MgSO_4$) and evaporated to give an oil, which was purified by column chromatography using 19:1 (DCM:MeOH) as eluant to provide the title product (43mg); ^1H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 6H), 1.02-1.28 (m, 37H), 2.22 (m, 1H), 4.13 (m, 1H), 5.42-5.97 (m, 3H).

Preparation 33

tert-Butyl 2-{[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate

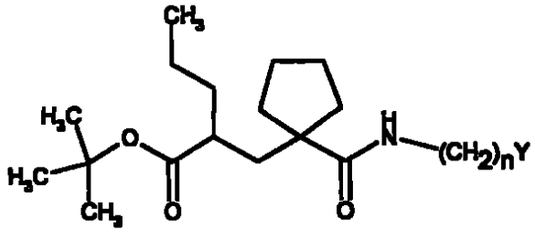


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (41mg, 0.21mmol), 1-hydroxybenzotriazole hydrate (27mg, 0.2mmol), N-methylmorpholine (35µl, 0.31mmol) and finally 1-amino-1-cyclopentanemethanol (ex Aldrich Chemical Co.) (25mg, 0.22mmol) were added to a solution of the acid from preparation 1 (150mg, 0.53mmol) in N,N-dimethylformamide (3ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (3x25ml), and brine (25ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product

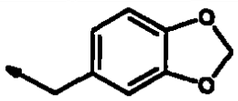
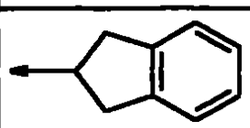
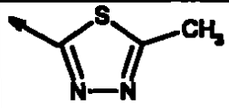
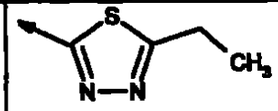
was purified by chromatography on silica gel, using ethyl acetate:pentane (30:70) as the eluant to afford the title compound, 38mg, 57%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.29 (m, 3H), 1.41-1.78 (m, 26H), 1.78-1.98 (m, 4H), 2.04 (m, 1H), 2.26 (m, 1H), 3.59 (dd, 1H), 3.70 (dq, 1H), 4.80 (t, 1H), 5.81 (s, 1H); LRMS : m/z 380 (MH^+).

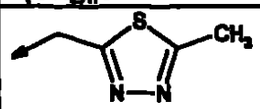
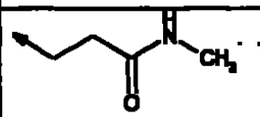
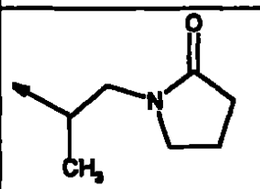
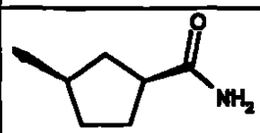
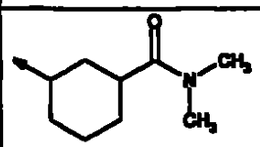
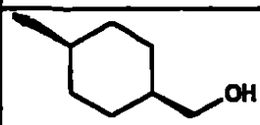
Preparations 34 to 43

Compounds of formula IVc, i.e. compounds of general formula IV where Prot is *tert*-butyl and R^1 is propyl, were prepared from the title product from Preparation 1 and the amine indicated, following a similar procedure to that described in Preparation 33.



(IVc)

Prep	-$(CH_2)_nY$	Prec. amine	Data
34		piperonylamine (ex Aldrich Chemical Co.)	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.26 (m, 4H), 1.42 (s, 9H), 1.46 (m, 2H), 1.59-1.75 (m, 5H), 1.95 (m, 2H), 2.06 (m, 1H), 2.22 (m, 1H), 4.26 (dd, 1H), 4.39 (dd, 1H), 5.95 (m, 3H), 6.78 (m, 3H). LRMS : m/z 418.3 (MH^+)
35[1]		2-aminoindan hydrochloride (ex Aldrich Chemical Co.)	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.25 (m, 3H), 4H), 1.42 (m, 12H), 1.56-1.70 (m, 4H), 1.90 (m, 2H), 2.02 (m, 1H), 2.22 (m, 1H), 2.80 (m, 2H), 3.35 (m, 2H), 4.76 (m, 1H), 5.86 (d, 1H), 7.19 (m, 4H). LRMS : m/z 400.3 (MH^+)
36[2]		2-amino-5-methyl-1,3,4-thiadiazole (ex Lancaster)	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.20-1.85 (m, 20H), 2.18 (m, 4H), 2.67 (s, 3H), 9.80 (bs, 1H). LRMS : m/z 382.3 (MH^+)
37[2]		2-amino-5-ethyl-1,3,4-thiadiazole (ex Lancaster)	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.20-1.80 (m, 22H), 1.84 (m, 1H), 2.20 (m, 4H), 3.04 (q, 2H), 9.10 (bs, 1H). LRMS : m/z 396.2 (MH^+)

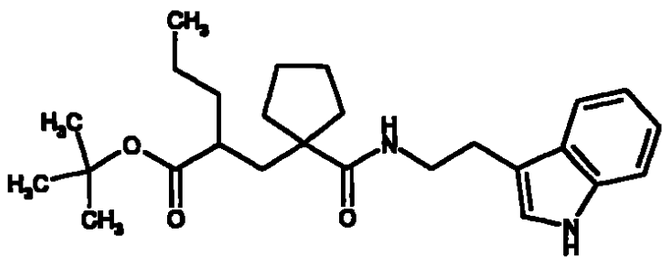
Prep	-$(CH_2)_nY$	Prec. amine	Data
38		Prep 22	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 4H), 1.42 (s, 9H), 1.44-1.76 (m, 7H), 1.95-2.12 (m, 3H), 2.20 (m, 1H), 2.76 (s, 3H), 4.74 (dd, 1H), 4.82 (dd, 1H), 6.54 (bs, 1H). LRMS : m/z 396.2 (MH^+)
39[1,2]		Prep 23	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.21-1.38 (m, 3H), 1.40-1.70 (m, 17H), 1.88-2.04 (m, 3H), 2.20 (m, 1H), 2.39 (t, 2H), 2.80 (d, 3H), 3.53 (m, 2H), 6.13 (bs, 1H), 6.40 (m, 1H). LRMS : m/z 369.5 (MH^+)
40[2]		Prep 24	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (m, 3H), 1.16 (2xd, 3H), 1.20-1.72 (m, 21H), 1.83 (m, 1H), 1.98 (m, 3H), 2.17 (m, 1H), 2.38 (m, 2H), 1.96 (m, 1H), 3.34 (m, 1H), 3.54-3.62 (m, 2H), 4.15-4.20 (m, 1H), 6.21-6.35 (2xbd, 1H). LRMS : m/z 409.3 (MH^+).
41[2]		Prep 20	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.19-1.38 (m, 4H), 1.42 (m, 12H), 1.60 (m, 3H), 1.74-2.02 (m, 10H), 2.18 (m, 1H), 2.78 (m, 1H), 4.38 (m, 1H), 5.32 (bs, 1H), 5.57 (bs, 1H), 7.28 (bs. 1H). LRMS : m/z 395 (MH^+)
42[2]		Prep 21	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.18-1.78 (m, 25H), 1.84-2.03 (m, 6H), 2.22 (m, 1H), 2.68 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.84 (m, 1H), 5.78 (m, 1H). LRMS : m/z 437.7 (MH^+)
43[2]		Prep 29	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.20-1.79 (m, 30H), 1.90 (m, 2H), 2.05 (m, 1H), 2.24 (m, 1H), 3.56 (m, 2H), 4.04 (m, 1H), 5.82 (bd, 1H). LRMS : m/z 396.4 (MH^+)

1 = reaction conducted at room temperature

2 = Methanol:dichloromethane was used as the column eluant

Preparation 44

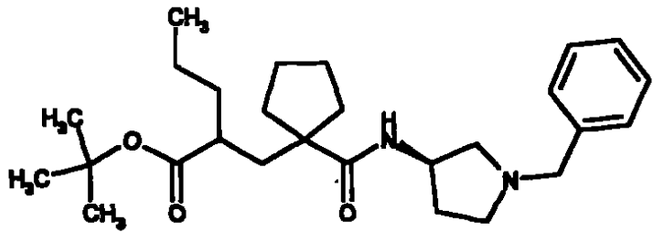
tert-Butyl 2-[[1-({[2-(1*H*-indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]-methyl]pentanoate



The title compound was obtained as a pale yellow oil in 80% yield from the acid from preparation 1 and tryptamine, following a similar procedure to that described in preparation 33, except the reaction was performed in dichloromethane at room temperature; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.86 (t, 3H), 1.26 (m, 3H), 1.42 (m, 11H), 1.50-1.69 (m, 6H), 1.83 (m, 1H), 1.90-2.05 (m, 2H), 2.22 (m, 1H), 2.99 (t, 3H), 3.60 (m, 2H), 5.78 (m, 1H), 7.06 (s, 1H), 7.14 (m, 1H), 7.20 (m, 1H), 7.38 (d, 1H), 7.63 (d, 1H), 8.02 (bs, 1H); LRMS : m/z 427.5 (MH^+).

Preparation 45

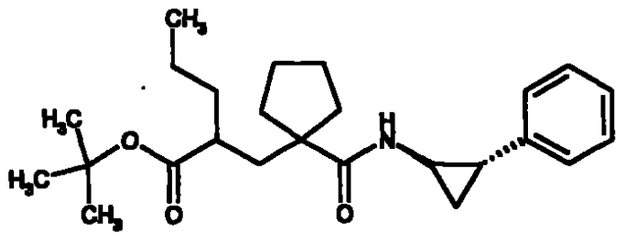
tert-Butyl 2-[(1-{[(3*S*)-1-benzylpyrrolidinyl]amino}cyclopentyl)methyl]pentanoate



The title compound was obtained quantitatively from the acid from preparation 1 and (3*S*)-1-benzyl-3-aminopyrrolidine (ex. Aldrich Chemical Co.), following a similar procedure to that described in preparation 44; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.10-1.78 (m, 21H), 1.90-2.05 (m, 3H), 2.20-2.38 (m, 3H), 2.58 (m, 2H), 2.84 (m, 1H), 3.62 (s, 2H), 4.45 (m, 1H), 6.02 (m, 1H), 7.33 (m, 5H).

Preparation 46

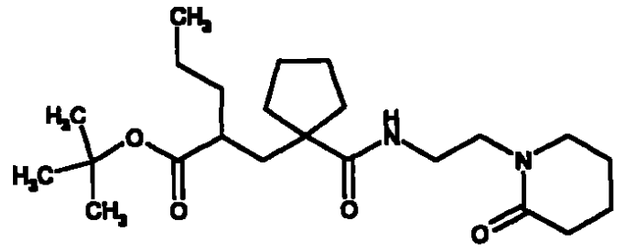
tert-Butyl 2-[[1-({[cis-2-phenylcyclopropyl]amino}carbonyl)-cyclopentyl]methyl]pentanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (81mg, 0.42mmol), N-methylmorpholine (0.15ml, 1.06mmol) and 1-*S*-amino-2-*R*-phenyl cyclopropane hydrochloride (*J. Med. Chem.*, 1988, 29, 2044) (60mg, 0.35mmol) were added to a solution of the acid from preparation 1 (100mg, 0.35mmol) in dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The reaction mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 95:5) to afford the title compound as a yellow oil, 85mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.16 (m, 1H), 1.20-1.58 (m, 16H), 1.63 (m, 5H), 1.90-2.14 (m, 4H), 2.23 (m, 1H), 2.90 (m, 1H), 6.00 (m, 1H), 7.19 (m, 3H), 7.24 (m, 2H); LRMS : m/z 400 (MH^+).

Preparation 47

tert-Butyl 2-[[1-({[2-(2-oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]-methyl]pentanoate

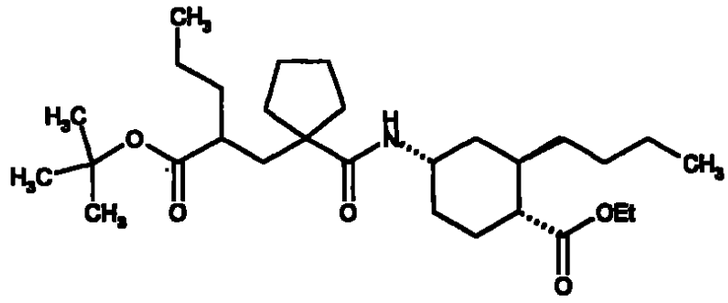


Hydrazine monohydrate (34µl, 0.70mmol) was added to a solution of the compound from preparation 6 (171mg, 0.63mmol) in ethanol (10ml), and the reaction heated under reflux for 5 hours. The cooled mixture was filtered, the filtrate concentrated under reduced pressure, the residue suspended in dichloromethane, and the suspension re-filtered. The resulting filtrate was concentrated under reduced pressure, and the residue purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (90:10:1) as eluant to give the amine, 16mg. The acid from preparation 1 (32mg,

0.11mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (25mg, 0.13mmol), 1-hydroxybenzotriazole hydrate (17mg, 0.13mmol), and N-methylmorpholine (25µl, 0.23mmol) were added to a solution of this amine in N,N-dimethylformamide (2ml), and the reaction stirred at room temperature for 18 hours. The mixture was partitioned between ethyl acetate and water, and the layers separated. The organic phase was washed with water (2x), dried ($MgSO_4$), and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98.5:1.5 to 95:5) to afford the title compound as an oil, 43mg, 17%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.22 (m, 3H), 1.38-1.65 (m, 17H), 1.58 (m, 4H), 1.95 (m, 3H), 2.17 (m, 1H), 2.37 (m, 2H), 3.30 (m, 2H), 3.38 (m, 2H), 3.50 (m, 2H), 6.76 (m, 1H); LRMS : m/z 409.2 (MH^+)

Preparation 48

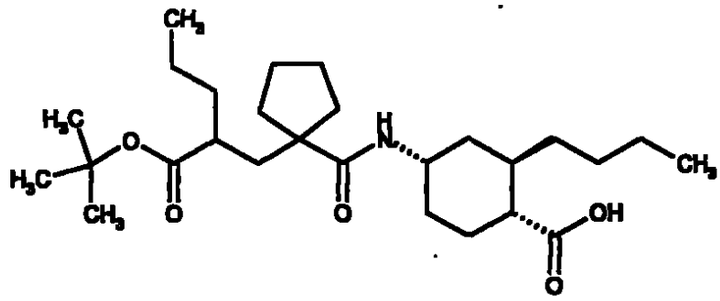
Ethyl (1*R*,2*R*,4*S*)-4-[({1-[2-(*tert*-butoxycarbonyl)pentyl]cyclopentyl}carbonyl)-amino]-2-butylcyclohexanecarboxylate



A mixture of the acid from preparation 1 (109mg, 0.38mmol), (1*R*,2*R*,4*S*)-4-amino-2-butyl-cyclohexanecarboxylic acid ethyl ester hydrochloride (WO, 9009374), (101mg, 0.38mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (95mg, 0.50mmol), 1-hydroxybenzotriazole hydrate (60mg, 0.40mmol) and triethylamine (0.12ml, 0.87mmol) in dichloromethane (3ml), was stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue treated with sodium bicarbonate solution and extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as eluant, and azeotroped with dichloromethane to afford the title compound, 190mg; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.88 (m, 6H), 1.20-1.40 (m, 13H), 1.40-2.10 (m, 25H), 2.16-2.30 (m, 2H), 4.18 (m, 3H), 5.83 (d, 1H).

Preparation 49

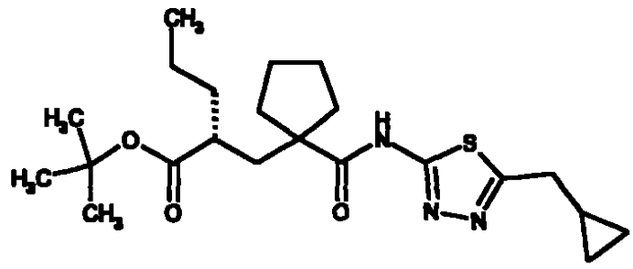
(1*R*, 2*R*,4*S*)-4-[({1-[2-(*tert*-Butoxycarbonyl)pentyl]cyclopentyl}carbonyl)amino]-2-butylcyclohexanecarboxylic acid



A mixture of the ethyl ester from preparation 48 (190mg, 0.39mmol) and 1N sodium hydroxide solution (0.85ml, 0.85mmol) in methanol (1.5ml) was stirred at room temperature for 22 hours. The reaction mixture was acidified to pH 1 using hydrochloric acid (2N), then partitioned between ethyl acetate and water. The layers were separated, and the organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to afford the title compound, 130mg, 72%; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.86 (m, 6H), 1.20-2.12 (m, 36H), 2.24 (m, 2H), 4.18 (m, 1H), 5.82 (d, 1H); LRMS : m/z 464 $(M-H)^-$.

Preparation 50

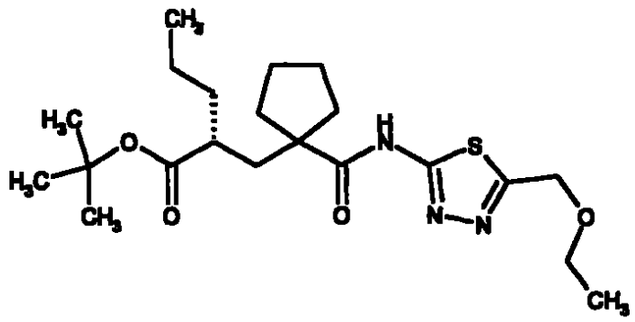
tert-Butyl (2*R*)-2-[[1-({[5-(cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]methyl]pentanoate



The title compound was prepared from the acid from preparation 2 and the amine from preparation 31, in 65% yield, following the procedure described in preparation 33; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.35 (m, 2H), 0.63 (m, 2H), 0.80 (m, 3H), 1.10 (m, 1H), 1.20-1.94 (m, 20H), 2.19 (m, 4H), 2.93 (t, 2H), 3.50 (s, 1H); LRMS : m/z 422.4 (MH^+); $[\alpha]_D = -14.15°$ (c = 0.082, methanol).

Preparation 51

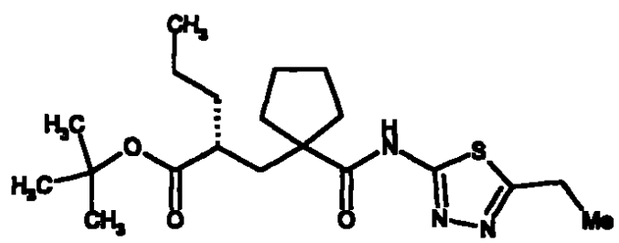
tert-Butyl (2*R*)-2-[[1-({[5-(ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)-cyclopentyl]methyl]pentanoate



The title compound was prepared from the acid from Preparation 2 and the title product from Preparation 97 in 51% yield, following the procedure described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.10-1.78 (m, 25H), 1.82 (m, 1H), 2.19 (m, 5H), 3.48 (s, 1H), 4.82 (s, 2H), 10.16 (brs, 1H); LRMS : m/z 426.4 (MH^+); $[\alpha]_D$ = -12.50° (c = 0.08, methanol).

Preparation 51a

tert-Butyl-(2*R*)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl) methyl]pentanoate

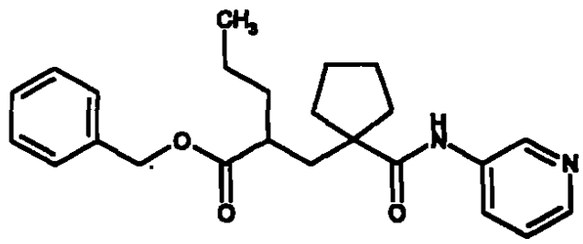


To a mixture of dichloromethane (2.39 L) and pyridine (2.39 L) at –14 °C was added thionyl chloride (135 ml, 1.85 mol, 1.1 eq) with stirring over a period of 1 hour under a nitrogen atmosphere. After stirring the resultant orange solution for 5 minutes, a solution of the product from Preparation 2 (477 g, 1.67 mol) in dichloromethane (477 ml) was added over a period of 45 minutes, causing the reaction mixture to become turbid. After stirring the reaction mixture for 3 hours, triethylamine (424 g, 4.19 mol) was added over a period of 20 minutes, followed by 4-dimethylaminopyridine (20.5 g, 168 mmol). To the resultant mixture was then added 2-amino-5-ethyl-1,3,4-thiadiazole (ex. Lancaster) (282 g, 2.18 mol) in 3 portions over a period of 10 minutes. The reaction was then allowed to warm to ambient temperature and was stirred for 43 hours. *n*-Heptane (2.4 L) and deionised water (1 L) were then added to the reaction mixture. Concentrated

hydrochloric acid (3.56 L) was then added with stirring over a period of 0.5 hours whilst cooling in an ice-water bath. The layers were then separated and to the organic layer was added deionised water (2 L) and concentrated hydrochloric acid (250 ml) with stirring. The layers were separated again, and to the organic layer was added deionised water (2 L) and concentrated hydrochloric acid (250 ml) with stirring. To the organic layer was then added saturated aqueous potassium carbonate solution (1 L) with stirring. The organic phase was collected and was then washed with saturated brine (2 x 1 L). The resultant solution was then concentrated under vacuum to give the title compound (546 g, 1.38 mol, 85% yield) as a viscous, dark brown oil that was used in the next step without further purification; LRMS (negative APCI) : m/z $[M-H]^-$ 394; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.76 (t, 3H), 1.08-1.23 (m, 3H), 1.23-1.27 (m, 2H), 1.32 (s, 9H), 1.34-1.42 (m, 2H), 1.42-1.53 (m, 4H), 1.53-1.68 (m, 2H), 1.87 (dd, 1H), 1.95-2.06 (m, 1H), 2.06-2.24 (m, 3H), 2.98 (q, 2H), 12.15 (bs, 1H).

Preparation 52

Benzyl 2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoate

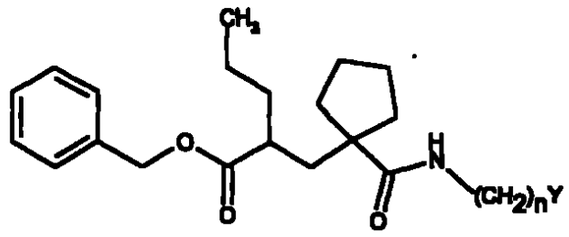


Triethylamine (0.11ml, 0.78mmol) was added to a mixture of the acid chloride from preparation 3 (200mg, 0.60mmol) and 2-aminopyridine (61mg, 0.65mmol) in dichloromethane (3ml), and the reaction stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue partitioned between sodium bicarbonate solution (5ml) and ethyl acetate (20ml), and the layers seperated. The organic phase was dried ($MgSO_4$), and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant, to afford the title compound, 130mg; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.21 (m, 3H), 1.40 (m, 1H), 1.43-1.72 (m, 6H), 1.81 (d, 1H), 1.98 (m, 1H), 2.18 (m, 1H), 2.24 (m, 1H), 2.46 (m, 1H), 4.98 (m, 2H), 7.20-7.38 (m, 6H), 7.42 (s, 1H), 8.06 (d, 1H), 8.35 (d, 1H), 8.56 (s, 1H).

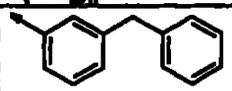
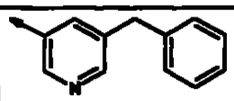
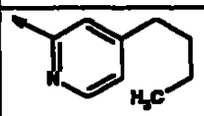
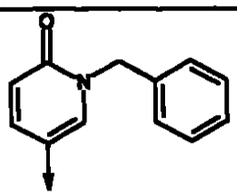
Preparations 53 to 56

Compounds of formula IVd, i.e. compounds of general formula IV where Prot is benzyl

and R^1 is propyl, were prepared from the acid chloride from Preparation 3 and the amine indicated, following a similar procedure to that described in preparation 52.



(IVd)

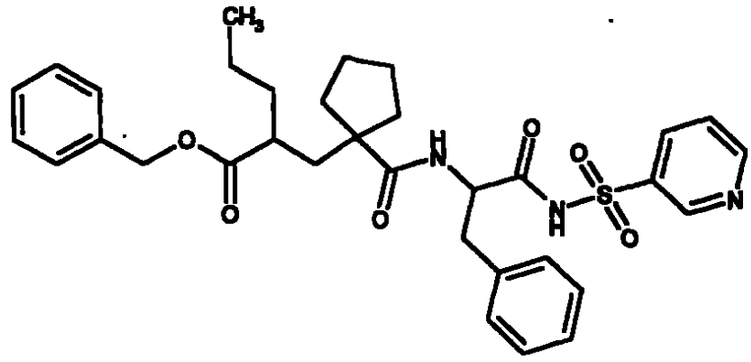
Prep	$-(CH_2)_nY$	Prec. amine	Data
53[1]		ex Trans World Chemicals	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.24 (m, 2H), 1.40-1.76 (m, 7H), 1.84 (dd, 1H), 1.98 (m, 1H), 2.19 (dd, 1H), 2.28 (m, 1H), 2.58 (m, 1H), 3.98 (s, 2H), 4.99 (dd, 2H), 6.98 (d, 1H), 7.19-7.42 (m, 15H).
54		Prep 27	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.24 (m, 3H), 1.39-1.78 (m, 6H), 1.82 (dd, 1H), 1.98 (m, 2H), 2.20 (dd, 1H), 2.25 (m, 1H), 2.50 (m, 1H), 3.98 (s, 2H), 4.98 (dd, 2H), 7.18-7.40 (m, 10H), 7.45 (s, 1H), 7.98 (s, 1H), 8.23 (s, 1H), 8.42 (s, 1H).
55		Prep 30	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 0.92 (t, 3H), 1.21 (m, 2H), 1.30-1.70 (m, 12H), 1.82 (dd, 1H), 2.04 (m, 1H), 2.20 (m, 2H), 2.50 (m, 1H), 2.58 (t, 2H), 4.98 (dd, 2H), 6.83 (d, 1H), 7.30 (m, 5H), 7.90 (s, 1H), 8.08 (s, 1H), 8.15 (d, 1H).
56[2]		Prep 28	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.25 (m, 2H), 1.27-1.99 (m, 10H), 2.07-2.30 (m, 2H), 2.47 (m, 1H), 4.99 (s, 2H), 5.10 (dd, 2H), 6.59 (d, 1H), 7.15 (d, 1H), 7.34 (m, 11H), 8.10 (s, 1H).

1 = dichloromethane used as the column eluant

2 = N-methylmorpholine was used as the base

Preparation 57

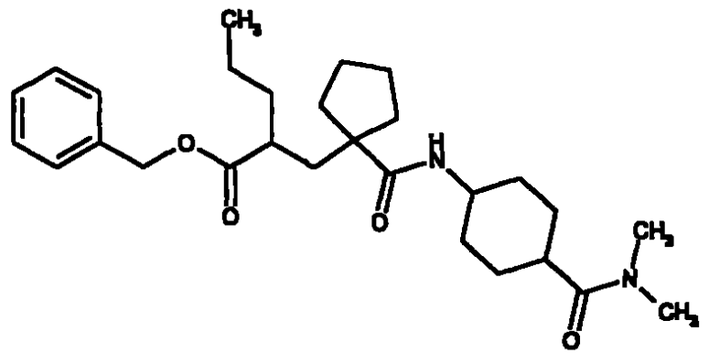
Benzyl 2-({1-[({1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoate



The amine hydrochloride from preparation 25 (828mg, 2.19mmol) and N-methylmorpholine (2.21g, 21.9mmol) were added to an ice-cold solution of the acid chloride from preparation 3 (737mg, 2.19mmol) in dichloromethane (50ml), and the reaction stirred at room temperature for 24 hours. The reaction mixture was evaporated under reduced pressure, the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The organic phase was washed with brine (25ml), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:methanol (100:0 to 95:5) to give the title compound as a cream foam, 975mg, 73%; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.72 (m, 3H), 0.94-2.20 (m, 17H), 2.84 (m, 1H), 3.00 (m, 1H), 4.18 (m, 1H), 5.00 (m, 2H), 6.95 (m, 2H), 7.02 (m, 3H), 7.38 (m, 6H), 8.06 (m, 1H), 8.60 (m, 1H), 8.87 (s, 1H).

Preparation 58

cis-Benzyl 2-({1-[({4-[(dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate

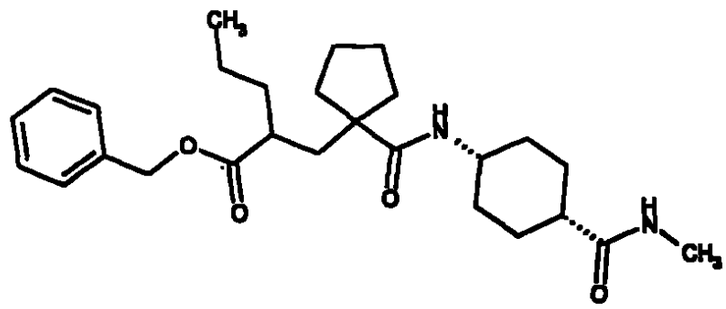


A mixture of cis-4-[[(1-[2-[(benzyloxy)carbonyl]pentyl]cyclopentyl)carbonyl]-

amino}cyclohexanecarboxylic acid (EP 274234, Example 310) (200mg, 0.45mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (112mg, 0.58mmol), 1-hydroxybenzotriazole hydrate (70mg, 0.46mmol) and dimethylamine (0.56ml, 2M in tetrahydrofuran, 1.12mmol) in dichloromethane (5ml) was stirred at room temperature for 16 hours. The mixture was concentrated under reduced pressure and the residue partitioned between sodium bicarbonate solution and ethyl acetate, and the layers separated. The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant to afford the title compound, 150mg; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.22 (m, 3H), 1.32-1.88 (m, H), 2.00 (m, 4H), 2.40 (m, 1H), 2.60 (m, 1H), 2.97 (s, 3H), 3.04 (s, 3H), 4.04 (m, 1H), 5.12 (s, 2H), 5.80 (bd, 1H), 7.37 (m, 5H).

Preparation 59

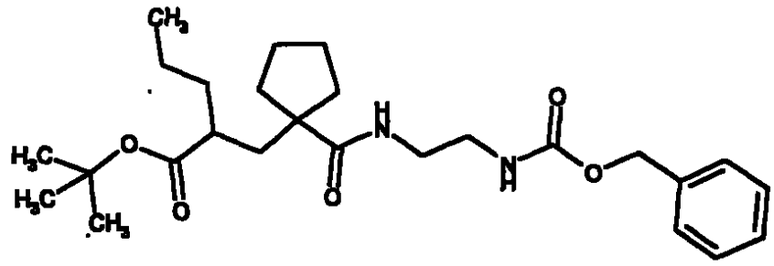
cis-Benzyl 2-({1-[({4-[(methylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate



The title compound was prepared in 49% yield from cis-4-[[(1-[2-[(benzyloxy)carbonyl]pentyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylic acid (EP 274234, Example 310) and methylamine (2M in tetrahydrofuran), following the procedure described in preparation 58; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.17-2.12 (m, 22H), 2.21 (m, 1H), 2.41 (m, 1H), 2.80 (d, 3H), 4.00 (m, 1H), 5.12 (s, 2H), 5.61 (m, 1H), 5.79 (d, 1H), 7.38 (m, 5H).

Preparation 60

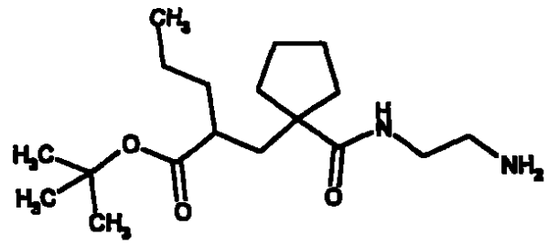
tert-Butyl 2-[(1-[[(2-[[(benzyloxy)carbonyl]amino]ethyl)amino]carbonyl]-cyclopentyl)methyl]pentanoate



The title compound was obtained as a yellow oil in 55% yield, from the acid from preparation 1 and N-benzyloxycarbonyl-1,2-diaminoethane (ex. Aldrich Chemical Co.) following a similar procedure to that described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.74 (m, 17H), 1.90 (m, 2H), 2.04 (m, 1H), 2.20 (m, 1H), 3.32 (m, 3H), 3.44 (m, 1H), 5.10 (s, 2H), 5.61 (m, 1H), 6.20 (m, 1H), 7.36 (m, 5H).

Preparation 61

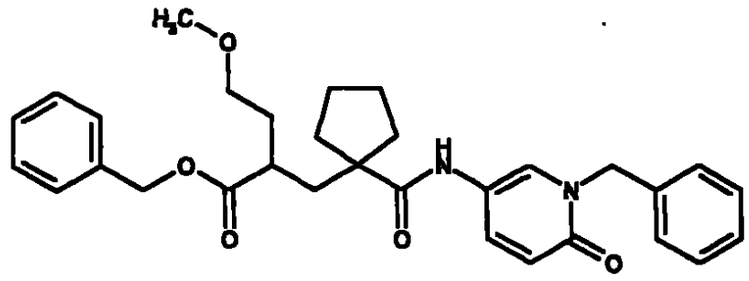
tert-Butyl 2-[(1-[[(2-aminoethyl)amino]carbonyl]cyclopentyl)methyl]pentanoate



A mixture of the carbamate from preparation 60 (1.43g, 3.10mmol) and 10% palladium on charcoal (200mg) in ethanol (8ml) was hydrogenated at room temperature and 1 atm for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to afford the title compound, 920mg, 92%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.54 (m, 12H), 1.61 (m, 5H), 1.92-2.12 (m, 3H), 2.20 (m, 1H), 2.98 (m, 2H), 3.38 (m, 1H), 3.42 (m, 1H), 3.97 (m, 2H), 6.65 (m, 1H); LRMS : m/z 326.8 (M^+).

Preparation 62

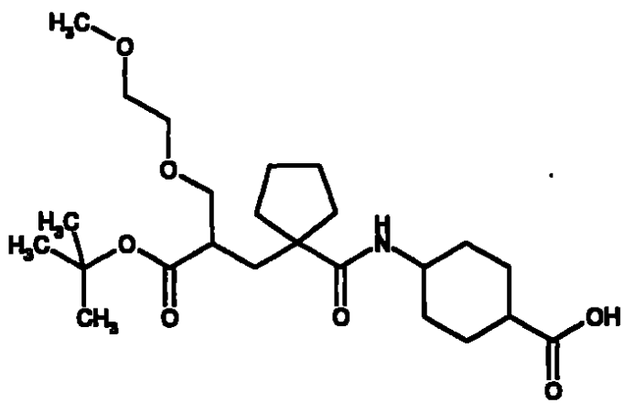
Benzyl 2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]cyclopentyl)-methyl]-4-methoxybutanoate



Oxalyl chloride (0.26ml, 3.0mmol) was added to an ice-cooled solution of 1-[2-[(benzyloxy)carbonyl]-4-methoxybutyl]cyclopentanecarboxylic acid (EP 274234, Example 15) (1.0g, 3.0mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (20ml), and the reaction stirred at room temperature for 2 hours. The solution was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x10ml). The product was dissolved in dichloromethane (20ml), then cooled in an ice-bath. The amine from preparation 28 (600mg, 3mmol) and N-methylmorpholine (0.6ml, 5.45mmol) were added and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced preasure, and partitioned between water and ether. The organic layer was washed with hydrochloric acid (2N), sodium bicarbonate solution, then water, dried ($MgSO_4$) and evaporated under reduced pressure. The residual green solid was purified by medium pressure column chromatography on silica gel using ethyl acetate:hexane (90:10) as eluant to afford the title compound, 880mg, 57%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.37-2.28 (m, 12H), 2.46-2.64 (m, 1H), 3.20 (s, 3H), 3.31 (m, 2H), 4.97 (dd, 2H), 5.08 (dd, 2H), 6.57 (d, 1H), 7.12 (m, 1H), 7.18-7.48 (m, 10H), 8.08 (d, 1H).

Preparation 63

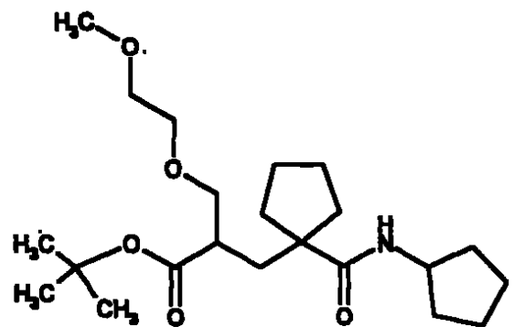
4-[[(1-(3-tert-Butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl)cyclopentyl)-carbonyl]amino]cyclohexanecarboxylic acid



A mixture of benzyl 4-[[(1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylate (EP 274234, Example 96), and 10% palladium on charcoal (250mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 18 hours. The reaction mixture was filtered through Solkafloc®, the filtrate concentrated under reduced pressure and the residue azeotroped with toluene (3x) and then dichloromethane (3x), to give the title compound, 2.0g, 96%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.48 (s, 9H), 1.53-1.84 (m, 14H), 1.94-2.10 (m, 5H), 2.60 (m, 2H), 3.40 (s, 3H), 3.41-3.63 (m, 5H), 3.96 (m, 1H), 5.90 (bd, 1H).

Preparation 84

tert-Butyl 3-{1-[(cyclopentylamino)carbonyl]cyclopentyl}-2-[(2-methoxyethoxy)methyl]-propanoate

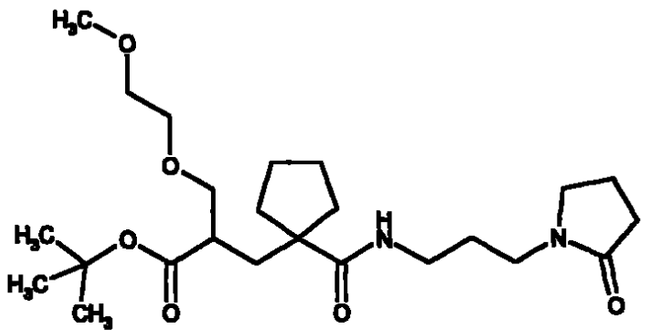


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (197mg, 1.07mmol), 1-hydroxybenzotriazole hydrate (139mg, 1.07mmol), N-methylmorpholine (0.18ml, 1.64mmol) and cyclopentylamine (101µl, 1.07mmol) were added to a solution of 1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}-cyclopentanecarboxylic acid (EP 274234, Example 42) (400mg, 1.07mmol) in dichloromethane (5ml), and the reaction stirred at room temperature for 22 hours. The reaction was quenched by the addition of

water, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to afford the title compound as a clear oil, 320mg, 78%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.22-2.02 (m, 27H), 2.58 (m, 1H), 3.38 (s, 3H), 3.40 (m, 1H), 3.46 (m, 2H), 3.57 (m, 3H), 4.10-4.20 (m, 1H), 5.80 (bs, 1H).

Preparation 65

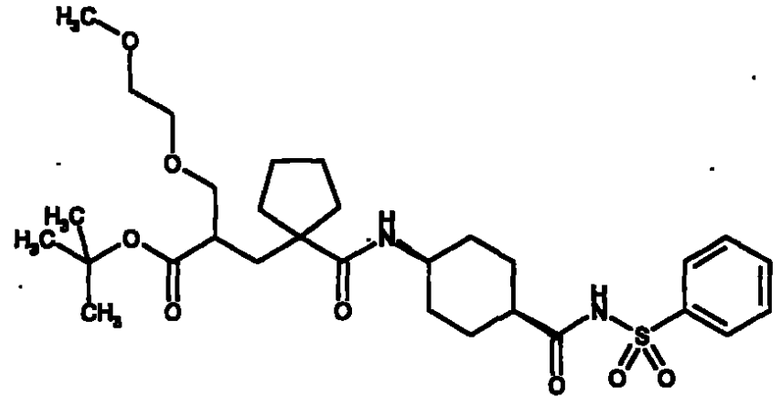
tert-Butyl 3-(2-methoxyethoxy)-2-[(1-{[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]methyl}propanoate



The title compound was obtained as a clear oil in 97% yield from 1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}-cyclopentanecarboxylic acid (EP 274234, Example 42) and 1-(3-aminopropyl)-2-pyrrolidinone pyrrolidinone (Ex. Aldrich Chemical Co.), following a similar procedure to that described in preparation 64, except dichloromethane:methanol (95:5) was used as the column eluant, ^1H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.50 (m, 2H), 1.60-1.70 (m, 7H), 1.78 (m, 1H), 1.90 (m, 1H), 2.20 (m, 4H), 2.40 (m, 2H), 2.58 (m, 1H), 3.14 (m, 1H), 3.20 (m, 1H), 3.38 (m, 6H), 3.42-3.60 (m, 6H), 7.00 (m, 1H).

Preparation 66

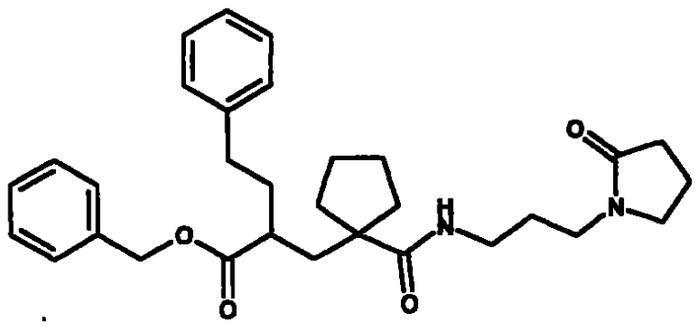
cis-tert-Butyl 3-(2-methoxyethoxy)-2-[(1-[[(4-[[(phenylsulfonyl)aminolcarbonyl]-cyclohexyl)aminolcarbonyl]cyclopentyl)methyl]propanoate



N,N'-Dicyclohexylcarbodiimide (199mg, 0.97mmol), 4-dimethylaminopyridine (118mg, 0.97mmol) and benzenesulphonamide (152mg, 0.97mmol) were added to an ice-cooled solution of the acid from preparation 63 (400mg, 0.878mmol) in dichloromethane (12ml) and N,N-dimethylformamide (0.5ml), and the reaction stirred at room temperature for 20 hours. The mixture was concentrated under reduced pressure and the residue suspended in cold ethyl acetate. The resulting insoluble material was filtered off, the filtrate washed with hydrochloric acid (1N), and water, then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound as a white foam, 480mg, 92%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.44 (s, 9H), 1.63 (m, 13H), 1.80 (m, 2H), 1.88 (m, 1H), 1.98 (m, 2H), 2.36 (m, 1H), 2.57 (m, 1H), 3.38 (s, 3H), 3.40 (m, 1H), 3.51 (t, 2H), 3.58 (m, 3H), 3.95 (m, 1H), 5.92 (d, 1H), 7.56 (m, 2H), 7.62 (m, 1H), 8.05 (d, 2H), 8.75 (bs, 1H); LRMS : m/z 618 (MNa^+).

Preparation 67

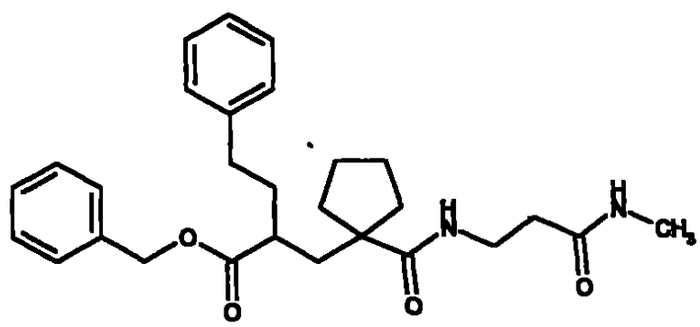
Benzyl 2-[[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]-methyl]-4-phenylbutanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.06g, 5.53mmol), 1-hydroxybenzotriazole hydrate (0.60g, 4.44mmol) and 4-methylmorpholine (0.56g, 5.54mmol) were added sequentially to a cooled solution of 1-[2-[(benzyloxy)carbonyl]-4-phenylbutyl]cyclopentanecarboxylic acid (EP 274234 Example 17) (1.5g, 3.94mmol) in dry dichloromethane (15ml) at room temperature, followed by N-(3-aminopropyl)-2-pyrrolidinone (Ex. Aldrich Chemical Co.) (0.56g, 3.94mmol), and the reaction stirred at room temperature for 18 hours. The mixture was washed with water, 2N hydrochloric acid, saturated aqueous sodium bicarbonate solution, and then dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as the eluent to provide the title compound as a clear gum, 800mg, 40%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.37-2.20 (m, 16H), 2.34-2.58 (m, 5H), 2.92-3.46 (m, 6H), 5.07 (d, 1H), 5.18 (d, 1H), 6.98-7.47 (m, 10H).

Preparation 68

Benzyl 2-[[1-({[3-(methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl]-4-phenylbutanoate

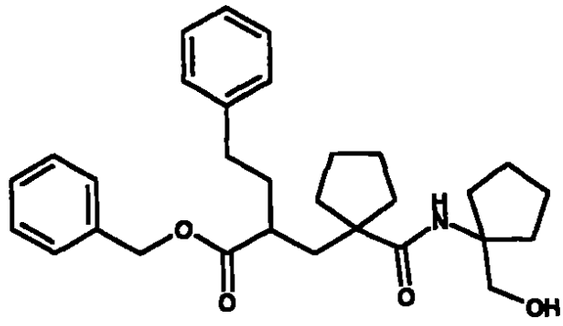


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (122mg, 0.64mmol), 1-

hydroxybenzotriazole hydrate (86mg, 0.64mmol) and 4-methylmorpholine (173µl, 1.59mmol) were added sequentially to a cooled solution of 1-[2-[(benzyloxy)carbonyl]-4-phenylbutyl]cyclopentane-carboxylic acid (EP 274234, Example 17) (202mg, 0.53mmol) in N,N-dimethylformamide (5ml) at room temperature, followed by the amine hydrochloride from preparation 23 (146mg, 1.06mmol), and the reaction stirred at 90°C for 18 hours. The cooled solution was concentrated under reduced pressure and the residue partitioned between water (20ml) and ethyl acetate (100ml). The layers were separated, the organic phase washed with water (3x30ml), brine (25ml) dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (98:2) as eluant to afford the title compound as a colourless oil, 162mg, 67%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.38-1.53 (m, 2H), 1.53-1.96 (m, 8H), 2.02 (m, 2H), 2.27 (t, 2H), 2.46 (m, 3H), 2.76 (d, 3H), 3.44 (m, 2H), 5.13 (s, 2H), 5.79 (bs, 1H), 6.38 (m, 1H), 7.06 (d, 2H), 7.18 (m, 1H), 7.22 (m, 2H), 7.38 (m, 5H); LRMS : m/z 465.5 (MH^+).

Preparation 69

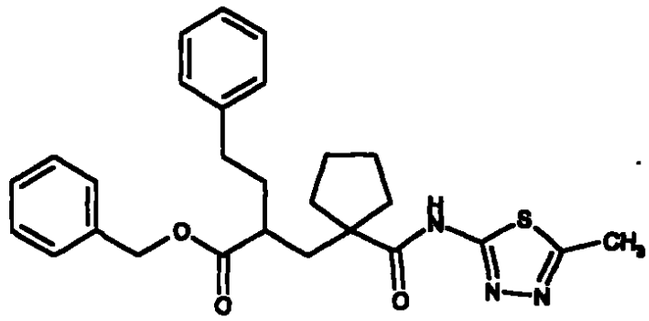
Benzyl 2-[[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl]-4-phenylbutanoate



The title compound was obtained as a crystalline solid (48%) from 1-[2-[(benzyloxy)carbonyl]-4-phenylbutyl]cyclopentane-carboxylic acid (EP 274234, Example 17) and 1-amino-1-cyclopentanemethanol, following a similar procedure to that described in preparation 68, except the reaction mixture was stirred at room temperature for 18 hours, and the crude product purified by column chromatography on silica gel using ethyl acetate:pentane as eluant; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.38 (m, 2H), 1.50-1.95 (m, 16H), 2.01 (m, 2H), 2.45 (m, 3H), 3.49 (dd, 1H), 3.60 (dd, 1H), 4.58 (m, 1H), 5.10 (s, 2H), 5.67 (s, 1H), 7.01 (d, 2H), 7.14 (m, 1H), 7.20 (m, 2H), 7.36 (m, 5H); LRMS : m/z 478.3 (MH^+).

Preparation 70

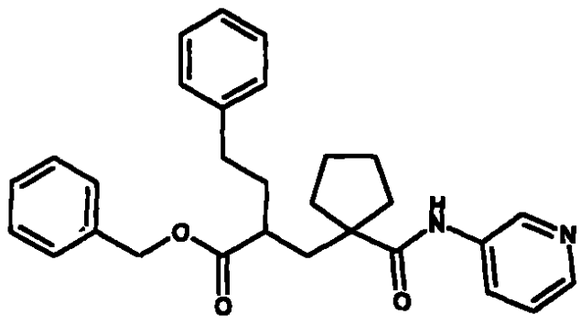
Benzyl 2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoate



The title compound was obtained as a clear oil in 74% yield from 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234, Example 17) and 2-amino-5-methyl-1,3,4-thiadiazole (ex Lancaster), following a similar procedure to that described in preparation 68; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.58-1.76 (m, 7H), 1.83-1.98 (m, 3H), 2.03 (m, 1H), 2.20 (m, 1H), 2.35 (m, 1H), 2.44 (m, 3H), 2.65 (s, 3H), 5.02 (dd, 2H), 7.00 (d, 2H), 7.15 (m, 1H), 7.19 (m, 2H), 7.35 (m, 5H); LRMS : m/z 478.7 (MH^+).

Preparation 71

Benzyl 4-phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoate

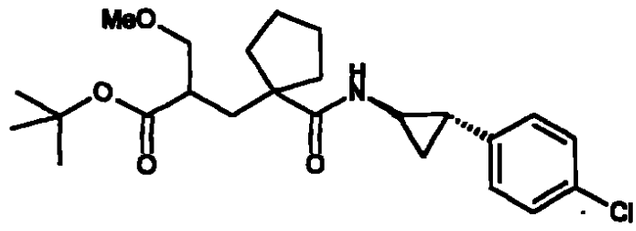


Oxalyl chloride (2.29ml, 26.3mmol) was added to a solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234, Example 17) (5.0g, 13.14mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (25ml), and the solution stirred for 2.5 hours. The mixture was evaporated under reduced pressure, the residue azeotroped with dichloromethane to give a yellow oil. This was then dissolved in dichloromethane (50ml) and a solution of this acid chloride (10ml, 2.45mmol) was added to an ice-cooled solution of triethylamine (248mg, 2.45mmol) and 3-aminopyridine (253mg, 2.70mmol) in dry dichloromethane (10ml), and the reaction

stirred at room temperature for 18 hours. The solution was washed with water (3x), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate:hexane (40:60) as eluant, and repeated using an elution gradient of ether:hexane (90:10 to 100:0). The product was crystallised from ethyl acetate:hexane to afford the title compound, 740mg, 66%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.38-2.07 (m, 10H), 2.10-2.37 (m, 2H), 2.42-2.63 (m, 3H), 5.02 (s, 2H), 6.94-7.44 (m, 10H), 7.50 (s, 1H), 8.03 (d, 1H), 8.36 (d, 1H), 8.52 (s, 1H).

Preparation 72

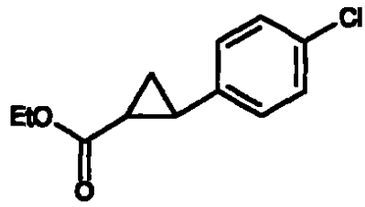
trans-tert-Butyl-3-[1-({[2-(4-chlorophenyl)cyclopropyl]amino}carbonyl)-cyclopentyl]-2-(methoxymethyl)propanoate



The product from Preparation 94 (286mg, 1mmol), the product from Preparation 76 (203mg, 1mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (211mg, 1.1mmol), triethylamine (1ml) and HOBt (148mg, 1.1mmol) in DCM (5 ml) were stirred at room temperature for 16h. The reaction mixture was washed with water (20mls), dried over $MgSO_4$ and purified by column chromatography using 1:8, then 1:5 EtOAc:pentane as eluant to provide the title product as a colourless film (122mg, 40%); R_f 1:5 (EtOAc:pentane) 0.2; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.05-1.2 (m, 2H), 1.4 (s, 9H), 1.55-1.75 (m, 4H), 1.9-2.0 (m, 4H), 2.4-2.5 (m, 1H), 2.75-2.85 (m, 1H), 3.3 (s, 3H), 3.4-3.5 (m, 1H), 6.3 (m, 1H), 7.05 (d, 2H), 7.2 (d, 2H); HRMS : m/z M+H, Found 436.2242. $C_{24}H_{35}NO_4Cl$ requires 436.2249.

Preparation 73

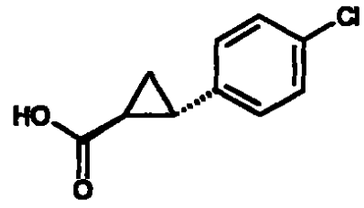
Ethyl-2-(4-chlorophenyl)cyclopropanecarboxylate



A mixture of 4-chlorostyrene (10.1ml, 86mmol) and rhodium acetate dimer (1g, 4.5mmol) in toluene (50ml) was heated to 85°C before adding ethyl diazoacetate (11.3mls, 94mmol) over 30mins and the whole then heated at 80°C for a further 1h before concentration *in vacuo*. The residue was then purified by column chromatography using 1:2 DCM:pentane as eluant to give the title product as a colourless oil (7.8g, 37%); R_f 1:2 (DCM:pentane) 0.35; ^{1}HNMR (400MHz, $CDCl_3$) δ 1.15-1.3 (m, 4H), 1.5-1.7 (m, 1H), 1.8-1.9 (m, 1H), 2.4-3.55 (m, 1H), 4.2 (q, 2H), 6.95 (d, 2H), 7.20-7.28 (m, 2H); and LRMS : m/z, $M+NH_4^+$ 242.

Preparation 74

trans-2-(4-Chlorophenyl)cyclopropanecarboxylic acid

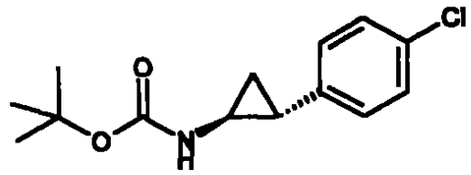


The product from preparation 73 (7.8g, 37mmol) was dissolved in EtOH (75mls) at room temperature under nitrogen and sodium methoxide (8.1g, 150mmol) was added portionwise over 15mins. After the addition was complete, the mixture was then refluxed for 18h. The reaction mixture was concentrated *in vacuo*, and the resulting residue diluted with DCM and water (150mls, 2:1 mixture). The organic layer was removed, and the aqueous layer re-extracted with DCM (2x50mls). The combined organic extracts were dried over $MgSO_4$ and evaporated to provide the *trans* ester (4.98g, 62%). Acidification of the aqueous layer with concentrated HCl to pH 1 resulted in a white precipitate, which was filtered and dried under vacuum to provide the hydrolysis product (the corresponding acid) as a white powder (1.95g, 27%). Dissolution of the ester in MeOH (50mls), water (50mls) and LiOH (1.34g, 32mmol) gave a clear solution which was heated at *ca.* 70°C overnight. The reaction mixture was cooled, concentrated *in vacuo*, and acidified with concentrated HCl to pH 1. The resulting white precipitate was

extracted with EtOAc (3x50mls) and the combined organic extracts were dried over $MgSO_4$ and evaporated to dryness, to provide the acid (4g, 96%). This acid was combined with the hydrolysed product from the previous step to give a total of 5.95g; ^{1}HNMR (400MHz, $CDCl_3$) δ 1.3-1.4 (m, 1H), 1.6-1.7 (m, 1H), 1.8-1.9 (m, 1H), 2.5-2.6 (m, 1H), 7.00 (d, 2H), 7.26 (d, 2H); LRMS : m/z, M-H 195.

Preparation 75

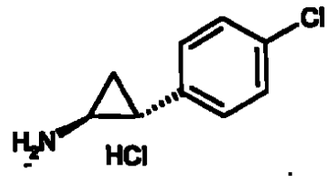
trans-tert-Butyl-2-(4-chlorophenyl)cyclopropylcarbamate



The product from preparation 74 (1.5g, 7.65mmol), DPPA (1.8ml, 8.4mmol) and triethylamine (1.25ml, 12mmol) in *tert*-butanol (20 ml) was heated at *ca.* 90°C under nitrogen for 48h. When cool, the mixture was diluted with EtOAc (20mls) and saturated Na_2CO_3 solution (20mls) and the organic layer was then removed. The aqueous layer was reextracted with EtOAc (20mls) and the combined organic layers were dried over $MgSO_4$, filtered and evaporated. The resulting residue was purified by column chromatography using 1:10, then 1:2 EtOAc:pentane as eluant to provide the title product as a white solid (1.7g, 83%); R_f 1:2 (EtOAc:pentane) 0.9; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.2-1.3 (m, 1H), 1.4 (s, 10H), 1.9-2.0 (m, 1H), 2.6 (br.s, 1H), 4.8 (br.s, 1H), 7.0 (d, 2H), 7.2 (d, 2H); HRMS : m/z M+Na, Found 290.0923. $C_{14}H_{18}NO_2ClNa$ requires 290.0918.

Preparation 76

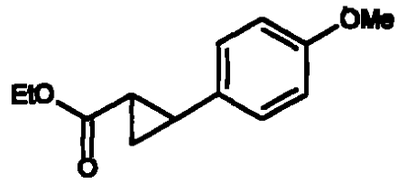
2-(4-Chlorophenyl)cyclopropylamine



The product from Preparation 75 was taken up in EtOAc, cooled to 0°C and hydrogen chloride gas was bubbled through the solution for 30mins. The solution was then concentrated *in vacuo* to give the title product (1.29g, 6.3mmol); R_f 1:3 (EtOAc:pentane) 0; ^{1}HNMR (400MHz, CD_3OD) δ : 1.3-1.4 (m, 1H), 1.4-1.5 (m, 1H), 2.3-2.4 (m, 1H), 2.8-2.9 (m, 1H), 7.15 (d, 2H), 7.3 (d, 2H).

Preparation 77

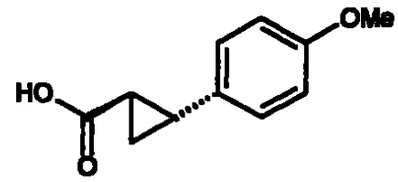
Ethyl-2-(4-methoxyphenyl)-cyclopropanecarboxylate



A mixture of 4-methoxystyrene (25.5mls, 192mmols), rhodium acetate dimer (2g, 4.5mmol) and toluene (100mls) was stirred at room temperature under nitrogen for 20mins and then heated to 85°C. Ethyl diazoacetate (19.8mls, 188mmols) was added dropwise over 50mins at a rate of one drop every 2 to 3 seconds to maintain the internal reaction temperature at around 95°C. After the addition was complete, the mixture was heated for 1h at 85°C and then cooled to room temperature. The mixture was filtered through Arbocel® and evaporated to an oil which was purified by column chromatography using DCM:pentane (1:2) as eluant to provide the title product (13g, 31%) which was a 3:1 mixture of the *trans*:*cis* isomers; R_f 0.22 (DCM:pentane) 1:2; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.20-1.38 (m, 5H), 1.83 (ddd, 1H), 2.50 (ddd, 1H), 3.80 (s, 3H), 4.16 (q, 2H), 6.82 (d, 2H), 7.03 (d, 2H).

Preparation 78

Trans-2-(4-methoxyphenyl)-cyclopropanecarboxylic acid



Sodium methoxide (14.8g, 273.8mmol) was added to a solution of the product from Preparation 77 (13g, 59mmol) in EtOH (135mls) while stirring at room temperature under a nitrogen atmosphere. After the addition was complete, the mixture was refluxed gently for 1h, by which time TLC analysis indicated that there was no *cis* isomer remaining. The reaction was cooled to room temperature and water (100mls) added in one portion. The whole was then stirred at room temperature for 63h and then evaporated to remove the MeOH before acidifying with concentrated HCl to pH 1. The suspension was extracted with EtOAc (2x100mls), and the extracts dried ($MgSO_4$) and evaporated to give a yellow solid, which was purified by column chromatography using 1:1 EtOAc:pentane to provide the title product (8.9g, 78%); R_f 1:1 (EtOAc:pentane) 0.4;

^{1}HNMR (400MHz, $CDCl_3$) δ : 1.30-1.41 (m, 1H), 1.58-1.69 (m, 1H), 1.79-1.90 (m, 1H), 2.53-2.62 (m, 1H), 6.83 (d, 2H), 7.04 (d, 2H).

Preparation 79

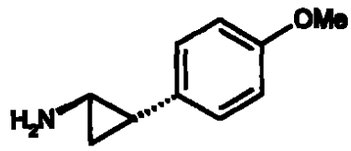
Trans-tert-Butyl-2-(4-methoxyphenyl)-cyclopropylcarbamate



DPPA (11mls, 50.9mmol) was added to a stirred mixture of the product from Preparation 78 (8.9g, 46.3mmol), triethylamine (10.1mls, 72.7mmol) and *tert*-BuOH (75mls). The mixture was heated at 90°C for 43h. When cool, the *tert*-BuOH was removed by evaporation and the resulting oily residue treated with saturated K_2CO_3 (120 ml) and then extracted with EtOAc (2x100mls). The combined organic extracts were then evaporated under reduced pressure to give a brown solid which was purified by column chromatography using DCM:MeOH (98:2) as eluant to provide the title product (5.8g, 48%); ^{1}HNMR (400MHz, $CDCl_3$) δ: 1.07-1.14 (m, 2H), 1.44 (s, 9H), 1.93-2.06 (m, 1H), 2.62-2.71 (m, 1H), 3.80 (s, 3H), 4.72-4.88 (m, 1H), 6.80 (d, 2H), 7.08 (d, 2H).

Preparation 80

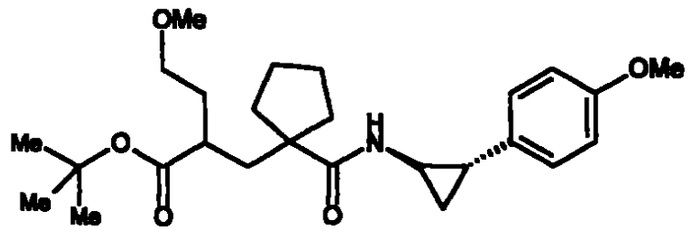
Trans-2-(4-methoxyphenyl)-cyclopropylamine



TFA (20mls) was added to a stirred mixture of the product from Preparation 79 (5.8g, 22.0mmol) and DCM (15mls) at room temperature under nitrogen. The reaction was stirred for 16h, after which time the solvent was removed under reduced pressure, and the resulting oil treated with saturated aqueous K_2CO_3 until a pH of 10 was reached (*ca.* 150mls required). This opaque solution was extracted with EtOAc (2x150mls) and the extracts were then dried over $MgSO_4$ and evaporated to give a biege solid. This solid was purified by column chromatography using 99:1, the 95:5 DCM:MeOH as eluant to give the title product as a white solid (3.2g, 89%); R_f DCM:MeOH (19:1) 0.18; ^{1}HNMR (400MHz, $CDCl_3$) δ: 0.87-1.04 (m, 2H), 1.79-1.90 (m, 1H), 2.43-2.54 (m, 1H), 3.80 (s, 3H), 6.80 (d, 2H), 6.98 (d, 2H).

Preparation 81

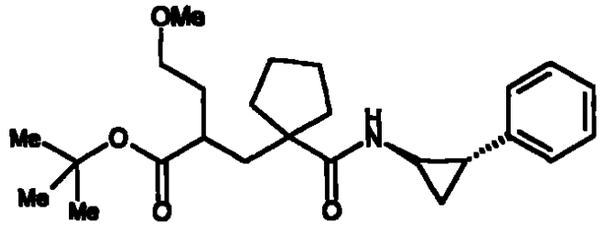
Tert-Butyl-4-methoxy-2-[[1-({[2-(4-methoxyphenyl)cyclopropyl]amino}carbonyl)-cyclopentyl]methyl]butanoate



The product from Preparation 99 (210mg, 0.66mmol), triethylamine (1ml), HOBt (140mg, 0.73mmol) and the product from Preparation 80 (107mg, 0.66mmol) were combined sequentially in DCM at room temperature under nitrogen. WSCDI (98mg, 0.73mmol) was then added to the mixture and the whole stirred overnight for some 16h. The reaction was diluted with water, the organic layer was separated and then washed with brine, dried over $MgSO_4$, filtered and evaporated to a yellow oil. This was purified by column chromatography using 1:3 EtOAc:pentane as eluant to provide the title product (113mg, 38%); R_f 1:10 (EtOAc:pentane) 0.2; ^{1}NMR (400MHz, $CDCl_3$) δ : 1.0-1.10 (m, 2H), 1.40 (s, 9H), 1.35-1.45 (m, 2H), 1.50-1.80 (m, 7H), 1.85-2.10 (m, 4H), 2.25-2.35 (m, 1H), 2.70-2.80 (m, 1H), 3.2 (s, 3H), 3.25-3.35 (m, 2H), 3.7 (s, 3H), 6.05 (br.s, 1H), 6.70 (d, 2H), 7.05 (d, 2H); LRMS: m/z 446 (M+H).

Preparation 81a

Tert-Butyl-4-methoxy-2-[[1-({[2-phenylcyclopropyl]amino}carbonyl)-cyclopentyl]methyl]butanoate

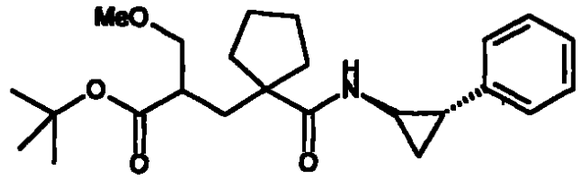


The title product was prepared in analogous fashion to Preparation 81 replacing the product from Preparation 80 with 1-*S*-amino-2-*R*-phenyl cyclopropane (*J. Med. Chem.*, 1986, 29, 2044); ^{1}NMR (400MHz, $CDCl_3$) δ : 1.1 (m, 1H), 1.2 (m, 1H), 1.4 (s, 9H), 1.6 (m, 8H), 1.7, (m, 1H), 1.9 (m, 2H), 2.1 (m, 2H), 2.4 (q, 1H), 2.8 (q, 1H), 3.2 (s, 3H), 3.3 (q, 2H), 6.1 (bs, 1H), 7.1 (d, 3H), 7.2 (d, 2H). ; LRMS: m/z 416 (M+H); HRMS m/z Found 416.2794. $C_{25}H_{32}NO_4$ requires 416.2796.

83

Preparation 82

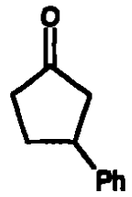
Tert-Butyl-3-methoxy-2-[(1-[[(2-phenylcyclopropyl)aminolcarbonyl]cyclopentyl)-methyl]propanoate



The product from Preparation 94 (200mg, 0.68mmol), triethylamine (1ml), HOBt (98mg, 0.73mmol) and 1-*S*-amino-2-*R*-phenyl cyclopropane (*J. Med. Chem.*, 1986, 29, 2044) (123mg, 0.73mmol) were combined sequentially in DCM (6mls) at room temperature under nitrogen. WSCDI (98mg, 0.73mmol) was then added to the mixture and the whole stirred overnight for some 16h. The reaction was diluted with water, the organic layer was separated and then washed with brine, dried over $MgSO_4$, filtered and evaporated to a yellow oil. This was purified by column chromatography using 1:3 EtOAc:pentane as eluant to provide the title product (184mg, 62%); R_f 1:10 (EtOAc:pentane) 0.2; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.0-1.1 (m, 2H), 1.4 (s, 9H), 1.5-1.8 (m, 6H), 1.8-2.05 (m, 4H), 2.3-2.4 (m, 1H), 2.7-2.8 (m, 1H), 3.2 (s, 3H), 3.25-3.35 (m, 1H), 3.7 (s, 3H), 6.05 (s, 1H), 6.7 (d, 2H), 7.05 (d, 2H); LRMS : m/z M+H, 446.

Preparation 83

3-Phenyl cyclopentanone

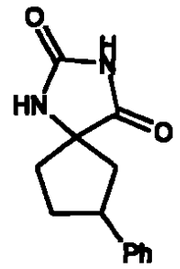


Phenyl magnesium bromide (0.27moles) was taken up in dry diethyl ether (200 ml) and cooled to 0°C under a nitrogen atmosphere. Copper (I) iodide (25.5g, 0.13moles) was added in one portion, and the suspension stirred at 0°C for 20mins. Cyclopenten-2-one was then added dropwise over 10-15mins and the resulting solution stirred at 0°C for 10mins, and then allowed to warm to room temperature over the course of 1h. The reaction mixture was added to 100mls of a mixture of saturated ammonium chloride solution and concentrated ammonia, the pH of which was initially measured at 9. The whole was stirred at room temperature for 30mins, and then filtered, and the layers of the filtrate were then separated. The aqueous layer was extracted with ether (2x70mls)

and the extracts combined with the original organic layer. The bulked ether layers were then washed with brine, dried over $MgSO_4$, filtered and evaporated to a pale yellow oil. This oil was then chromatographed using 1:3 ether/pentane to give the title product (2.9g, 14%); R_f EtOAc:pentane (1:2) 0.85; ^{1}HNMR (300MHz, $CDCl_3$) δ : 1.91-2.10 (m, 1H), 2.18-2.59 (m, 4H), 2.67 (dd, 1H), 3.38-3.52 (m, 1H), 7.19-7.47 (m, 5H).

Preparation 84

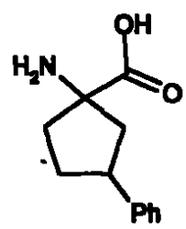
7-Phenyl-1,3-diazaspiro[4.4]nonane-2,4-dione



The product from Preparation 83 (5.8g), potassium cyanide (2.75g) and ammonium carbonate (9.1g) were heated in 80mls of 50% aqueous EtOH for 7h, and then at room temperature for 48h. The mixture was filtered, and the solid washed thoroughly with water (3x50mls). The filtrate was concentrated, the pH adjusted to 2 using concentrated HCl and the resulting suspension filtered off and washed with water (3x50mls). The bulked solids were recrystallised from EtOH and water (300ml:100ml) to give the title product as 1:1 mixture of diastereoisomeric pairs (6.32g); R_f 0.6 in EtOAc; ^{1}HNMR (300MHz, $CDCl_3$) δ : 1.60-2.58 (m, 6H), 3.08-3.37 (m, 1H), 6.83-7.44 (m, 5H), 8.33 (d, 1H), 10.60 (s, 1H); Anal. Found: C, 68.09; H, 6.21; N, 12.25%. $C_{13}H_{14}N_2O_2$ requires C, 67.81; H, 6.13; N, 12.17%.

Preparation 85

1-Amino-3-phenyl-cyclopentane carboxylic acid

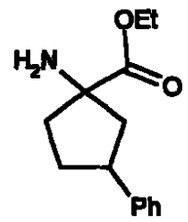


The title product from Preparation 84 (6.2g), barium hydroxide octahydrate (17.2g) and water (100 ml) were heated together in a bomb at 160°C for 7h, and then allowed to stand overnight at room temperature. The reaction mixture was acidified to pH 1 using

concentrated H_2SO_4. The resulting suspension was then filtered and the solid was washed with water (100mls). The filtrate was then basified to ca. pH 6 using concentrated ammonia solution, the suspension cooled in a ice bath, and then filtered. The solid was washed with water, and dried under vacuum to give the title product (2.9g); m.p. 265°C (dec.); R_f 0.5 in *methyl isobutyl* ketone: acetic acid: water (2:1:1); ^{1}HNMR (300MHz, $CDCl_3$) δ : 2.06-3.14 (m, 6H), 3.42-3.73 (m, 1H), 7.12-7.44 (m, 5H); Anal. Found C, 69.54; H, 7.26; N, 6.73%. $C_{12}H_{15}NO_2$ requires C, 70.22; H, 7.37; N, 6.82%.

Preparation 86

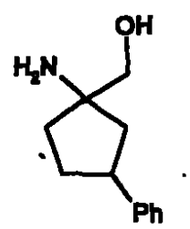
Ethyl-1-amino-3-phenyl-cyclopentanecarboxylate



The title product from Preparation 85 (500mg, 2.4mmol) was taken up in EtOH saturated with hydrogen chloride (70 ml) at 0°C and then stirred at room temperature for 16h. Nitrogen gas was then bubbled through the solution for 10mins, and the solvent evaporated to give a biege solid. This was treated with saturated aq. $NaHCO_3$ solution (10mls) and extracted with EtOAc (2x10mls). The combined organic extracts were dried and evaporated to give the title product (360mg, 63%); R_f DCM:MeOH (97:3) 0.23; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.31 (t, 3H), 1.50-2.37 (m, 4H), 2.38-2.44 (m, 1H), 2.63 (dd, 1H), 3.22-3.36 and 3.43-3.57 (m, 1H), 4.20 (q, 2H), 7.20-7.35 (m, 5H).

Preparation 87

(1-Amino-3-phenylcyclopentyl)methanol

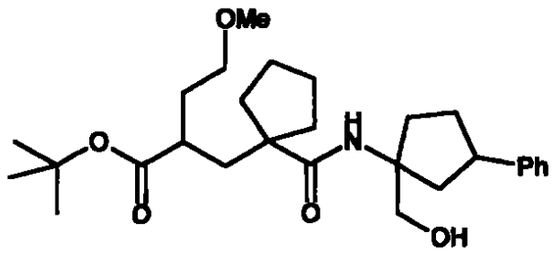


Sodium borohydride (190mg) was added portionwise to a stirred solution of the product from Preparation 86 (390mg, 1.67mmol) in 8mls of a 50% solution of aqueous EtOH and then heated at 50°C for 3h. The reaction mixture was then evaporated to give a

suspension of an oil in water. This oil was extracted with EtOAc (20mls), and evaporated under reduced pressure to provide the title product (295mg); R_f (1:2 ether:pentane) 0.65; ^{1}HNMR (400MHz, $CDCl_3$) δ: 1.30-2.36 (m, 6H), 3.02-3.17 (m, 1H), 3.33-3.50 (m, 2H), 7.14-7.37 (m, 5H).

Preparation 88

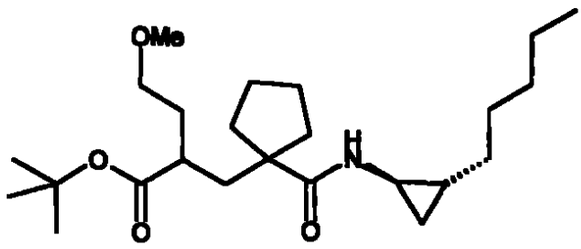
Tert-Butyl-2-[[1-({[1-(hydroxymethyl)-3-phenylcyclopentyl]amino}carbonyl)-cyclopentyl]methyl]-4-methoxybutanoate



The title product was prepared by a similar method to that described in preparation 33 from the product from Preparation 99 and the title product from preparation 87; R_f EtOAc: pentane (1:4) 0.25; ^{1}HNMR (400MHz, $CDCl_3$) δ : 0.80-0.88 (m, 3H), 1.16-2.60 (m, 21H), 1.42 (s, 9H), 3.04-3.32 (m, 1H), 3.57-3.84 (m, 2H), 4.56-4.77 (m, 1H), 5.94 (br.t, 1H), 7.16-7.28 (m, 5H).

Preparation 89

Tert-Butyl-4-methoxy-2-[(1-{[(2-pentylcyclopropyl)amino]carbonyl}cyclopentyl)-methyl]butanoate

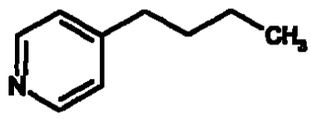


The title product from preparation 99 (105mg, 0.33mmol), triethylamine (0.5ml), HOBt (49mg, 0.36mmol) and 1-amino-2-pentyl-cyclopropane (100mg, 0.33mmol) were combined sequentially in DCM at room temperature under nitrogen. WSCDI (70mg, 0.36mmol) was then added to the mixture and the mixture stirred overnight for 16h. The reaction was diluted with water, the organic layer was separated and then washed with brine, dried over $MgSO_4$, filtered and evaporated to a yellow oil. This was purified by column chromatography using 1:3 EtOAc:pentane as eluant to give the title product

(96mg, 71%); R_f 1:6 (EtOAc:pentane) 0.2; ^1H NMR (400MHz, $CDCl_3$) δ : 0.4-0.6 (m, 2H), 0.7-0.9 (m, 4H), 1.05-1.15 (m, 1H), 1.2-1.3 (m, 4H), 1.3-1.5 (m, 14H), 1.5-2.0 (m, 10H), 2.2-2.4 (m, 2H), 3.2-3.35 (m, 5H), 5.7-5.9 (br.s, 1H); LRMS : m/z, M+H, 410.

Preparation 90

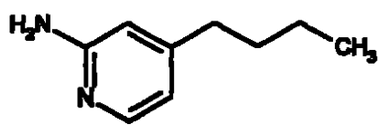
4-Butylpyridine



Lithium di*iso*propylamide was formed by the addition *n*-butyllithium (43ml of a 2.5M solution in hexanes) to a stirred solution of di*iso*propylamine (10.9g) in dry THF (100mls) at -30°C under nitrogen. After 1h of stirring at this temperature, the solution was cooled to -78°C and a solution of 4-methyl pyridine was added (10g) in dry THF (20 ml), followed by continued stirring at -78°C for 1h. Iodopropane (20g) was added dropwise over 45mins as a solution in 20mls dry THF, followed by continued stirring of the whole mixture for 1h. Saturated aqueous ammonium chloride solution (20 ml) was added, and the reaction was then extracted with ether (2x100mls). The combined ether extracts were washed with water (75mls), dried over $MgSO_4$ and evaporated to give a clear oil. This oil was purified by distillation under water aspiration vacuum (ca. 30mmHg) and the title product was collected as the fraction which distilled over at 84-90°C; ^1H NMR (400MHz, $CDCl_3$) δ : 0.93 (t, 3H, Me), 1.30-1.42 (m, 2H), 1.57-1.66 (m, 2H), 2.60 (t, 2H), 7.06 (d, 2H), 8.46 (d, 2H).

Preparation 90a

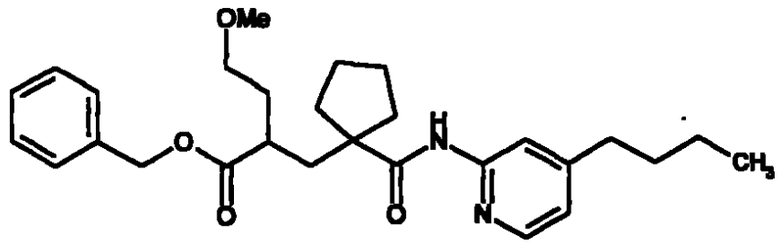
2-Amino-4-butyl pyridine



The title product was prepared from the product from Preparation 90 according to the method described in the Journal of the Chemical Society, 1946, p936.

Preparation 91

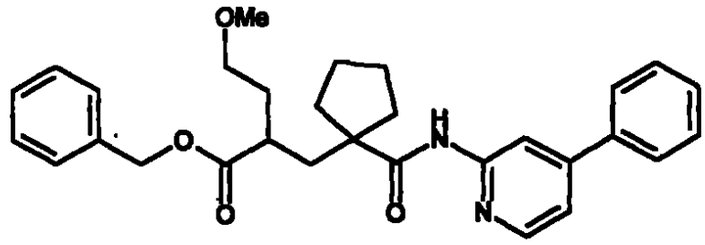
Benzyl-2-[(1-{[(4-butyl-2-pyridinyl)-aminolcarbonyl]cyclopentyl)methyl]-4-methoxy-butanoate



The title product was prepared by a similar procedure to that described in preparation 62 from 1-{2-[(benzyloxy)carbonyl]-4-methoxybutyl}-cyclopentanecarboxylic acid (EP 274234, Example 15) and the product from preparation 90a; ^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.89 (t, 3H, Me), 1.36 (q, 2H, CH_2), 1,47-2.26 (m, 14H), 2.55-2.68 (m, 3H), 3.17 (s, 3H, OMe), 3.24 (t, 2H, CH_2OMe), 4.91 (d, 1H, CHPh), 5.00 (d, 1H, CHPh), 6.83 (d, 1H, Ar), 7.27-7.35 (m, 5H), 7.94 (brs, 1H, NH), 8.07 (s, 1H, Ar), 8.13 (d, 1H, Ar).

Preparation 92

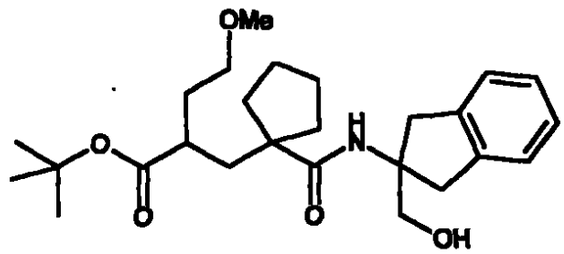
Benzyl-2-[(1-{[(4-phenyl-2-pyridinyl)-aminolcarbonyl]cyclopentyl)methyl]-4-methoxy-butanoate



The title product was prepared by a similar procedure to that described in preparation 62 from 1-{2-[(benzyloxy)carbonyl]-4-methoxybutyl}-cyclopentanecarboxylic acid (EP 274234, Example 15) and 2-amino-4-phenyl pyridine (see Journal of Medicinal Chemistry, 1978, p874); ^{1}HNMR ($CDCl_3$, 400MHz) δ : 1.43-2.34 (m, 10H), 2.60-2.68 (m, 1H), 3.17 (s, 3H, OMe), 3.26 (t, 2H, CH_2), 4.93 (d, 1H, CHPh), 5.02 (d, 1H, CHPh), 7.18-7.32 (m, 5H, Ph), 7.38-7.46 (m, 3H), 7.61-7.69 (m, 2H), 8.02 (brs, 1H, NH), 8.29 (d, 1H, Ar), 8.57 (s, 1H, Ar).

Preparation 93

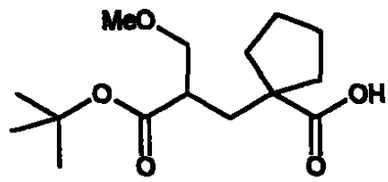
Tert-Butyl-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-methoxybutanoate



The title product was prepared by a similar procedure to that described in preparation 33 from the product from preparation 99 and and 2-amino-2-hydroxymethyl-2,3-dihydroindene (WO9110644; Example 8a); ^{1}HNMR ($CDCl_3$, 400MHz) δ: 1.40 (s, 9H), 1.44-2.00 (m, 12H), 2.37-2.43 (m, 1H), 2.99 (d, 1H), 3.08 (d, 1H), 3.20-3.38 (m, 7H), 3.65 (dd, 1H), 3.84 (dd, 1H), 4.40 (t, 1H), 6.00 (s, 1H), 7.10-7.18 (m, 4H).

Preparation 94

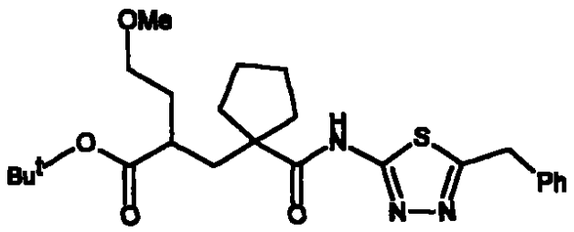
3-(1-Carboxycyclopentyl)-2-(methoxymethyl)propanoic acid *tert*-butyl ester



The title product from Preparation 2, stage b) (10g, 41.3mmol) was taken up in THF at -78°C and lithium diisopropylamide (43ml, 86.7mmol, 2M solution in THF) added dropwise. The mixture was stirred at -78°C for 40min, after which time chloromethyl methyl ether (4.7ml, 62mmol) was added dropwise. The solution was then allowed to warm slowly to room temperature overnight and was quenched by the addition of 2N HCl (100ml). The organics were extracted with EtOAc (2x100ml), dried ($MgSO_4$) and purified by column chromatography using 2%, then 3%, and then 5% MeOH in DCM to provide the title product as a yellow oil (6.2g, 53%); ^{1}HNMR ($CDCl_3$, 400MHz) δ 1.40 (9H, s), 1.40-1.50 (4H, m), 1.20-1.80 (1H, m), 1.80-1.90 (1H, m), 2.00 (1H, dd), 2.00-2.05 (3H, m), 2.20 (1H, dd), 2.50-2.60 (1H, m), 3.30 (1H, s), 3.30-3.40 (1H, m), 3.40 (1H, t); LRMS: m/z, MNH_4^+ 304.

Preparation 95

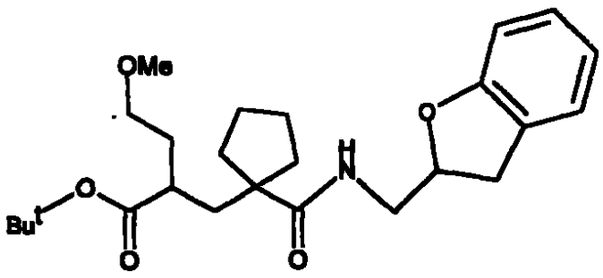
Tert-Butyl-2-[(1-[[(5-benzyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)methyl]-4-methoxybutanoate



The product from Preparation 98 was reacted with the title product from Preparation 99 using a similar procedure to that described in Preparation 33 to provide the amide as a white foam; ^{1}HNMR ($CDCl_3$, 400MHz) δ 0.80 (t, 3H), 1.20-1.35 (m, 2H), 1.37 (s, 9H), 1.40-2.20 (m, 13H), 4.30 (s, 2H), 7.30 (m, 5H); LRMS m/z 459 (M+H).

Preparation 96

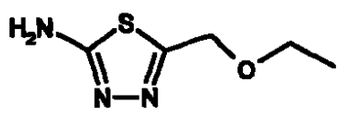
Tert-Butyl-2-[(1-[[(2,3-dihydro-1-benzofuran-2-ylmethyl)amino]carbonyl]cyclopentyl)methyl]-4-methoxybutanoate



The title compound was prepared from 2-aminomethyl-2,3-dihydrobenzofuran (J. Med. Chem., 1968, 11(4), page 644) and the title product from Preparation 99 using a similar procedure to that described in Preparation 33; ^{1}HNMR ($CDCl_3$, 400MHz) δ 1.4 (s, 10H), 1.45-2.00 (m, 12H), 2.05 (m, 1H), 2.30 (m, 1H), 2.90 (m, 1H), 3.05 (m, 5H), 3.30 (m, 2H), 3.55-3.65 (m, 1H), 4.80 (m, 1H), 6.15 (m, 1H), 6.70 (d, 1H), 6.80 (t, 1H), 7.00 (d, 1H), 7.05 (m, 1H); LRMS m/z 432 (M+H).

Preparation 97

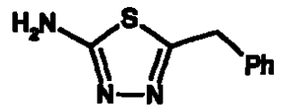
5-(Ethoxymethyl)-1,3,4-thiadiazol-2-amine



Prepared following a similar procedure to preparation 31 from ethoxyacetic acid.

Preparation 98

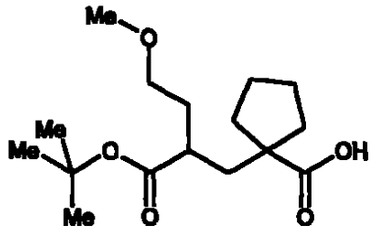
5-Benzyl-1,3,4-thiadiazol-2-amine



The title compound was prepared following a similar procedure to preparation 31 from phenyl acetic acid; ^{1}HNMR ($CDCl_3$; 400MHz) δ 4.10 (m, 2H), 7.30 (m, 5H); Anal. Found C, 56.73; H, 4.72; N, 21.67%. $C_9H_9N_3S$ requires C, 56.62; H, 4.74; N, 21.97%.

Preparation 99

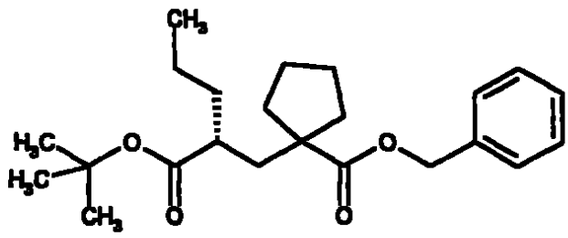
1-[2-(*tert*-Butoxycarbonyl)-4-methoxybutyl]cyclopentanecarboxylic acid



A solution of the title product from Preparation 2, stage b) in dry tetrahydrofuran (100ml) was added to a stirred solution of lithium diisopropylamide (130ml) in a mixture of hexane (52ml) and tetrahydrofuran (200ml) at –78°C under nitrogen. After 1 hour a solution of 2-bromoethyl methyl ether in tetrahydrofuran (100ml) was added maintaining the temperature at –78°C. The reaction mixture was allowed to warm up to room temperature overnight. The mixture was quenched with water (100ml) and acidified to pH 1 with 2M hydrochloric acid, and extracted with ethyl acetate (2x 150ml). The combined organic extracts were dried over magnesium sulphate and concentrated *in vacuo* to give the crude acid which was chromatographed on silica. Elution with increasing proportions of methanol in dichloromethane (neat dichloromethane to 1:50) gave an oil (7.7g, 25.6mmol, 52%). Rf 0.3 methanol, dichloromethane 1:20. ^{1}H NMR ($CDCl_3$ 400MHz) δ: 1.4 (s, 9H), 1.4-1.7 (m, 7H), 1.75-1.95 (m, 2H), 2.0-2.15 (m, 3H), 2.3-2.4 (m, 1H), 3.3 (s, 3H), 3.3-3.4 (m, 2H). LRMS: m/z 299 ($M-H^+$).

Preparation 100

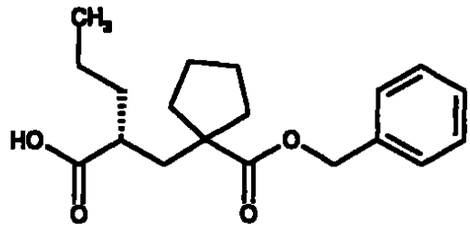
Benzyl-1-[2-(tert-butoxycarbonyl)pentyl]cyclopentanecarboxylate



The product from Preparation 2 (513mg, 1.80mmol) was dissolved in methanol (5mls of 75% aqueous methanol) and cesium carbonate (300mg, 0.95mmol) was added in one portion at room temperature. After 5mins, the solvents were removed under reduced pressure, and the residue azeotroped with toluene (2x5mls) and then redissolved in 7mls of dry DMF under a nitrogen atmosphere. Benzyl bromide was taken up in 3mls of dry DMF, and added slowly with stirring, before the reaction mixture was stirred at room temperature for 3h. The mixture was poured into ethyl acetate (40mls) and washed with water (40mls), 1N HCl (20mls) and water (2x20mls). The organic layer was dried ($MgSO_4$) and evaporated to a thick oil, which was purified by column chromatography using 1:2 DCM:pentane, then 1:2 EtOAc:pentane as eluant to provide the title product (430mg, 64%); ^{1}HNMR (400MHz, $CDCl_3$) 0.83 (t, 3H), 1.17-1.32 (m, 3H), 1.42 (s, 9H), 1.36-1.68 (m, 7H), 1.80 (dd, 1H), 1.97-2.12 (m, 4H), 5.10 (app. q, 2H), 7.36 (m, 5H).

Preparation 101

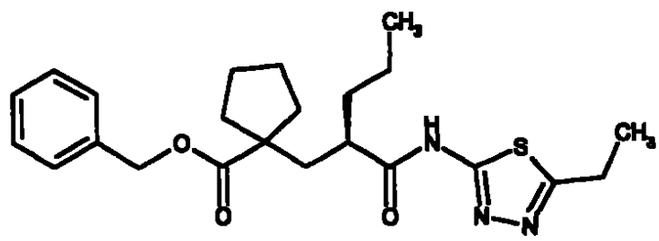
2-({1-[(Benzyloxy)carbonyl]cyclopentyl}methyl)pentanoic acid



The product from Preparation 100 (430mg, 1.15mmol) was taken up in TFA (2 ml) under a nitrogen atmosphere and stirred for 16h. The mixture was evaporated to dryness, and the residue then purified by column chromatography, using 95:5 DCM:MeOH to provide the title product (353, 97%); ^{1}HNMR (400MHz, $CDCl_3$) 0.82 (t, 3H), 1.20-1.74 (m, 10H), 1.80 (dd, 1H), 2.04-2.13 (m, 3H), 2.24-2.41 (m, 1H), 5.10 (app q., 2H), 7.36 (m, 5H).

Preparation 102

Benzyl-1-(2-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]pentyl)-cyclopentanecarboxylate



The product from Preparation 101 (353mg, 1.11mmol), 2-amino-5-ethyl-1,3,4-thiadiazole (ex Lancaster) (150mg, 1.15mmol), WSCDI (255mg, 1.20mmol), HOBt (173mg, 1.20mmol) and 4-methylmorpholine (0.24mls, 1.20mmol) were all mixed together in 5mls of acetonitrile and stirred under nitrogen for 16h at room temperature. After this time, the mixture was warmed to 50°C for 3h, and then at 80°C for 3h. The mixture was cooled to room temperature, evaporated, dissolved in EtOAc (10mls) and washed with $NaHCO_3$ (10mls). The organic layer was dried ($MgSO_4$) and evaporated to provide a gum which was purified by column chromatography to give the title product (430mg, 90%); ^{1}HNMR (400MHz, $CDCl_3$) 0.79 (t, 3H), 1.15-1.24 (m, 3H), 1.37 (t, 3H), 1.42-1.63 (m, 7H), 1.83 (dd, 1H), 2.00-2.20 (m, 3H), 2.42-2.51 (m, 1H), 2.97 (q, 2H), 5.01 (app. q, 2H), 7.30 (m, 5H).

NEP Assay

The Preparation and Assay of Soluble Neutral Endopeptidase (NEP) from Canine, Rat, Rabbit and Human Kidney Cortex.

Soluble NEP is obtained from the kidney cortex and activity is assayed by measuring the rate of cleavage of the NEP substrate Abz-D-Arg-Arg-Leu-EDDnp to generate its fluorescent product, Abz-D-Arg-Arg.

Experimental Procedure:-

1 Materials

All water is double de ionised.

1.1 Tissues:

Human Kidney	IIAM (Pennsylvania. U.S.A.)
Rat Kidney	In house tissue supply
Rabbit Kidney	In house tissue supply
Canine Kidney	In house tissue supply

1.2 Homogenisation medium:

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.3 Tris buffer (NEP buffer):

50ml of 50mM Tris pH 7.4 (Sigma T2663) is diluted in 950ml of water.

1.4 Substrate (Abz-D-Arg-Arg-Leu-EDDnp):

Made to order from SNPE, and is stored as a powder at –20°C. A 2mM stock is made by gently re-suspending the substrate in Tris buffer, this should not be vortexed or sonicated. 600µl aliquots of the 2mM stock are stored at –20 for up to one month. (Medeiros, M.A.S., Franca, M.S.F. et al., (1997), Brazilian Journal of Medical and Biological Research, 30, 1157-1162).

1.5 Total product:

Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined. The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.6 Stock solution:

A 300µM stock of Phosphoramidon (Sigma R7385) is made up in NEP buffer and

stored in 50µl aliquots at -20.

1.7 Dimethyl sulphoxide (DMSO).

1.8 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.9 Black 96 well flat bottom assay plates (Costar 3915).

1.10 Topseal A (Packard 6005185).

1.11 Centrifuge tubes

2 Specific Equipment

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 Fluostar galaxy.

2.5 Weebart 1589 shaking incubator.

3 Methods

3.1 Tissue Preparation

3.2 Dog, rat, rabbit, and human NEP is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approximately 10 volumes of homogenisation buffer (1.2) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.8) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer containing magnesium chloride (9mg MgCl in 5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,830rpm) for 12 minutes in a Beckman centrifuge (2.3) before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge (2.1) and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer containing magnesium chloride (0.9mg MgCl in 0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer (2.2). This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4 Determination of NEP Activity

The activity of the previously aliquoted NEP is measured by its ability to cleave the NEP specific peptide substrate.

4.1 A 4% DMSO/NEP buffer solution is made (4mls DMSO in 96mls NEP buffer).

4.2 Substrate, total product, enzyme, and Phosphoramidon stocks are left on ice to thaw.

4.3 50µl of 4% DMSO/NEP buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:40 to make a 50µM solution. 100µl of 50µM substrate is added to each well (final concentration 25µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of NEP buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:80 to make a 25µM solution. 200µl of 25µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37 degC in a shaking incubator for 60 minutes.

4.8 The 300µM Phosphoramidon stock is diluted 1:100 to 300nM. The reaction is stopped by the addition of 100µl 300nM Phosphoramidon and incubated at 37°C in a shaking incubator for 20 minutes before being read on the Fluostar (ex320/em420).

5 NEP Inhibition Assays

5.1 Substrate, total product, enzyme and Phoshoramidon stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in NEP buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO solution (4mls DMSO in 96mls NEP buffer).

5.3 50µl of compound in duplicate is added to the 96 well plate and 50µl of 4% DMSO/NEP buffer is added to control and blank wells (see appendix for plate

90

layout). Alternatively see appendix for robotic dilutions.

5.4 The 2mM substrate stock is diluted 1:40 in NEP buffer to make a 50µM solution (275µl 2mM substrate to 10.73ml buffer is enough for 1 plate).

5.5 The enzyme stock diluted in NEP buffer (determined from activity checks).

5.6 The 2mM total product stock is diluted 1:80 in NEP buffer to make a 25µM solution. 200µl is added to the first four wells of a separate plate.

5.7 The 300µM Phosphoramidon stock is diluted 1:1000 to make a 300nM stock (11µl Phosphoramidon to 10.99ml NEP buffer.

5.8 To each well in the 96 well plate the following is added:

Table: Reagents to be added to 96 well plate.

	Compound/ DMSO	Tris Buffer	Substrate	NEP enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.9 The reaction is initiated by the addition of the NEP enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.10 The reaction is stopped with 100µl 300nM Phosphoramidon and incubated at 37°C for 20 minutes in a shaking incubator before being read on the Fluostar (ex320/em420).

6 Calculations

The activity of the NEP enzyme is determined in the presence and absence of compound and expressed as a percentage.

% Control activity (turnover of enzyme) =

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

% Activity with inhibitor =

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

Activity expressed as % of control =

$$\frac{\text{\% Activity with Inhibitor}}{\text{\% Control activity}} \times 100$$

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC50 values calculated using LabStats fit-curve in Excel.

ACE Assay

The Preparation and Assay of Soluble Angiotensin Converting Enzyme (Ace), from Porcine and Human Kidney Cortex.

Soluble ACE activity is obtained from the kidney cortex and assayed by measuring the rate of cleavage of the ACE substrate Abz-Gly-p-nitro-Phe-Pro-OH to generate its fluorescent product, Abz-Gly.

1 Materials

All water is double de ionised.

1.1 Human Kidney: IIAM (Pennsylvania. U.S.A.) or UK Human Tissue Bank (UK HTB)

1.2 Porcine kidney ACE Sigma (A2580)

1.3 Homogenisation buffer-1

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.4 Homogenisation buffer-2

100mM Mannitol, 20mM Tris @ pH7.1 and 10mM $MgCl_2.6H_2O$ (Fisher M0600/53)

To 500ml of the homogenisation buffer 1 (1.4) 1.017g of $MgCl_2$ is added.

1.5 Tris buffer (ACE buffer).

50mM Tris and 300mM NaCl @ pH 7.4

50ml of 50mM Tris pH 7.4 (Sigma T2663) and 17.52g NaCl (Fisher S/3160/60) are made up to 1000ml in water.

1.6 Substrate (Abz-D-Gly-p-nitro-Phe-Pro-OH) (Bachem M-1100)

ACE substrate is stored as a powder at –20°C. A 2mM stock is made by gently re-suspending the substrate in ACE buffer, this must not be vortexed or

sonicated. 400µl aliquots of the 2mM stock are stored at –20°C for up to one month.

1.7 Total product

Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined (see calculations). The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.8 Stop solution.

0.5M EDTA (Promega CAS[6081/92/6]) is diluted 1:250 in ACE buffer to make a 2mM solution.

1.9 Dimethyl sulphoxide (DMSO).

1.10 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.11 Black 96 well flat bottom assay plates (Costar 3915 or Packard).

1.12 Topseal A (Packard 6005185).

1.13 Centrifuge tubes

2 Specific Equipment

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 BMG Fluostar Galaxy.

2.5 Wesbart 1589 shaking incubator.

3 Methods

3.1 Tissue Preparation

3.2 Human ACE is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approxdmately 10 volumes of homogenisation buffer-1 (1.4) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.11) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge

tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer-2 (1.5) (5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,630rpm) for 12 minutes in a Beckman centrifuge before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer-2 (0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer. This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4 Determination Of ACE Activity

The activity of the previously aliquoted ACE is measured by its ability to cleave the ACE specific peptide substrate.

Porcine ACE (1.2) is defrosted and resuspended in ACE buffer (1.6) at 0.004U/µl, this is frozen down in 50µl aliquots.

4.1 A 4% DMSO/ACE buffer solution is made (4mls DMSO in 96mls ACE buffer).

4.2 Substrate (1.7), total product (1.8) and enzyme (1.1, 1.2, 1.3), are left on ice to thaw.

4.3 50µl of 4% DMSO/ACE buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:100 to make a 20µM solution. 100µl of 20µM substrate is added to each well (final concentration in the assay 10µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of ACE buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:200 to make 10µM solution. 200µl 10µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37°C in a shaking incubator for 60 minutes.

4.8 The enzyme reaction is stopped by the addition of 100µl 2mM EDTA in ACE buffer and incubated at 37°C in a shaking incubator for 20 minutes before being read on the BMG Fluostar Galaxy (ex320/em420).

5 ACE Inhibition Assays

5.1 Substrate, total product, and enzyme stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in ACE buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO/ACE buffer solution (4mls DMSO in 96mls ACE buffer).

5.3 50µl of compound, in duplicate, is added to the 96 well plate and 50µl of 4% DMSO/ACE buffer is added to control and blank wells (see appendix-1 for plate layout).

5.4 Steps 5.2 and 5.3 can be carried out either by hand or using the Packard multiprobe robots (see appendix-2 for details)

5.5 The 2mM substrate stock is diluted 1:100 in ACE buffer to make a 20µM solution (10µM final concentration in the assay) (110µl of 2mM substrate added to 10.89ml buffer is enough for 1 plate).

5.6 The enzyme stock is diluted in ACE buffer, as determined from activity checks (4.0).

5.7 The 2mM total product stock is diluted 1:200 in ACE buffer to make a 10µM solution. 200µl is added to the first four wells of a separate plate.

5.8 The 0.5mM EDTA stock is diluted 1:250 to make a 2mM stock (44µl EDTA to 10.96ml ACE buffer).

5.9 To each well of the 96 well plate the following reagents are added:

Table 1: Reagents added to 96 well plate.

	Compound/ DMSO	Tris Buffer	Substrate	ACE enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.10 50µl of the highest concentration of each compound used in the assay is added in duplicate to the same 96 well plate as the totals (5.7). 150µl of ACE buffer is added to determine any compound fluorescence.

5.11 The reaction is initiated by the addition of the ACE enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.12 The reaction is stopped by the addition of 100µl 2mM EDTA and incubated at

37°C for 20 minutes in a shaking incubator, before being read on the BMG Fluostar Galaxy (ex320/em420).

6 Calculations

The activity of the ACE enzyme is determined in the presence and absence of compound and expressed as a percentage. (FU = Fluorescence units)

(i) % Control activity (turnover of enzyme) =

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(ii) % Activity with inhibitor =

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(iii) Activity expressed as % of control =

$$\frac{\text{\% Activity with inhibitor}}{\text{\% Control activity}} \times 100$$

or $$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of controls} - \text{Mean FU of blanks}} \times 100$$

(iv) % Inhibition = 100 - % control

(v) For fluorescent compounds the mean FU of blanks containing compound (5.10) is deducted from the mean FU of compound values used to calculate the % Activity.

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC_{50} values calculated using LabStats fit-curve in Excel.

93

The specific examples herein had an IC50 against NEP of less than 5000nM.

In addition many of the examples tested had a selectivity for NEP over ACE of at least 300 fold.

Animal Model of arousal response

We have developed an animal model that mimics the physiological arousal response observed during female sexual arousal and directly reflects the clinical data obtained in human volunteers. The model uses Laser Doppler technologies to record small changes in vaginal and clitoral blood flow induced by pelvic nerve stimulation or vasoactive neurotransmitters. During sexual arousal there is an increase in genital blood flow resulting from increased innervation from the pelvic nerve. This increase in genital blood flow leads to increased genital lubrication and genital sensitivity which are associated with sexual arousal. The major cause of FSAD is decreased genital blood flow and this manifests itself as reduced vaginal, labial and clitoral engorgement. Treatment of women with FSAD is achievable by restoration of the *normal* sexual arousal response. This can be achieved by enhancing genital blood flow.

The pelvic nerve-stimulated increase in vaginal and clitoral blood flow, observed in the animal model, represents the endogenous vascular effects observed during female sexual arousal. Therefore this model can be used to firstly, identify the mechanisms involved in the regulation of vaginal and clitoral blood flow and secondly, use the model to validate novel approaches for the enhancement of genital blood flow.

The title product from Example 29 was administered using this animal model according to the following protocol to show an enhancement in pelvic nerve stimulated increases in genital blood flow in the rabbit.

Two routes of administration were studied: a) intravenous administration and b) topical administration. Both studies used the anesthetised rabbit model according to the following protocol.

Methods

Anaesthetic Protocol

Female New Zealand rabbits (~2.5kg) were pre-medicated with a combination of Medetomidine (Domitor®) 0.5ml/kg *I.m.*, and Ketamine (Vetalar®) 0.25ml/kg *I.m.* whilst maintaining oxygen intake via a face mask. The rabbits were tracheotomised using a Portex™ uncuffed endotracheal tube 3 ID, connected to ventilator and maintained a

ventilation rate of 30-40 breaths per minute, with an approximate tidal volume of 18-20 ml, and a maximum airway pressure of 10 cm H_2O. Anaesthesia was then switched to Isoflurane and ventilation continued with O_2 at 2l/min. The right marginal ear vein was cannulated using a 23G or 24G catheter, and Lactated Ringer solution perfused at 0.5ml/min. The rabbit was maintained at 3% Isoflurane during invasive surgery, dropping to 2% for maintenance anaesthesia.

Stimulation of the Pelvic Nerve

A ventral midline incision was made into the abdominal cavity. The incision was about 5cm in length just above the pubis. The fat and muscle was *bluntly* dissected away to reveal the hypogastric nerve which runs down the body cavity. It was essential to keep close to the side curve of the pubis wall in order to avoid damaging the femoral vein and artery which lie above the pubis. The sciatic and pelvic nerves lie deeper and were located after further dissection on the dorsal side of the rabbit. Once the sciatic nerve is identified, the pelvic nerve was easily located. The term *pelvic nerve* is loosely applied; anatomy books on the subject fail to identify the nerves in sufficient detail. However, stimulation of the nerve causes an increase in vaginal and clitoral blood flow, and innervation of the pelvic region. The pelvic nerve was freed away from surrounding tissue and a *Harvard* bipolar stimulating electrode was placed around the nerve. The nerve was slightly lifted to give some tension, then the electrode was secured in position. Approximately 1ml of light paraffin oil was placed around the nerve and electrode. This acts as a protective lubricant to the nerve and prevents blood contamination of the electrode. The electrode was connected to a *Grass* S88 Stimulator. The pelvic nerve was stimulated using the following parameters:- 5V, pulse width 0.5ms, duration of stimulus 10 seconds and a frequency range of 2 to 16Hz. Reproducible responses were obtained when the nerve was stimulated every 15-20 minutes.

A frequency response curve was determined at the start of each experiment in order to determine the optimum frequency to use as a sub-maximal response, normally 4Hz. The compound(s) to be tested were infused, via the femoral vein, using a *Harvard* 22 infusion pump allowing a continuous 15 minute stimulation cycle.

Positioning of the Laser Doppler Probes

A ventral midline incision was made, at the caudal end of the pubis, to expose the pubic

area. Remove any connective tissue, and expose the tunica of the clitoris, ensuring that the wall is free from small blood vessels. The external vaginal wall was also exposed by removing any connective tissue. One laser Doppler flow probe was inserted 3cm into the vagina, so that half the probe shaft is still visible. A second probe was positioned so that it lies just above the external clitoral wall. The position of these probes was then adjusted until a signal was obtained. A second probe was placed just above the surface of a blood vessel on the external vaginal wall. Both probes were clamped in position.

Vaginal and clitoral blood flow was recorded either as numbers directly from the Flowmeter using *Po-ne-mah* data acquisition software (Ponemah Physiology Platform, Gould Instrument Systems Inc), or indirectly from Gould chart recorder trace. Calibration is set at the beginning of the experiment (0-125ml/min/100g tissue).

Administration of Inhibitors

a) Intravenous administration

Cannulation of Blood Vessels

The left groin area of the rabbit was shaved and a vertical incision was made approximately 5cm in length along the thigh. The femoral vein and artery were exposed, isolated and then cannulated with a PVC catheter (17G) for the infusion of drugs and compounds. Cannulation was repeated for the femoral artery, inserting the catheter to a depth of 10cm to ensure that the catheter reached the abdominal aorta. This arterial catheter was linked to a Gould system to record blood pressure. Samples for blood gas analysis were also be taken via the arterial catheter. Systolic and diastolic pressures were measured, and the mean arterial pressure calculated using the formula (diastolic x2 + systolic) ÷3. Heart rate was measured via the pulse oxymeter and *Po-ne-mah* data acquisition software system (Ponemah Physiology Platform, Gould Instrument Systems Inc).

The title compound form Example 29 was made up in saline or 5% glucose solution (200µl 50% glucose in 1.8ml water for injection). The inhibitor and vehicle controls were infused using a Harvard 22 pump, infusing at 500µl/min via a 3-way tap into the femoral vein. After the infusion, the catheter was flushed with heparinised saline (Hepsaline) so that no NEP inhibitor was left in the catheter.

b) *Topical administration of Inhibitors*

A topical formulation was prepared mixing the product from Example 29 at 90% saturation in 50% propylene glycol/50% water. The mixture was made viscous with carboxymethyl cellulose (CMC) giving a final concentration of approximately 2.5mg/ml. The administration of 0.2ml provides a maximal dose of 0.5mg.

This formulation was applied topically to the internal vaginal wall via an in house designed applicator. Basically, pieces of tubing (ID 3mm, OD 4mm) 10cm in length were cut and attached to a 1ml syringes. The syringes were each filled with a control gel (containing no active ingredient) or the formulation described above. The tubing was inserted 2cm into the vagina and 0.2ml of the gel was gently injected to avoid disturbing the laser Doppler probe. The addition of gel caused no major distension to the vagina and there was no excessive leakage of the gels from within the vagina during non-stimulated or stimulated periods.

Results and Discussion

Animal model of sexual arousal

We have developed a robust reproducible model of the physiology of sexual arousal. Using this anaesthetised rabbit model, we are capable of measuring small changes in genital blood flow using Laser Doppler technology. Stimulation of the pelvic nerve is used to simulate the neuronal effects of sexual arousal.
FSAD is associated with and may result from reduced genital blood flow.

Our results demonstrate that the title compound of Example 29 significantly enhanced pelvic nerve stimulated increases in genital blood flow at clinically relevant doses. This enhancement is seen with both intravenous and topical administration.

Figure 1 shows the effect on vaginal and clitoral blood flow of intravenous administration of the title product from Example 29. Intravenous administration enhanced pelvic nerve stimulated (PNS) increases in genital blood flow in the anaesthetised rabbit model of sexual arousal. Repetitive PNS at 15 minute intervals induced reproducible increases in genital blood flow (Hatched Bars). Intravenous administration of the title product from Example 29 (Grey bar) enhanced the peak increase in clitoral and vaginal blood flow induced by submaximal stimulation frequencies (eg 4Hz) compared to increases observed during time matched control stimulations or vehicle controls (Hatched bar).

The following simultaneous enhancements were observed following a 1.0mg/kg iv bolus infusion – a 131% increase in clitoral and a 92% increase in vaginal blood flow (n=3). Data expressed as mean ± sem; all changes were monitored using laser Doppler technologies.

Figure 2 shows the effect on vaginal blood flow over time of administering the title product from Example 29 topically. There was no observable change to non-stimulated/basal vaginal blood flow, neither were any changes observed on stimulated vaginal blood flow after the initial insertion of the tubing or application of the 0.2ml of the vehicle gel.

Repetitive pelvic nerve stimulation at submaximal stimulation frequencies at 15 minute intervals induces reproducible increases in vaginal blood flow (filled circle). Intravaginal application of a set concentration of the tile product from Example 29 (0.2mg/ml) enhanced the peak increase in vaginal blood flow (open circles) compared to the mean control flow increases. The title product from Example 29 had no effect on basal (non-stimulated) vaginal blood flow (open squares) compared to control flow (filled square). All changes were monitored using laser Doppler technologies and data is expressed as mean + s.e.mean (n=4), *** P<0.001 Student's t-test.

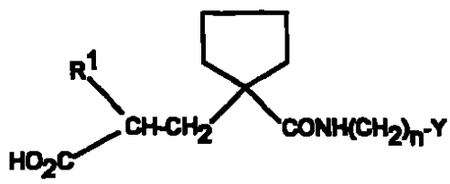
This study demonstrates that the title product from Example 29, when applied topically to the vagina significantly enhanced pelvic nerve-stimulated increases in vaginal blood flow. The degree of enhancement is comparable to increases observed after intravenous infusion of the compound. Interestingly, the enhancement occurred at free plasma concentration of the title product from Example 29 which would not be expected to cause a potentiation of vaginal blood flow.

In conclusion, this study demonstrates that intravaginal, topical application of the compounds of the invention enhances pelvic nerve stimulated increases in vaginal blood flow.

96

Claims

1 The use of a compound of formula (I), pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, in the preparation of a medicament for the treatment of female sexual dysfunction;



(I)

wherein

R^1 is C_{1-8}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6} alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-8}alkyl; or R^1 is C_{1-8} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or $-NR^2R^3$ wherein R^2 and R^3 are as previously defined;

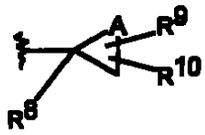
R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycl...alkyl, aryl or heterocyclyl; p is 0, 1, 2 or 3;

n is 0, 1 or 2;

the $-(CH_2)_n-$ linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

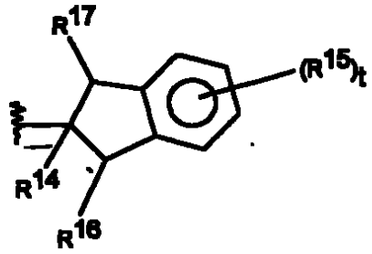
Y is the group



wherein A is $-(CH_2)_q-$ where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, $-CH_2OH$, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl (optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy, or R^9 and R^{10} together form a dioxolane; R^{11} and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

Y is the group, $-C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

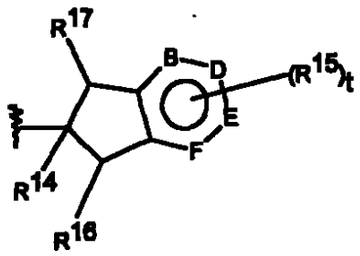
Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as

previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl; and u is 0, 1, 2 or 3.

2 A compound of formula (I), pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^1, n and Y are as defined in claim 1

with the proviso that Y is not the group -C(O)NR^{11}R^{12} and when R^1 is propyl or phenylethyl, R^{14} is not -CH$_2$OH.

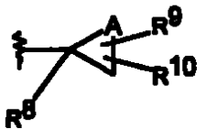
3 A compound of formula (I), pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^1, n and Y are as defined in claim 1 with the proviso that Y is not the group -C(O)NR^{11}R^{12} and R^{14} is not H or -CH$_2$OH.

4 A compound according to claims 2 or 3, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^1 is C$_{1-8}$alkyl, C$_{1-8}$alkoxy, C$_{1-8}$alkoxy(C$_{1-3}$)alkyl, C$_{1-8}$alkoxyC$_{1-8}$alkoxyC$_{1-3}$alkyl or C$_{1-8}$alkyl substituted with aryl.

5 A compound according to claim 4, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^1 is C$_{1-8}$alkyl, C$_{1-8}$alkoxy, C$_{1-8}$alkoxy(C$_{1-3}$)alkyl or C$_{1-8}$alkoxyC$_{1-8}$alkoxyC$_{1-3}$alkyl.

6 A compound according to claim 5, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^1 is C$_{1-4}$alkyl or C$_{1-8}$alkoxy(C$_{1-3}$)alkyl.

7 A compound according to any one of claims 2 to 6, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein when Y is the group



and the carbocyclic ring is fully saturated, then preferably one of R^9 or R^{10} is -CH$_2$OH; -C(O)NR^{11}R^{12}; C$_{1-8}$alkyl; phenyl optionally substituted by C$_{1-4}$alkyl; or phenyl(C$_{1-4}$alkyl) wherein the phenyl group is optionally subatituted by C$_{1-4}$alkyl.

8 A compound according to claim 7, pharmaceutically acceptable salts, solvates,

polymorphs or prodrugs thereof, wherein the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, is $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl; phenyl optionally substituted by C_{1-4}alkyl; or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl.

9 A compound according to claims 7 or 8, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^9 and R^{10} are attached to adjacent carbon atoms in the ring.

10 A compound according to any one of claims 7 to 9, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^8 is CH_2OH.

11 A compound according to any one of claims 2 to 6, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein when Y is the group $-NR^{18}S(O)_uR^{19}$, preferably R^{18} is H.

12 A compound according to any one of claims 2 to 6 or 11, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^{19} is benzyl or phenyl.

13 A compound according to any one of claims 2 to 6 or 11 or 12, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein u is 2.

14 A compound according to any one of claims 2 to 6, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein Y is an optionally substituted 5-7 membered heterocyclic ring.

15 A compound according to claim 14, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein the 5-7 membered heterocyclic ring is an optionally substituted aromatic ring.

16 A compound according to claim 15, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein said aromatic ring is pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazolyl, thiazolyl,

thiadiazolyl, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, pyridonyl, quinoxalinyl or quinazolinyl each of which may be substituted as defined in claim 1.

17 A compound according to claim 16, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein the aromatic ring is oxadiazole, pyridone or thiadiazole each of which may be substituted as defined in claim 1.

18 A compound according to claim 17, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein the aromatic ring is 1,2,5-oxadiazole, 1,3,4-oxadiazole, 2-pyridone or 1,3,4-thiadiazole each of which may be substituted as defined in claim 1.

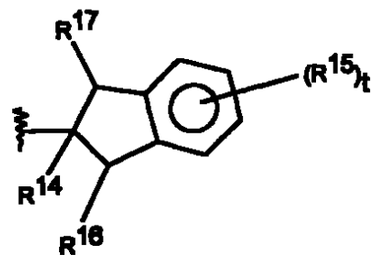
19 A compound according to any one of claims 14 to 18, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein the 5-7 membered heterocyclic ring is substituted by one or more C_{1-8}alkyl, phenyl or phenylC_{1-4}alkyl.

20 A compound according to claim 19, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein the 5-7 membered heterocyclic ring is substituted by C_{1-4}alkyl or benzyl.

21 A compound according to any one of claims 17 to 20, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein when Y is a pyridone said pyridone is *N*-substituted pyridone.

22 A compound according to claim 14, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein Y is a lactam linked at the nitrogen.

23 A compound according to any one of claims 2 to 6, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein Y is

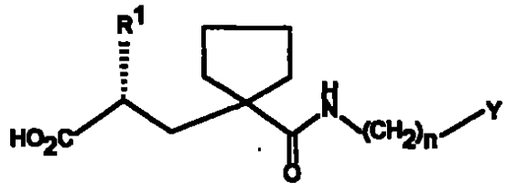


wherein R^{14} is CH_2OH or $C(O)NR^{11}R^{12}$

24 A compound according to any one of claims 2 to 6 or 23, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein R^{16} and R^{17} are hydrogen.

25 A compound according to any one of claims 2 to 6, 23 or 24, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, wherein t is 0.

26 A compound of formula Ie, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof,



(Ie)

wherein R^1, Y and n are defined in any one of claims 2 to 25.

27 A compound, pharmaceutically acceptable salts, solvates, polymorphs or prodrugs thereof, selected from the group consisting of:

2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]cyclopentyl)-methyl]-4-methoxybutanoic acid (Example 35);

2-[[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (Example 40);

(+)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid (Example 44);

2-[(1-[[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)methyl]-4-phenylbutanoic acid (Example 43);

cis-3-(2-methoxyethoxy)-2-[(1-[[(4-[[(phenylsulfonyl)amino]carbonyl]cyclohexyl)-amino]carbonyl]cyclopentyl)methyl]propanoic acid (Example 38);

(+)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl]pentanoic acid (Example 31);

(2*R*)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)-methyl]pentanoic acid or (-)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)-methyl]pentanoic acid (Example 29);

(2*S*)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)-methyl]pentanoic acid or (+)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)-methyl]pentanoic acid (Example 30);

2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21);

2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]cyclopentyl)-methyl]pentanoic acid (Example 22);

2-[[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 9);

trans-3-[1-({[2-(4-chlorophenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxymethyl)propanoic acid (Example 46);

trans-3-[1-({[2-(4-methoxyphenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 47);

trans-3-[1-({[2-pentylcyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 48);

3-[1-({[5-benzyl-[1,3,4]-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 49);

3-[1-({[4-butylpyridin-2-yl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 50);

3-[1-({[4-phenylpyridin-2-yl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 51);

3-[1-({[1-hydroxymethyl-3-phenylcyclopentyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 52);

2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}-4-methoxybutanoic acid (Example 53);

trans-3-[1-({[2-phenylcyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 54);

(*R*)- 2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}-4-methoxybutanoic acid (Example 55); and
(*S*)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}-4-methoxybutanoic acid (Example 56).

28 The use according to claim 1 wherein the female sexual dysfunction treated includes at least female sexual arousal dysfunction (FSAD).

29 The use according to claims 1 or 28 wherein the medicament is administered systemically.

30 The use according to claim 29 wherein the medicament is administered orally.

31 The use of a compound as defined in any one of claims 2 to 27 in the preparation of a medicament for the treatment or prophylaxis of a condition for which a beneficial therapeutic response can be obtained by the inhibition of neutral endopeptidase.

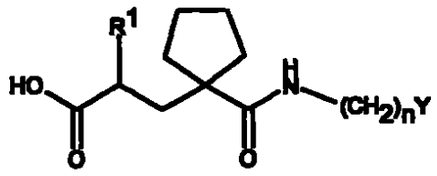
32 A compound as defined in any one of claims 2 to 27 for use in medicine.

33 A pharmaceutical formulation including a compound as defined in any one of claims 2 to 27 together with a pharmaceutically acceptable excipient.

34 A method for the treatment or prophylaxis of female sexual dysfunction including administering to the patient a therapeutically effective amount of a compound as defined in any one of claims 1 to 27.

35 A female sexual dysfunction pharmaceutical formulation including a therapeutically effective amount of a compound as defined in any one of claims 2 to 27 together with a pharmaceutically acceptable excipient.

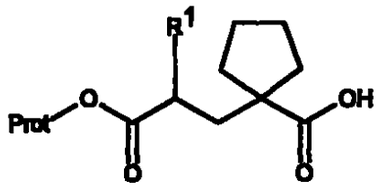
36 A process for preparing a compound of formula I or salts thereof



(I)

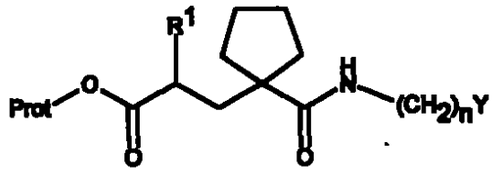
wherein R^1, n and Y are as defined in any one of claims 2 to 27, comprising the steps of:

a) reacting a compound of formula II



(II)

wherein Prot is a suitable protecting group, with a compound of formula $Y(CH_2)_nNH_2$ (III), to give a compound of formula IV,



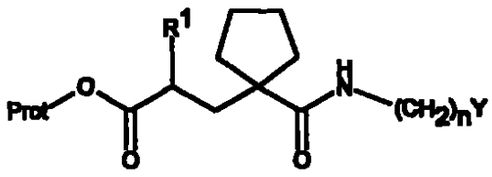
(IV)

then

b) reacting the compound of formula IV under suitable deprotecting conditions to give the compound of formula I; then

c) optionally forming a salt.

37 A compound of formula IV



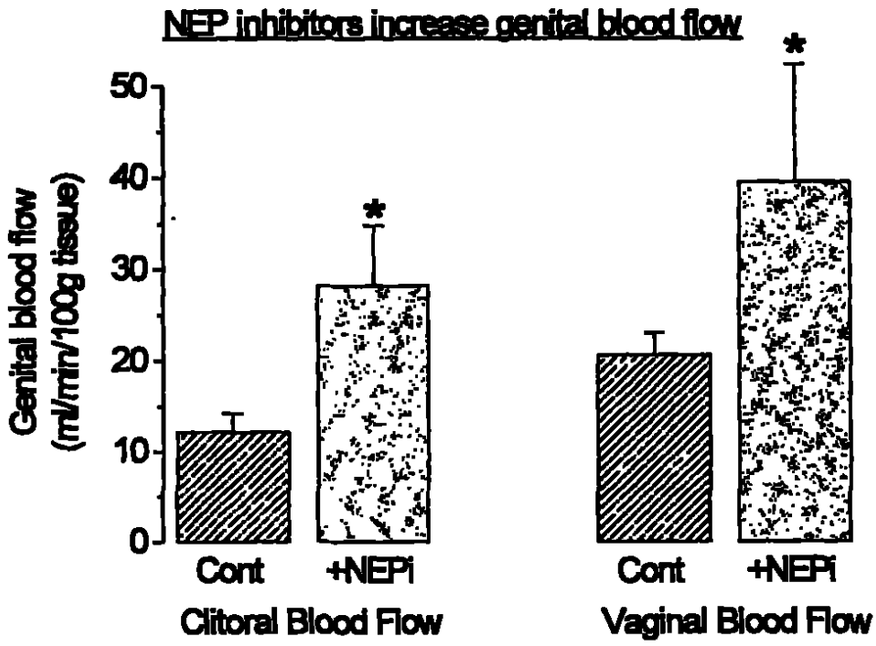
(IV)

wherein R^1, n, and Y are as defined in any one of claims 2 to 27 and wherein Prot is a protecting group.

101

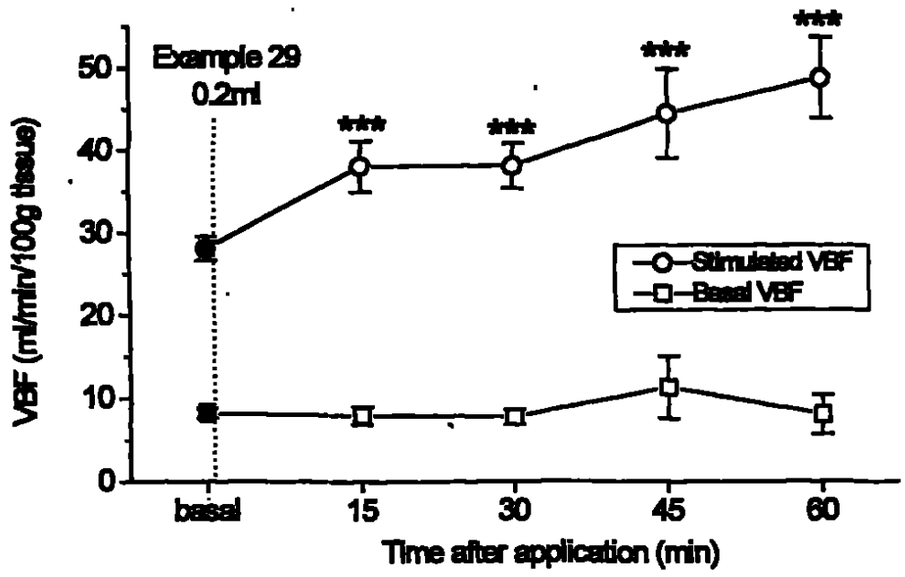
102

Figure 1



5

Figure 2



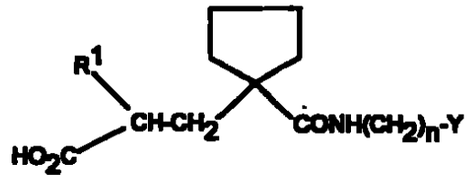
102

Derivados de ciclopentil-glutaramida como inhibidores de endopeptidasa neutra

Esta invención se refiere a inhibidores de enzima endopeptidasa neutra (NEP), sus aplicaciones, procedimientos para su preparación, intermedios utilizados en su preparación, y composiciones que contienen dichos inhibidores. Estos inhibidores tienen utilidad en diversos campos terapéuticos, incluido el tratamiento de la disfunción sexual femenina (FSD), especialmente el trastorno de excitación sexual femenina (FSAD).

Los inhibidores de NEP están descritos en los documentos WO 91/07386 y WO 91/10644.

Según un primer aspecto, la presente invención proporciona el uso de un compuesto de fórmula (I), sus sales, solvatos o profármacos farmacéuticamente aceptables, en la preparación de un medicamento para el tratamiento de disfunción sexual femenina:



(I)

en la que:

R^1 es alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: halo, hidroxi, alcoxi C_{1-6}, hidroxialcoxi C_{2-6}, alcoxi C_{1-6}-(alcoxi C_{1-6}), cicloalquilo C_{3-7}, cicloalquenilo C_{3-7}, arilo, ariloxi, (alcoxi C_{1-4})-ariloxi, heterociclilo, heterociclil-oxi, $-NR^2R^3$, NR^4COR^5, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ y $-CO_2$-(alquilo C_{1-4}); o R^1 es cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes de dicha lista, sustituyentes que pueden ser iguales o diferentes, cuya lista incluye además alquilo C_{1-6}; o R^1 es alcoxi C_{1-6}, $-NR^2R^3$ o $-NR^4SO_2R^5$;

donde

R^2 y R^3 son cada uno independientemente H, alquilo C_{1-4}, cicloalquilo C_{3-7} (opcionalmente sustituido con hidroxi o alcoxi C_{1-4}), arilo, (alquil C_{1-4})-arilo, alcoxi C_{1-6}-arilo o heterociclilo; o R^2 y R^3 junto con el

nitrógeno al que están unidos forman un grupo pirrolidinilo, piperidino, morfolino, piperazinilo o N-(alquil C_{1-4})piperazinilo;

R^4 es H o alquilo C_{1-4};

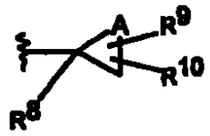
R^5 es alquilo C_{1-4}, CF_3, arilo, (alquil C_{1-4})-arilo, (alcoxi C_{1-4})-arilo, heterociclilo, alcoxi C_{1-4} o $-NR^2R^3$, donde R^2 y R^3 son como se han definido previamente;

R^6 es alquilo C_{1-4}, arilo, heterociclilo o NR^2R^3, donde R^2 y R^3 son como se han definido previamente; y

R^7 es alquilo C_{1-4}, cicloalquilo C_{3-7}, arilo o heterociclilo; p es 0, 1, 2 ó 3; n es 0, 1 ó 2;

el enlace $-(CH_2)_n-$ está opcionalmente sustituido con alquilo C_{1-4}, alquilo C_{1-4} sustituido con uno o más grupos fluoro o fenilo, alcoxi C_{1-4}, hidroxi, hidroxi-(alquilo C_{1-3}), cicloalquilo C_{3-7}, arilo o heterociclilo;

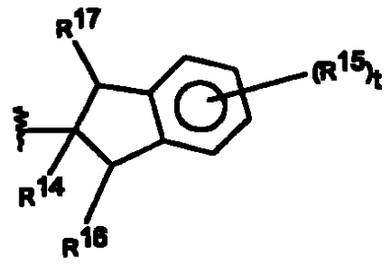
Y es el grupo



donde A es $-(CH_2)_q-$, donde q es 1, 2, 3 ó 4 para completar un anillo carbocíclico de 3 a 7 miembros que puede ser saturado o insaturado; R^8 es H, alquilo C_{1-6}, $-CH_2OH$, fenilo, fenil-(alquilo C_{1-4}) o $CONR^{11}R^{12}$; R^9 y R^{10} son cada uno independientemente H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, alquilo C_{1-6}, fenilo (opcionalmente sustituido con alquilo C_{1-4}, halo o alcoxi C_{1-4}), o fenil-(alquilo C_{1-4}), (donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}, halo o alcoxi C_{1-4}) o R^9 y R^{10} juntos forman un dioxolano; R^{11} y R^{12}, que pueden ser iguales o diferentes, son H, alquilo C_{1-4}, R^{13} o $S(O)_rR^{13}$, donde r es 0, 1 ó 2 y R^{13} es fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-alquilo C_{1-4} donde el fenilo está opcionalmente sustituido con alquilo C_{1-4}; o

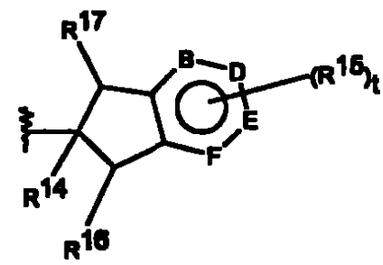
Y es el grupo $-C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se han definido previamente, excepto que R^{11} y R^{12} no son ambos H; o

Y es el grupo



donde R^{14} es H, CH_2OH o $C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se han definido previamente; cuando está presente R^{15}, que puede ser igual o diferente a cualquier otro R^{15}, es OH, alquilo C_{1-4}, alcoxi C_{1-4}, halo o CF_3; t es 0, 1, 2, 3 ó 4; y R^{16} y R^{17} son independientemente H o alquilo C_{1-4}; o

Y es el grupo



donde uno o dos de B, D, E o F es un nitrógeno, siendo los demás carbono; y R^{14} a R^{17} y t son como se han definido previamente; o

Y es un anillo heterocíclico de 5 a 7 miembros opcionalmente sustituido, que puede ser saturado, insaturado o aromático y que contiene un nitrógeno, oxígeno o azufre y opcionalmente uno, dos o tres átomos de nitrógeno adicionales en el anillo y que puede estar opcionalmente condensando con benceno y opcionalmente sustituido con:

alcoxi C_{1-6}; hidroxi, oxo; amino; mono- o di-(alquil C_{1-4})-amino; alcanoil C_{1-4}-amino; o

alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: alcoxi C_{1-6}, haloalcoxi C_{1-6}, alquil C_{1-6}-tio, halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo; o

cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar

sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: alquilo C_{1-6}, alcoxi C_{1-6}, haloalcoxi C_{1-6}, alquil C_{1-6}-tio, halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo;

donde, cuando hay una sustitución oxo en el anillo heterocíclico, el anillo contiene solamente uno o dos átomos de nitrógeno y la sustitución oxo es adyacente a un átomo de nitrógeno del anillo; o

Y es $-NR^{18}S(O)_uR^{19}$, donde R^{18} es H o alquilo C_{1-4}; R^{19} es arilo, aril-alquilo C_{1-4} o heterociclilo (preferiblemente piridilo); y u es 0, 1, 2 ó 3.

Algunos de los compuestos de fórmula I están descritos en los documentos WO 91/10664 y WO 91/07386, pero no se desprende de estos documentos que dichos compuestos puedan ser útiles en el tratamiento de la disfunción sexual femenina. Los compuestos restantes de fórmula I son nuevos.

Por lo tanto, conforme a un segundo aspecto, la invención proporciona un compuesto (nuevo) de fórmula I, o sus sales, solvatos o profármacos del mismo farmacéuticamente aceptables, donde R^1, n e Y son como se han definido en el primer aspecto con la condición de que Y no sea el grupo $-C(O)NR^{11}R^{12}$ y, cuando R^1 es propilo o feniletilo, R^{14} no sea H o $-CH_2OH$.

De acuerdo con un tercer aspecto, la invención proporciona un (nuevo) compuesto de fórmula (I), o una sal, un solvato o un profármaco del mismo farmacéuticamente aceptable, en el que R^1, n e Y son como se han definido en el primer aspecto con la condición de que Y no sea el grupo $-C(O)NR^{11}R^{12}$ y R^{14} no sea H o $-CH_2OH$.

En la definición anterior, salvo indicación en contrario, los grupos alquilo que tienen tres o más átomos de carbono pueden ser de cadena lineal o ramificada. El término arilo, como se utiliza aquí, representa un grupo hidrocarbonado aromático tal como fenilo o naftilo que puede estar opcionalmente sustituido con, por ejemplo, uno o más de OH, CN, CF_3, alquilo C_{1-4}, alcoxi C_{1-4}, halo, carbamoílo, aminosulfonilo, amino, mono o di(alquil C_{1-4})amino o (alcanoil C_{1-4})amino.

Halo significa fluoro, cloro, bromo o yodo.

En la definición anterior, salvo indicación en contrario, el término heterociclilo representa un grupo heterocíclico de 5 ó 6 miembros que contiene nitrógeno, oxígeno o azufre que, salvo indicación en contrario, puede ser saturado, insaturado o aromático y que puede incluir opcionalmente un átomo más de oxígeno o de uno a

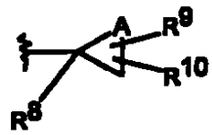
tres átomos adicionales de nitrógeno en el anillo y que, opcionalmente, pueden estar condensado con benceno o sustituido con, por ejemplo, uno o más radicales halo, alquilo C_{1-4}, hidroxi, carbamoílo, bencilo, oxo, amino o mono- o di-(alquil C_{1-4})amino o (alcanoil C_{1-4})amino. Ejemplos particulares de heterociclos incluyen piridilo, piridonilo, pirazinilo, pirimidinilo, piridazinilo, pirrolilo, imidazolilo, pirazolilo, triazolilo, tetrazolilo, furanilo, tetrahidrofuranilo, tetrahidropiranilo, dioxanilo, tienilo, oxazolilo, isoxazolilo, tiazolilo, oxadiazolilo, tiadiazolilo, indolilo, isoindolinilo, quinolilo, isoquinolilo, quinoxalinilo, quinazolinilo y bencimidazolilo, estando cada uno de llos opcionalmente sustituido como se ha definido anteriormente.

Sustituyentes R^1 preferidos son alquilo C_{1-6}, alcoxi C_{1-6}, alcoxi C_{1-6}-alquilo (C_{1-3}), alcoxi C_{1-6}-alcoxi C_{1-6}-alquilo C_{1-3} o alquilo C_{1-6} sustituido con arilo.

Sustituyentes R^1 más preferidos son alquilo C_{1-6}, alcoxi C_{1-6}, alcoxi C_{1-6}-alquilo (C_{1-3}) (especialmente metoxietilo) o alcoxi C_{1-6}-alcoxi C_{1-6}-alquilo C_{1-3} (preferiblemente metoxietoximetilo).

Sustituyentes R^1 más preferidos todavía son alquilo C_{1-4} (preferiblemente propilo) o alcoxi C_{1-6}-alquilo C_{1-3} (preferiblemente metoxialquilo, más preferiblemente metoxietilo).

Cuando Y es el grupo



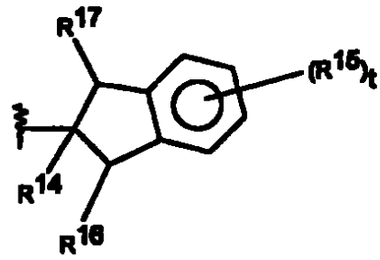
y el anillo carbocíclico está totalmente saturado, entonces preferiblemente uno de R^9 o R^{10} es $-CH_2OH$, $-C(O)NR^{11}R^{12}$, alquilo C_{1-6}, fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-(alquilo C_{1-4}) donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}. Más preferiblemente, el anillo carbocíclico tiene 5, 6 ó 7 miembros, donde uno de R^9 o R^{10} es $-C(O)NR^{11}R^{12}$, siendo el otro alquilo C_{1-6}; fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-(alquilo C_{1-4}) donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}. Más preferiblemente, R^9 y R^{10} están unidos a los átomos de carbono adyacentes en el anillo. Más preferiblemente, R^8 es CH_2OH.

Cuando Y es el grupo $-NR^{18}S(O)_uR^{19}$, R^{18} es preferiblemente H. Más preferiblemente, R^{19} es bencilo o fenilo. Más preferiblemente, u es 2.

Preferiblemente, Y es un anillo heterocíclico de 5 a 7 miembros opcionalmente sustituido. Más preferiblemente, el anillo es un anillo aromático opcionalmente sustituido, particularmente piridilo, pirazinilo, pirimidinilo, piridazinilo, pirazolilo, triazolilo, tetrazolilo, oxadiazolilo, tiazolilo, tiadiazolilo, oxazolilo, indolilo, isoindolinilo, quinolilo, isoquinolilo, piridonilo, quinoxalinilo o quinazolinilo [especialmente oxadiazol (preferiblemente 1,2,5- ó 1,3,4-oxadiazol), piridona (preferiblemente 2-piridona) o tiadiazol (preferiblemente 1,3,4-tiadiazol)], cada uno de los cuales puede estar sustituido como se ha definido en el primer aspecto. Preferiblemente, el anillo heterocíclico está sustituido con uno o más alquilo grupos C_{1-6}, fenilo o fenil-alquilo C_{1-4}, más preferiblemente con alquilo C_{1-4} o bencilo. Preferiblemente, Y es una piridona N-sustituida, preferiblemente con bencilo o alquilo C_{1-4}.

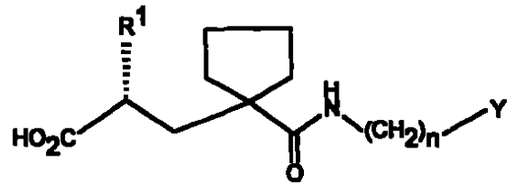
Preferiblemente, Y es una lactama unida al nitrógeno.

Preferiblemente, Y es



donde R^{14} es preferiblemente CH_2OH o $C(O)NR^{11}R^{12}$, especialmente $C(O)NR^{11}R^{12}$. Preferiblemente, R^{16} y R^{17} son hidrógeno. Preferiblemente, t es 0.

Son compuestos preferidos los de fórmula Ie:



(Ie)

Son compuestos particularmente preferidos de la invención:

ácido 2-[(1-{[(1-bencil-6-oxo-1,6-dihidro-3-piridinil)amino]carbonil}ciclopentil)-metil]-4-metoxibutanoico (Ejemplo 35),

p8

ácido 2-{[1-({[3-(2-oxo-1-pirrolidinil)propil]amino}carbonilciclopentil]metil}-4-fenilbutanoico (Ejemplo 40),

ácido (+)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}-4-fenilbutanoico (Ejemplo 44),

ácido 2-[(1-{[(5-metil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]-4-fenilbutanoico (Ejemplo 43),

ácido cis-3-(2-metoxietoxi)-2-[(1-{[(4-{[(fenilsulfonil)amino]carbonil}ciclo-hexil)-amino]carbonil}ciclopentil)metil]propanoico (Ejemplo 38),

ácido (+)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}pentanoico (Ejemplo 31),

ácido (2*R*)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)-metil]pentanoico o ácido (-)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}-ciclopentil)metil]pentanoico (Ejemplo 29);

ácido (2S)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]-pentanoico o ácido (+)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)-metil]pentanoico (Ejemplo 30) ,

ácido 2-({1-[(3-bencilanilino)carbonil]ciclopentil}metil)pentanoico (Ejemplo 21),

ácido 2-[(1-{[(1-bencil-6-oxo-1,6-dihidro-3-piridinil)amino]carbonil}ciclopentil)-metil]pentanoico (Ejemplo 22),

ácido 2-{[1-({[(1R,3S,4R)-4-(aminocarbonil)-3-butilciclohexil]amino}carbonil)-ciclopentil]metil}pentanoico (Ejemplo 9),

ácido *trans*-3-[1-({[2-(4-clorofenil)ciclopropil]amino}carbonil)ciclopentil]-2-(metoximetil)propanoico (Ejemplo 46);

ácido *trans*-3-[1-({[2-(4-metoxifenil)ciclopropil]amino}carbonil)ciclopentil]-2-(metoxietil)propanoico (Ejemplo 47);

ácido *trans*-3-[1-({[2-pentilciclopropil]amino}carbonil)ciclopentil]-2-(metoxi-etil)propanoico (Ejemplo 48);

ácido 3-[1-({[5-bencil-[1,3,4]-tiadiazol-2-il]amino}carbonil)ciclopentil]-2-(metoxietil)propanoico (Ejemplo 49);

ácido 3-[1-({[4-butilpiridin-2-il]amino}carbonil)ciclopentil]-2-(metoxietil)-propanoico (Ejemplo 50);

ácido 3-[1-({[4-fenilpiridin-2-il]amino}carbonil)ciclopentil]-2-(metoxietil)-propanoico (Ejemplo 51);

109 · 2

ácido 3-[1-({[1-hidroximetil-3-fenilciclopentil]amino}carbonil)ciclopentil]-2-(metoxietil)propanoico (Ejemplo 52);

ácido 2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}-4-metoxibutanoico (Ejemplo 53);

ácido *trans*-3-[1-({[2-fenilciclopropil]amino}carbonil)ciclopentil]-2-(metoxietil)-propanoico (Ejemplo 54);

ácido (*R*)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}-4-metoxibutanoico (Ejemplo 55); y

ácido (*S*)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}-4-metoxibutanoico (Ejemplo 56).

Para evitar dudas, salvo indicación en contrario, el término "sustituido" significa sustituido con uno o más grupos definidos. En cado de que los grupos puedan seleccionarse entre varios grupos alternativos, los grupos seleccionados pueden ser iguales o diferentes.

Para evitar dudas, el término "independientemente" significa que cuando se seleccionan más de un sustituyente entre los diversos sustituyentes posibles, esos sustituyentes pueden ser iguales o diferentes.

Las sales farmacéutica o veterinariamente aceptables de los compuestos de fórmula I que contienen un centro básico son, por ejemplo, sales de edición de ácido no tóxicas formadas con ácidos inorgánicos tales como ácido clorhídrico, bromhídrico, yodhídrico, sulfúrico y fosfórico, con ácidos carboxílicos o con ácidos organosulfónicos. Ejemplos incluyen las sales de HCl, HBr, HI, sulfato o bisulfato, nitrato, fosfato o hidrógenofosfato, acetato, benzoato, succinato, sacarato, fumarato, maleato, lactato, citrato, tartrato, gluconato, camsylato, metanosulfonato, etanosulfonato, bencenosulfonato, p-toluenosulfonato y pamoato. Los compuestos de la invención también pueden formar sales metálicas farmacéutica o veterinariamente aceptables, en particular sales no tóxicas de metales alcalinos y alcalinotérreos, con bases. Ejemplos incluyen las sales de sodio, potasio, aluminio, calcio, magnesio, zinc, dietolamina, olamina, etilendiamina, trometamina, cloína, megulamina y dietanolamina. Para una revisión de sales farmacéuticas adecuadas véase Berge y col., J. Pharm. Sci., 66, 1-19, 1977; P.L. Gould, International Journal of Pharmaceutics, 33 (1986), 201-217; y Bighley y col., Encyclopedia of Pharmacuetical Technology, Marcel Dekker Inc., New3 York 1996, volumen 13, página 453-497. Una sal preferida es la sal de sodio.

110

Los solvatos farmacéuticamente aceptables de los compuestos de la invención incluyen sus hidratos.

Aquí en lo sucesivo, los compuestos, sus sales farmacéuticamente aceptables, sus solvatos y polimorfos, definidos en cualquier aspecto de la invención (excepto los compuestos intermedios en procedimientos químicos) se denominan "compuestos de la invención".

Los compuestos de la invención pueden poseer uno o más centros quirales, así que existen en diversas formas estereoisómeras. Todos los estereoisómeros y sus mezclas están incluidos dentro del alcance de la presente invención. Los compuestos racémicos pueden separarse utilizando HPLC preparativa y una columna con una fase estacionaria quiral, o bien pueden resolverse para dar enantiómeros individuales utilizando métodos conocidos por los expertos en la técnica. Además, los compuestos intermedios quirales pueden resolverse y utilizar para preparar compuestos quirales.

En los casos en que los compuestos de la invención existen como isómeros E y Z, la invención incluye isómeros individuales así como sus mezclas.

En los casos en que los compuestos de la invención existen como isómeros tautómeros, la invención incluye tautómeros individuales así como sus mezclas.

En los casos en que los compuestos de la invención existen como isómeros ópticos, la invención incluye isómeros individuales así como sus mezclas.

En los casos en que los compuestos de la invención existen como diastereoisómeros, la invención incluye diastereoisómeros individuales así como sus mezclas.

La separación de diastereoisómeros o isómeros E y Z se puede efectuar por técnicas convencionales, p.ej. por cristalización fraccionada, cromatografía o HPLC (véanse los Ejemplos 29 y 30 de esta memoria). También se puede preparar un enantiómero individual de un compuesto de la invención a partir de un intermedio correspondiente ópticamente puro o por resolución tal como por HPLC del racemato correspondiente utilizando un soporte quiral adecuado, o por cristalización fraccionada de las sales diastereoisómeras formadas por reacción del correspondiente racemato con un ácido o base ópticamente activo adecuado, según sea apropiado. Una base ópticamente activa preferida es la pseudoefedrina (véase la preparación 2 de esta memoria).

Los compuestos de la invención pueden existir en una o más formas tautómeras. Todos los tautómeros y sus mezclas están incluidos en el alcance de la

111

presente invención. Por ejemplo, una reivindicación de 2-hidroxipiridinilo también incluiría su forma tautómera, α-piridonilo.

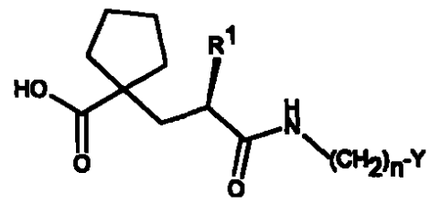
Los expertos en la técnica apreciarán que ciertos derivados protegidos de los compuestos de la invención, que pueden ser preparados antes de una etapa de desprotección final, pueden no poseer actividad farmacológica tal cuales, y sin embargo en ciertas circunstancias pueden administrarse oral o parenteralmente y ser posteriormente metabolizados en el cuerpo para formar compuestos de la invención que son farmacológicamente activos. Tales derivados pueden describirse, por lo tanto, como "profármacos". Además, ciertos compuestos de la invención pueden actuar como profármacos de otros compuestos de la invención.

Todos los derivados protegidos, y profármacos, de los compuestos de la invención están incluidos dentro del alcance de la invención. Los ejemplos de profármacos adecuados para los compuestos de la presente invención están descritos en Drugs of Today, volumen 19, número 9, 1983, págs. 499 - 538 y en Topics in Chemistry, Capítulo 31, págs. 306 - 316 y en "Design of Prodrugs" por H. Bundgaard, Elsevier, 1985, Capítulo 1 (las descripciones en dichos documentos se incorporan aquí por referencia).

Además, los expertos en la técnica apreciarán que ciertos restos, conocidos por los expertos en la técnica como "pro-restos", por ejemplo como describe H. Bundgaard en "Design of Prodrugs" (la descripción de cuyo documento se incorpora aquí por referencia) pueden ser puestos en funcionalidades apropiadas cuando dichas funcionalidades están presentes dentro de los compuestos de la invención.

Los profármacos preferidos para los compuestos de la invención incluyen: ésteres, ésteres de carbonato, hemiésteres, ésteres de fosfato, nitroésteres, ésteres de sulfato, sulfóxidos, amidas, carbamatos, compuestos azoicos, fosfamidas, glicósidos, éteres, acetales y cetales.

Los estudios del metabolismo de los fármacos han demostrado que, *in vivo*, los compuestos de fórmula I pueden formar compuestos de fórmula XXIII, compuestos que también son inhibidores de NEP.



(XXIII)

En particular, los autores de esta invención han demostrado que el ácido (2*R*)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]pentanoico (Ejemplo 29) forma *in vivo* ácido (2R)-1-(2-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}-pentil)-ciclopentanocarboxílico.

La invención también incluye todas las variaciones isotópicas adecuadas de los compuestos de la invención. Una variación isotópica se define como una en la que al menos un átomo ha sido sustituido por un átomo que tiene el mismo número atómico pero una masa atómica diferente de la masa atómica encontrada habitualmente en la naturaleza. Ejemplos de isótopos que se pueden incorporar en los compuestos de la invención incluyen los isótopos de hidrógeno, carbono, nitrógeno, oxígeno, fósforo, azufre, flúor y cloro tales ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F y ^{36}Cl, respectivamente. Ciertas variaciones isotópicas de los compuestos de la invención y sus sales farmacéuticamente aceptables, por ejemplo, aquellas en los que se incorpora un isótopo radiactivo tal como ^{3}H o ^{14}C, son útiles en estudios de distribución de fármacos y/o substratos en tejidos. El tritio, es decir ^{3}H y el carbono-14, es decir ^{14}C, son isótopos particularmente preferidos por su facilidad de preparación y detectabilidad. Además, la sustitución con isótopos tales como deuterio, es decir ^{2}H, puede producir ciertas ventajas terapéuticas como resultado de su mayor estabilidad metabólica, por ejemplo, semiduración *in vivo* incrementada o requisitos de dosificación disminuida y, por tanto, pueden ser preferidos en ciertas circunstancias. Las variaciones isotópicas en los compuestos de la invención y sus sales farmacéuticamente aceptables se pueden preparar generalmente por procedimientos convencionales tales como por los métodos ilustrativos o por las preparaciones descritas en los Ejemplos y Preparaciones aquí más adelante utilizando variaciones isotópicas apropiadas de reactivos adecuados.

Los compuestos de la invención son inhibidores de la endopeptidasa neutra EC.3.4.24.11, dependiente de zinc, y se propone que los compuestos de la invención

113

servirán para tratar las enfermedades enumeradas a continuación. Esta enzima está implicada en la ruptura de varios oligopéptidos bioactivos, al escindir enlaces peptídicos en el lado amino de los restos de aminoácidos hidrófobos. Los péptidos metabolizados incluyen los péptidos natriuréticos auriculares (ANP), bombesina, bradiquinina, péptido relacionado con el gen de la calcitonina, endotelinas, encefalinas, neurotensina, sustancia P y péptido intestinal vasoactivo. Algunos de estos péptidos tienen potentes funciones vasodilatadoras y neurohormonales, actividad diurética y natriurética o son mediadores de efectos de comportamiento. Así, los compuestos de la invención, al inhibir la endopeptidasa neutra EC.3.4.24.11, pueden potenciar los efectos biológicos de los péptidos bioactivos.

De este modo, en particular, los compuestos tienen utilidad en el tratamiento de diversos trastornos, entre ellos hipertensión, insuficiencia cardíaca, angina de pecho, insuficiencia renal, edema cíclico, síndrome de Ménière, hiperaldosteronismo (primario y secundario) e hipercalciuria. Además, debido a su capacidad de potenciar los efectos de ANF, los compuestos tienen utilidad en el tratamiento de glaucoma. Como resultado adicional de su capacidad de inhibir la endopeptidasa neutra EC.3.4.24.11, los compuestos de la invención pueden tener actividad en otros campos terapéuticos, entre ellos, por ejemplo, el tratamiento de los trastornos menstruales, parto prematuro, pre-eclampsia, endometriosis y trastornos de la reproducción (especialmente esterilidad masculina y femenina, síndrome ovárico policístico, e implantación fallida). También, los compuestos de la invención tratarán asma, inflamación, leucemia, dolor, epilepsia, trastornos afectivos, demencia y confusión senil, obesidad y trastornos gastrointestinales (especialmente diarrea y síndrome de intestino irritable), curación de heridas (especialmente úlceras del diabético y venosas y úlceras por decúbito), choque séptico, la modulación de la secreción de ácidos gástricos, el tratamiento de hiperreninemia, fibrosis quística, restenosis, complicaciones de la diabetes y aterosclerosis. En una realización preferida, los compuestos de la invención son útiles en el tratamiento de disfunción sexual femenina. (FSD), preferiblemente FSAD.

Se apreciará que todas las referencias hechas aquí a tratamiento, incluyen tratamiento curativo, paliativo o profiláctico.

Se ha encontrado que los compuestos de la invención inhiben la enzima endopeptidasa neutra. Por lo tanto, de acuerdo con un aspecto adicional, la invención proporciona el uso de un compuesto de la invención, en la preparación de un

114

medicamento para el tratamiento o la profilaxis de un proceso para el que se puede obtener una respuesta terapéutica beneficiosa por inhibición de endopeptidasa neutra.

De acuerdo con la invención, la FSD puede definirse como la dificultad o imposibilidad de que una mujer encuentre satisfacción en la expresión sexual. FSD es un término colectivo para varios trastornos sexuales femeninos diversos (Leiblum, S.R. (1998). Definition and classification of female sexual disorders, *Int. J.Impotence Res.*, 10, S104-S106; Berman, J.R., Berman, L & Goldstein, I. (1999). Female sexual dysfunction: Incidence, pathophysiology, evaluations and treatment options. *Urology*, 54, 358-391). La mujer puede tener falta de deseo, dificultad para experimentar excitación u orgasmo, dolor sexual o una combinación de estos problemas. Varios tipos de enfermedades, medicaciones, lesiones o problemas psicológicos pueden causar FSD. Los tratamientos en desarrollo están dirigidos a tratar subtipos específicos de FSD, principalmente trastornos de deseo y excitación sexual.

La mejor manera de definir las categorías de FSD es contrastarlas con las fases de la respuesta sexual femenina normal: deseo, excitación y orgasmo (Leiblum, S.R. (1998). Definition and classification of female sexual disorders. *Int. J.Impotence Res.*, 10, S104-S106). El deseo o la líbido es el estímulo para la expresión sexual. Sus manifestaciones suelen incluir pensamientos sexuales, ya sea cuando se está en compañía del compañero por el que se siente interés o cuando se expone a otro estímulo erótico. La excitación es la respuesta vascular a la estimulación sexual siendo un componente importante de la misma el aumento de tamaño de los genitales e incluye un aumento de la lubricación vaginal, alargamiento de la vagina y sensación/sensibilidad incrementada de los genitales. El orgasmo es la liberación de la tensión sexual que ha culminado durante la excitación.

Por tanto, ocurre FSD cuando una mujer tiene una respuesta inadecuada o insatisfactoria en cualquiera de estas fases, habitualmente deseo, excitación u orgasmo. Las categorías de FSD incluyen trastorno de deseo sexual hipoactivo, trastorno de excitación sexual, trastornos orgásmicos y trastornos de dolor sexual. Aunque los compuestos de la invención mejorarán la respuesta genital a la estimulación sexual (como en el trastorno de excitación sexual femenina), al hacerlo también puede mejorar el dolor, la tensión y el malestar asociados con el coito y así tratar otros trastornos sexuales femeninos.

Así pues, de acuerdo con un aspecto preferido de la invención, se proporciona el uso de un compuesto de la invención en la preparación de un

115

medicamento para el tratamiento o la profilaxis del trastorno de deseo sexual hipoactivo, trastorno de excitación sexual, trastorno orgásmicos y trastorno del dolor sexual, más preferiblemente para el tratamiento o la profilaxis de trastorno de excitación sexual, trastorno orgásmicos y trastorno del dolor sexual, y lo más preferiblemente en el tratamiento o la profilaxis del trastorno de excitación sexual.

El trastorno del deseo sexual hipoactivo ocurre si una mujer carece de deseo o tiene poco deseo de mantener una relación sexual y tiene pocos pensamientos o fantasías sexuales o ninguno. Este tipo de FSD puede estar causado por bajos niveles de testosterona, debido o bien a la menopausia natural o a la menopausia quirúrgica. Otras causas incluyen enfermedad, medicaciones, fatiga, depresión y ansiedad.

El trastorno de la excitación sexual femenina (FSAD) se caracteriza por una inadecuada respuesta genital a la estimulación sexual. Los genitales no experimentan el aumento de tamaño que caracteriza a la excitación sexual normal. Las paredes de la vagina apenas se lubrican, de manera que el coito es doloroso. Los orgasmos pueden estar impedidos. El trastorno de excitación puede estar causado por una disminución de los estrógenos en la menopausia o después del nacimiento de los hijos y durante la lactancia, así como por enfermedad, con componentes vasculares tales como diabetes y aterosclerosis. Otras causas son el resultado del tratamiento con diuréticos, antihistamínicos, antidepresivos, p.ej., SSRI o agentes antihipertensivos.

Los trastornos de dolor sexual (incluyen dispareunia y vaginismo) se caracterizan por el dolor resultante de la penetración y pueden estar causados por medicaciones que reduzcan la lubricación, endometriosis, enfermedad inflamatoria pélvica, enfermedad inflamatoria intestinal o problemas del tacto urinario.

La prevalencia de FSD es difícil de estimar debido a que este término abarca diversos tipos de problemas, algunos de los cuales son difíciles de cuantificar, y debido a que el interés en el tratamiento de FSD es relativamente reciente. Muchos problemas sexuales de las mujeres están asociados o bien directamente con el proceso del envejecimiento femenino o con las enfermedades crónicas tales como diabetes e hipertensión.

Ya que FSD consta de varios subtipos que expresan síntomas en fases separadas del ciclo de respuesta sexual, no hay una sola terapia. El tratamiento actual de FSD se centra principalmente en aspectos psicológicos o de relación. El tratamiento de FSD está sobresaliendo gradualmente a medida que hay más estudios clínicos y científicos básicos que se dedican a la investigación de este problema médico. Las

116

dolencias sexuales femeninas no tienen todas una patofisiología psicológica, especialmente en las mujeres en las que puede haber un componente de disfunción vasculógena (p.ej., FSAD) que contribuya a la dolencia sexual femenina global. Actualmente no hay ningún fármaco permitido para el tratamiento de FSD. La terapia farmacológica empírica incluye la administración de estrógenos (tópicamente o como terapia hormonal sustitutoria), andrógenos o agentes para cambiar el humor tales como buspirona o trazodona. Estas opciones de tratamiento suelen ser insatisfactorias debido a la escasa eficacia o los efectos secundarios inaceptables.

Como el interés para tratar farmacológicamente la FSD es relativamente reciente, la terapia consiste en lo siguiente: asistencia psicológica, lubricantes sexuales de venta sin receta y fármacos experimentales, incluidos fármacos aprobados para otros procesos. Esta medicaciones consisten en agentes hormonales, ya sea testosterona o combinaciones de estrógenos y testosterona y más recientemente fármacos vasculares, que han resultado eficaces en la disfunción eréctil. Ninguno de estos agentes ha demostrado ser muy eficaz en el tratamiento de FSD.

Como se ha discutido, los compuestos de la invención son particularmente útiles para el tratamiento o el trastorno de excitación sexual femenina (FSAD).

El Manual Diagnóstico y Estadístico (DSM) IV de la Asociación Americana de Psiquiatras define el trastorno de excitación sexual femenina (FSAD) como:

> "una imposibilidad persistente o recurrente de conseguir o mantener hasta el final de la actividad sexual una respuesta adecuada de excitación sexual mediante lubricación-aumento de tamaño. La alteración debe producir una acusada ansiedad o dificultad en las relaciones de pareja".

La respuesta de excitación consiste en la vasocongestión de la pelvis, lubricación vaginal y expansión y aumento de volumen de los genitales externos. La alteración provoca una acusada ansiedad y/o dificultad en las relaciones de pareja.

FSAD es un trastorno sexual de alta prevalencia que afecta a las mujeres pre-, peri- y post-menopáusicas (±HRT). Está asociado con trastornos simultáneos tales como depresión, enfermedades cardiovasculares, diabetes y trastornos UG.

Las consecuencias principales de FSAD son falta de congestión/aumento de tamaño, falta de lubricación y falta de sensación genital placentera. Las consecuencias secundarias de FSAD son disminución del deseo sexual, dolor durante el coito y dificultad para conseguir un orgasmo.

Recientemente, se ha establecido la hipótesis de que hay una base vascular

para, al menos, una proporción de pacientes con síntomas de FSAD (Goldstein *y col.*, Int. J. Impot. Res., 10, S84-S90, 1998), existiendo datos con animales que avalan este punto de vista (Park *y col.*, Int. J. Impot. Res., 9, 27-37, 1997).

Los fármacos experimentales para tratar FSAD, que están ahora siendo investigados para determinar su eficacia, son principalmente terapias de disfunción eréctil que estimulan la circulación de los genitales masculinos. Consisten en dos tipos de formulación, medicaciones orales o sublinguales (apomorfina, fentolamina, inhibidores de fosfodiesterasa tipo 5 (PDE5), p.ej., Sildenafil), y prostaglandina (PGE_1) que se inyectan o administran transuretralmente en hombres, y tópicamente a los genitales en mujeres.

Los compuestos de la invención son ventajosos ya que proporcionan un medio para recuperar una respuesta de excitación sexual normal - a saber un flujo de sangre genital incrementado - que conduce a congestión vaginal, clitoral y labial. Esto dará como resultado una mayor lubricación vaginal a través de la transudación de plasma, una mayor elasticidad vaginal y una mayor sensibilidad genital. Por lo tanto, la presente invención proporciona un medio para recuperar, o potenciar, la respuesta de excitación sexual normal.

Sin estar vinculados a ninguna teoría, los autores de la presente invención creen que los neuropéptidos tales como el péptido intestinal vasoactivo (VIP) son los principales neurotransmisores experimentales en la regulación de la respuesta de excitación sexual femenina, especialmente en la regulación del flujo sanguíneo genital. VIP y otros neuropéptidos son degradados/metabolizados por NEP EC.3.4.24.11. Así, los inhibidores de NEP potenciarán el efecto vasorrelajante endógeno de VIP liberado durante la excitación. Esto conducirá a un tratamiento de FSAD, tal como a través del aumento del flujo sanguíneo genital y, por tanto, de la congestión genital. Los autores de la invención han demostrado que los inhibidores selectivos de NEP EC.3.4.24.11 intensifican incrementos estimulados por los nervios pélvicos e inducidos por VIP en el flujo sanguíneo vaginal y clitoral. Además, los inhibidores selectivos de NEP intensifican VIP y las relajaciones mediadas por los nervios de pared vaginal aislada.

Así, la presente invención es ventajosa ya que ayuda a proporcionar un medio para recuperar una respuesta de excitación sexual normal, a saber flujo sanguíneo genital incrementado que conduzca a congestión vaginal, clitoral y labial. Esto dará como resultado un aumento de la lubricación vaginal vía transudación de plasma, un aumento de la elasticidad vaginal y un aumento de la sensibilidad vaginal. De aquí que

118

la presente invención proporcione un medio para recuperar, o potenciar, la respuesta de excitación sexual normal.

Han sido presentadas doctrinas antecedentes sobre NEP por Victor A. McKusick y col. en http://www3.ncbi.nlm.nih.gov/Omim/searchomin.htm. La siguiente información referente a NEP ha sido extraída de esa fuente.

"El antígeno de la leucemia linfocítica aguda común es un importante marcador de superficie celular en la diagnosis de la leucemia linfocítica aguda humana (ALL). Está presente en células leucémicas de fenotipo pre-B, que constituyen el 85% de casos de ALL. Sin embargo, CALLA no se limita a células leucémicas, y se encuentra en muy diversos tejidos normales. CALLA es una glicoproteína que es particularmente abundante en el riñón, donde está presente en el borde en cepillo de los túbulos proximales y en el epitelio glomerular. Letarte y col. (1988) clonaron un cDNA codificante de CALLA y demostraron que la secuencia de aminoácidos deducida de la secuencia de cDNA es idéntica a la secuencia de endopeptidasa neutra asociada con membranas humanas (NEP; EC 3.4.24.11), también conocida como encefalinasa. NEP escinde péptidos en el lado amino de los restos hidrófobos e inactiva varias hormonas peptídicas incluidas en glucagón, las encefalinas, la sustancia P, la neurotensina, la oxitocina y la bradiquinina. Por análisis de transfección de cDNA, Shipp y col. (1989) confirmaron que CALLA es una endopeptidasa neutra funcional del tipo que se había denominado previamente encefalinasa. Barker y col. (1989) demostraron que el gen de CALLA, que codifica una glicoproteína transmembranal de tipo II, de 100 kD, existe en una copia individual mayor que 45 kb que no se redistribuye en tumores que expresan CALLA de la superficie celular. El gen se localizó en el cromosoma 3 humano por estudios de híbridos de células somáticas y por hibridación in situ regionalizó la posición a 3q21-q27. Tran-Paterson y col. (1989) también asignaron el gen al cromosoma 3 por análisis de hibridación Southern de DNA procedente de híbridos celulares somáticos humanos-murinos. D'Adamio y col. (1989) demostraron que el gen CALLA abarca de 80 kb y está compuesto por 24 exones".

1. Barker, P. E.; Shipp, M. A.; D'Adamio, L.; Masteller, E. L.; Reinherz, E. L. The common acute lymphoblastic leukemia antigen gene maps to chromosomal region 3 (q21-q27). J. Immun. 142: 283-287, 1989.

2. D'Adamio, L.; Shipp, M. A.; Masteller, E. L.; Reinherz, E. L. : Organization of the

gene encoding common acute lymphobastic leukemia antigen (neutral endopeptidase 24.11): multiple miniexons and separate 5-prime untranslated regions. Proc. Nat. Acad. Sci. 86: 7103-7107, 1989.

3. Letarte, M.; Vera, S.; Tran, R.; Addis, J. B. L.; Onizuka, R. J.; Quackenbush, E. J.; Jongeneel, C. V.; McInnes, R. R. : Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. J. Exp. Med. 168: 1247-1253, 1988.

4. Shipp, M. A.; Vijayaraghavan, J.; Schmidt, E. V.; Masteller, E. L.; D'Adamio, L.; Hersh, L. B.; Reinherz, E. L. : Common acute lymphoblastic leukemia antigen (CALLA) is active neutral endopeptidases 24.11 ("enkephalinase"): direct evidence by cDNA transfection analysis. Proc. Nast. Acad. Sci. 86: 297-301, 1989.

5. Tran-Paterson, R.; Willard, H. F.; Letarte, M. : The common acute lymphoblastic leukemia antigen (neutral endopeptidase--3.4.24.11) gene is located on human chromosome 3. Cancer Genet. Cytogenet. 42: 129-134, 1989.

"Los órganos genitales se clasifican en órganos genitales internos y órganos genitales externos. Los órganos internos están situados dentro de la pelvis y comprenden los ovarios, los tubos uterinos, el útero y la vagina. Los órganos externos son superficiales al diafragma urogenital y están situados debajo del arco pélvico. Comprenden el monte púbico, los labios mayores y los labios menores, el clítoris, el vestíbulo, el bulbo del vestíbulo y las glándulas vestibulares mayores" (Gray's Anatomy, C.D. Clemente, 13ª Edición Americana).

Los compuestos de la invención encuentran aplicación en las siguientes subpoblaciones de pacientes con FSD: las mujeres jóvenes, ancianas, pre-menopáusicas, peri-menopáusicas, post-menopáusicas con o sin terapia hormonal sustitutoria.

Los compuestos de la invención encuentran aplicación en pacientes con FSD a consecuencia de:

i) Etiologías vasculógenas, p.ej. enfermedades cardiovasculares o ateroscleróticas, hipercolesterolemia, hábito de fumar, diabetes, hipertensión, radiación y traumatismo perineal, lesión por traumatismo en el sistema vascular pudendo iliohipogástrico.

ii) Etiologías neurógenas tales como lesiones en la médula espinal o enfermeda-

120

des del sistema nervioso central, entre ellas la esclerosis múltiple, diabetes, enfermedad de Parkinson, accidentes cerebrovasculares, neuropatías periféricas, cirugía pélvica radical o traumática.

iii) Etiologías hormonales/endocrinas tales como disfunción del eje hipotalámico/pituitario/gonadal, o disfunción de los ovarios, disfunción del páncreas, castración quirúrgica o médica, deficiencia de andrógenos, altos niveles circulantes de prolactina, p.e., hiperprolactinemia, menopausia natural, insuficiencia ovárica prematura, hiper- e hipo-tiroidismo.

iv) Etiologías psicógenas tales como depresión, trastorno obsesivo compulsivo, trastorno de ansiedad, depresión postnatal/depresión puerperal, problemas emocionales y familiares, angustia de comportamiento, diferencias matrimoniales, actitudes disfuncionales, fobias sexuales, inhibición religiosa o experiencias vividas traumatizantes .

v) Disfunción sexual inducida por fármacos a consecuencia de una terapia con inhibidores de la reabsorción selectiva de serotonina (SSRi) y otras terapias antidepresivas (tricíclicos y neurolépticos), terapias anti-hipertensivas, fármacos simpatolíticos, terapia crónica con píldoras anticonceptivas orales.

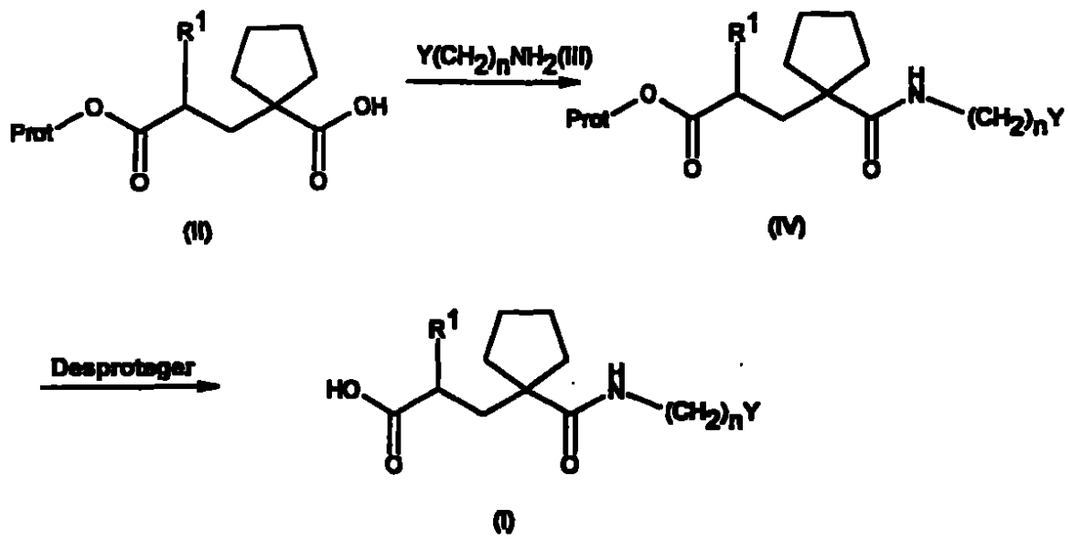
Los compuestos de la invención se pueden preparar de manera conocida y de varios modos. En los siguientes esquemas de reacción y aquí en lo sucesivo, salvo indicación en contrario R^1, n e Y son como se han definido para el primer aspecto. Estos procedimientos constituyen aspectos adicionales de la invención.

En toda la memoria descriptiva, las fórmulas generales están designadas por números romanos I, II, III, IV, etc. Los subgrupos de estas fórmulas generales se definen como Ia, Ib, Ic, etc.,...IVa, IVb, IVc, etc.

Los compuestos de fórmula general I se pueden preparar de acuerdo con el Esquema de reacción 1, haciendo reaccionar un compuesto de fórmula II (en la que Prot es un grupo protector adecuado) con una amina primaria de fórmula III para dar un compuesto de fórmula IV. La desprotección da compuestos de fórmula I.

121

Esquema 1



La etapa de acoplamiento ácido/amina se puede llevar a cabo haciendo reaccionar compuestos de fórmula II con compuestos de fórmula III (o su sal de amina) en presencia de un agente de acoplamiento, opcionalmente un catalizador, y un exceso de un aceptor de ácido, en un disolvente adecuado. Típicamente, el tratamiento de una mezcla de compuestos de fórmula II y compuestos de fórmula III con un agente de acoplamiento (por ejemplo diciclohexilcarbodiimida (DCC), 1-(3-dimetilaminopropil)-3-etilcarbodiimida (WSCDI), fosfato de benzotriazol-1-ildietilo, oxicloruro de fósforo, tetracloruro de titanio, clorofluoruro de sulfurilo, reactivo de Lawesson, PPACA, PYBOP o reactivo de Mukaiyama) opcionalmente en presencia de una amina terciaria como base (por ejemplo trietilamina, base de Hunig, piridina o NMM) durante un período de hasta 24 horas a temperaturas entre -78 y 100°C. Condiciones preferidas de reacción comprenden hacer reaccionar compuestos de fórmula II (1-1,5 equivalentes) con compuestos de fórmula III (o sus sales, 1-1,5 equivalentes), en presencia de hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (WSCDI) o N,N'-diciclohexilcarbodiimida (DCC) (1,1-1,3 equivalentes), 1-hidroxibenzotrazol hidratado (HOBT) o dimetilaminopiridina (DMAP= (1,05-1,2 equivalentes), N-metilmorfolina)(NMM) o trietilamina (2,3-3 equivalentes) en dimetilformamida o diclorometano a una temperatura entre la del ambiente y 90°C durante 16 a 18 horas.

Alternativamente, la etapa de acoplamiento ácido/amina puede transcurrir vía un intermedio activado (tal como una acil-imidazolida, un anhídrido mixto o un cloruro de ácido) en presencia de un exceso de aceptor de ácido en un disolvente adecuado. Las condiciones típicas de reacción comprenden el tratamiento de compuestos de fórmula II con un agente activante (por ejemplo N,N'-carbonildiimidazol, triflato de N,N'-carbonilbis(3-metilimidazolio), cloruro de tionilo o cloruro de oxalilo) opcionalmente en presencia de una amina terciaria como base (por ejemplo, trietilamina, base de Hunig, piridina o NMM) durante un período de hasta 24 horas, seguida por reacción con compuestos de fórmula III (o su sal), opcionalmente en presencia de un catalizador (por ejemplo, 4-dimetilaminopiridina) o un aditivo (por ejemplo, hidroxibenzotriazol) en un disolvente adecuado (por ejemplo diclorometano, THF, acetato de etilo, acetonitrilo, DMF o tolueno) opcionalmente en presencia de una amina adicional como base a temperaturas entre -78°C y 150°C durante un período de hasta 48 horas.

Las condiciones preferidas de reacción comprenden hacer reaccionar el cloruro de ácido de compuestos de fórmula II (1-1,1 equivalentes) con compuestos de fórmula III (o sus sales, 1 a 1,5 equivalentes) en presencia de trietilamina o N-metilmorfolina (1,4-10 equivalentes) en diclorometano como disolvente, a temperatura ambiente durante 24 horas. Alternativamente, los compuestos de fórmula II se pueden convertir en el cloruro de ácido *in situ* por tratamiento con cloruro de oxalilo en diclorometano, en presencia de una cantidad catalítica de dimetilformamida durante 2 horas a temperatura ambiente, o por tratamiento de compuestos de fórmula II con cloruro de tionilo en una mezcla de diclorometano y piridina a -10°C durante 3 horas, seguido por adición de trietilamina, 4-dimetilaminopiridina y el compuesto de fórmula III y dejando reaccionar la mezcla durante 48 horas a 20°C.

Los compuestos de fórmula I se pueden preparar a partir de compuestos de fórmula IV por desprotección. Los métodos para la desprotección de un grupo ácido dependen del grupo protector. Para ilustrarse con ejemplos de la metodología de protección/desprotección puede verse "Protective groups in Organic synthesis", TW Greene y PGM Wutz.

Por ejemplo, cuando Prot es un grupo terc-butilo, las condiciones de desprotección comprenden hacer reaccionar IV con ácido trifluoroacético/diclorometano (1:1-1,5 en volumen), a temperatura ambiente durante 2-18 horas, opcionalmente en presencia de un captador de carbocationes, p.ej. anisol (10 equivalentes). Cuando Y contiene un grupo hidroxi, puede ser necesaria la hidrólisis

básica del éster de ácido trifluoroacético intermedio. La metodología alternativa para la desprotección cuando Prot es terc-butilo comprende tratar IV con ácido clorhídrico en diclorometano a temperatura ambiente durante 3 horas. Para evitar dudas, Prot cuando se dice que es terc-butilo se da a título de Ejemplo y no pretende limitarse a terc-butilo.

Alternativamente, cuando Prot es terc-butilo, la desprotección se puede efectuar por tratamiento de compuestos de fórmula IV con un ácido fuerte (por ejemplo, cloruro de hidrógeno gaseoso o ácido clorhídrico concentrado, ácido bromhídrico, ácido fosfórico, ácido nítrico o ácido sulgfúrico, ácido trifluoroacético, ácido cloroacético, ácido para-toluenosulfónico, ácido trifluorometanosulfónico o ácido acético glácial) en cantidades comprendeidas entre una cantidad catalítica y un exceso, opcionalmente en un disolvente adecuado (por ejemplo tolueno, diclorometano, éter dietílico, etanol, THF o hexano) y opcionalmente en presencia de agua a temperaturas entre 20°C y 150°C durante un período de hasta 48 horas.

Las condiciones preferidas de desprotección cuando Prot es terc-butilo son el tratamiento de compuestos de fórmula IV con un exceso 10:1 de ácido trifluoroacético en diclorometano a temperatura ambiente durante 24 horas.

Cuando Prot es bencilo, las condiciones de desprotección comprenden hacer reaccionar IV con paladio sobre carbón vegetal (5-10%) en etanol acuoso (40-95%) a 1,05-4,20 kg/cm^2 a temperatura ambiente durante 2 h a 3 días.

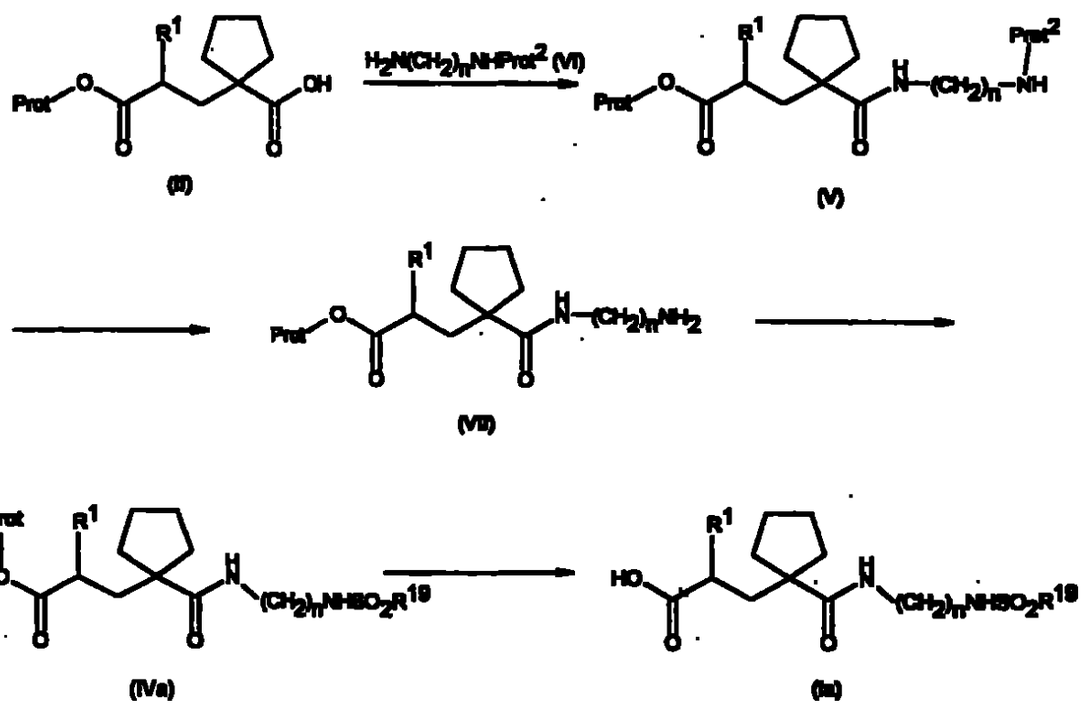
Estos procedimientos constituyen aspectos adicionales de la invención.

Los compuestos de fórmula IV son nuevos y constituyen un aspecto adicional de la invención.

Los compuestos de fórmula Ia, es decir los compuestos de fórmula general I donde Y es -NHSO$_2$R^{19}, se pueden preparar de acuerdo con el Esquema de reacción 2. Los compuestos de fórmula V se preparan primero haciendo reaccionar compuestos de fórmula II con compuestos de fórmula VI donde Prot^2 es un grupo protector de amina apropiado. Las condiciones de reacción preferidas son análogas a las descritas en la etapa de acoplamiento de ácido/amina para el Esquema 1 anterior. La desprotección selectiva de amina de los compuestos de fórmula VI da compuestos de fórmula VII. Los compuestos de fórmula VII se hacen reaccionar con R^{19}SO$_2$Cl en presencia de un aceptor de ácido en un disolvente apropiado para formar compuestos de fórmula VIII. La desprotección de los compuestos de fórmula VIII en condiciones análogas a las descritas para la etapa de desprotección del Esquema 1 da compuestos

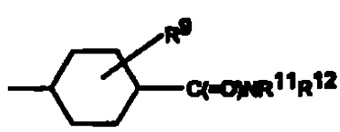
de fórmula Ia.

Esquema 2



Los métodos para la desprotección de un grupo amino dependen del grupo protector. Para ejemplos de la metodología de protección/desprotección véase "Protective groups in Organic Synthesis", TW Greene y PGM Wutz. Por ejemplo, cuando $Prot^2$ es benzoiloxicarbonilo, las condiciones de desprotección comprenden hacer reaccionar V con paladio sobre carbón vegetal (10%) en etanol a temperatura ambiente durante 18 horas.

Los métodos preferidos para la preparación de los compuestos de fórmula VIII comprenden la reacción de VII con $R^{19}SO_2Cl$ (1 equivalente) en presencia de trietilamina (1,5-2,5 equivalentes) en diclorometano a temperatura ambiente durante 2 a 3 días.

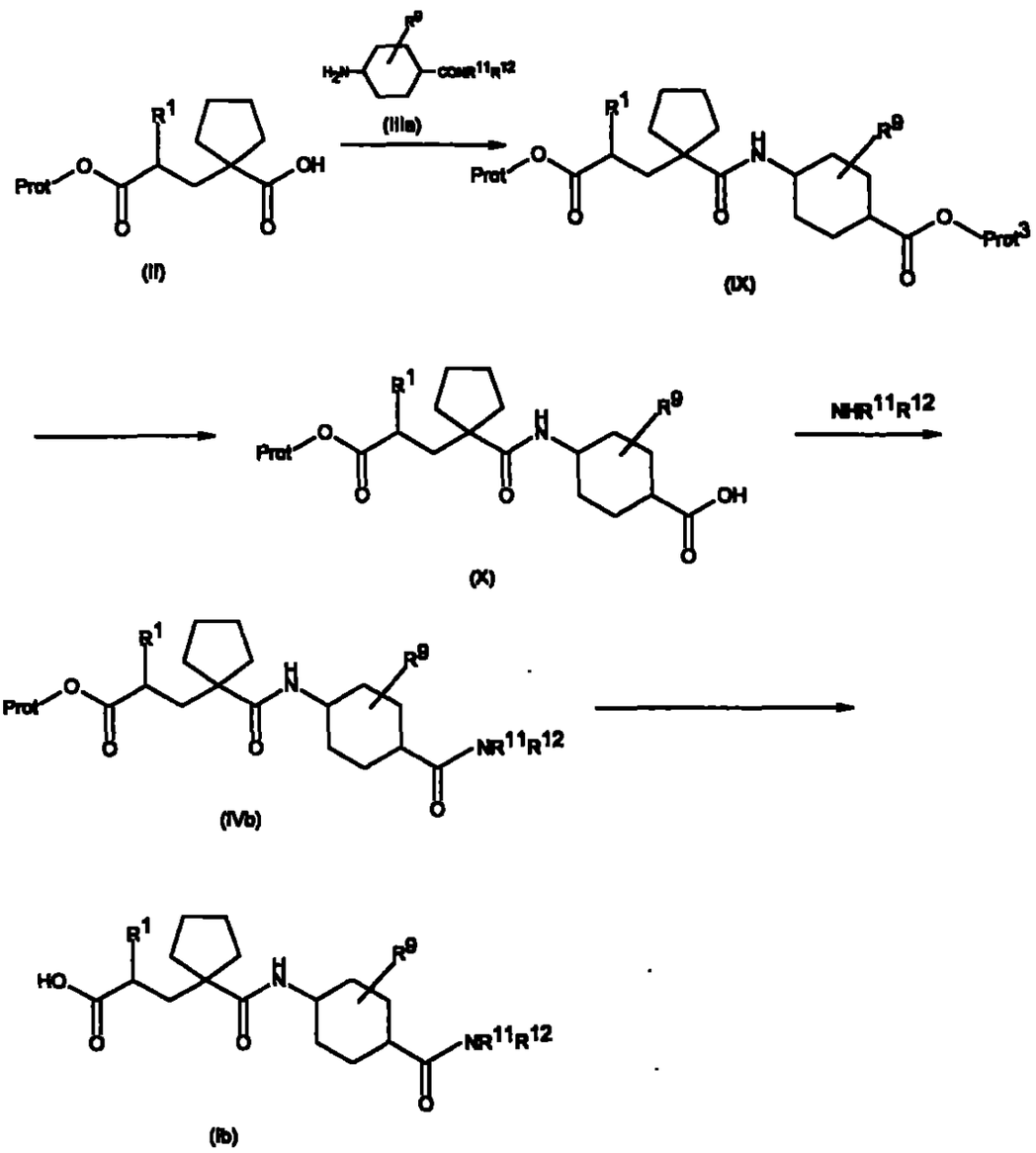
Los compuestos de fórmula Ib, es decir compuestos de fórmula I en la que n es 0 e Y es  , se pueden preparar de acuerdo con el

Esquema de reacción 3. Los compuestos de fórmula II se hacen reaccionar con compuestos de fórmula IIIa en condiciones análogas a las condiciones de acoplamiento de ácido/amina del Esquema 1 para dar compuestos de fórmula IX, donde $Prot^3$ es un grupo protector que puede ser separado selectivamente en presencia del grupo protector Prot. Un grupo protector $Prot^3$ preferido es un grupo éster lábil básico. Consecuentemente, el tratamiento del compuesto de fórmula IX en condiciones básicas da compuestos de fórmula X. Los compuestos de fórmula X se hacen reaccionar con compuestos de fórmula $NHR^{11}R^{12}$ en condiciones análogas a las condiciones de acoplamiento de ácido/amina del Esquema 1 para formar compuestos de fórmula XI. La desprotección de los compuestos de fórmula XI en condiciones análogas a la etapa de desprotección en el Esquema 1 de los compuestos de fórmula Ib.

Las condiciones preferidas para separar el grupo protector $Prot^3$ de IVb comprenden el tratamiento de IVb con hidróxido de sodio (1 N) en metanol a temperatura ambiente durante 22 horas.

126

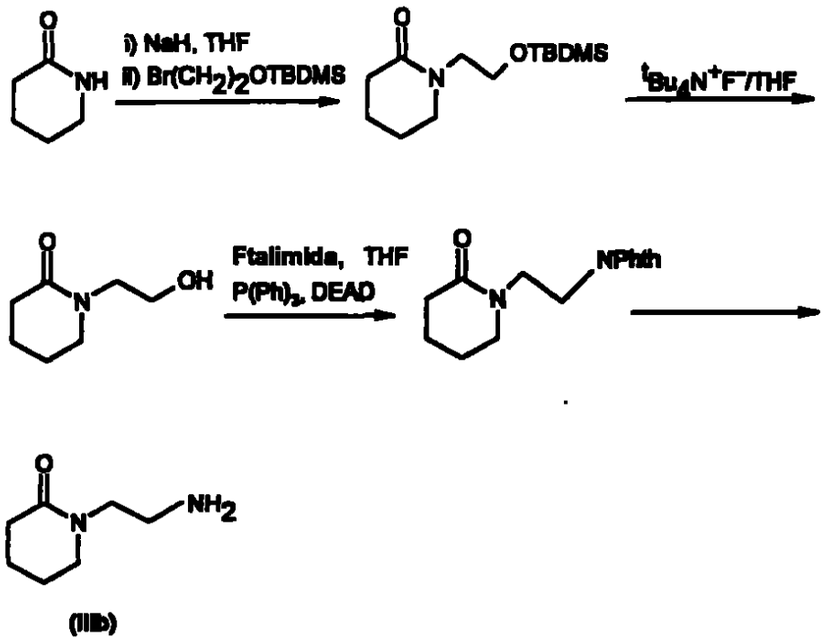
Esquema 3



Los compuestos de fórmula IIIb, es decir los compuestos de fórmula general III en la que n es 2, Y es 2-oxopiperidinio, se pueden preparar de acuerdo con el Esquema de reacción 4.

127 ~~22~~

Esquema 4

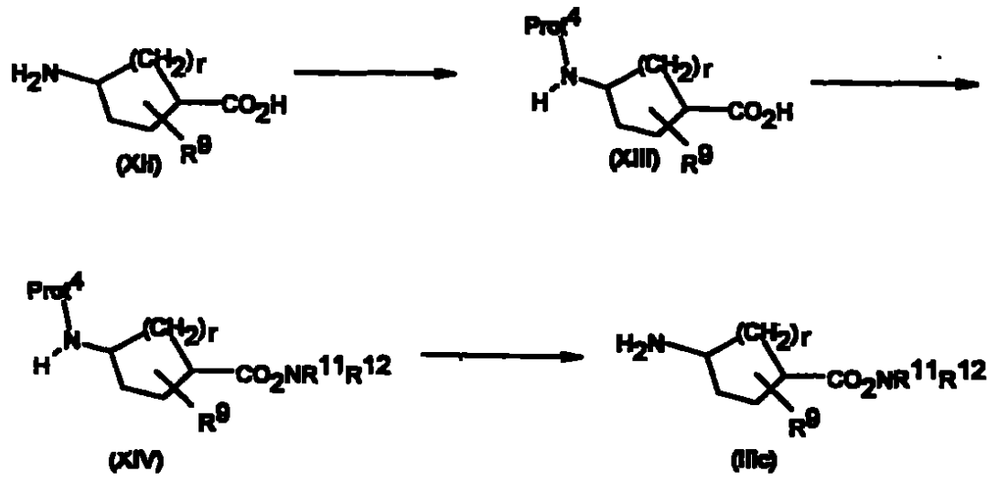


(IIIb)

Los compuestos de fórmula IIIc en la que *r* es 1 ó 2, se pueden preparar de acuerdo con el Esquema de reacción 5. Los compuestos de fórmula XII se protegen en el resto amina con un grupo protector apropiado $Prot^4$ para formar los compuestos de fórmula XIII. Un grupo protector preferido es terc-butiloxicarbonilo. Los compuestos de fórmula XIII se hacen reaccionar en condiciones típicas de acoplamiento de ácido/amina con $NHR^{11}R^{12}$ para formar compuestos de fórmula XIV, cuya desprotección forma compuestos de fórmula IIIc.

128

Esquema 5



Las condiciones de reacción típicas para introducir el grupo protector terc-butiloxicarbonilo comprenden tratar compuestos de fórmula XII con (terc-butiloxicarbonil)$_2$O en dioxano e hidróxido de sodio 2 N a temperatura ambiente durante 18 horas.

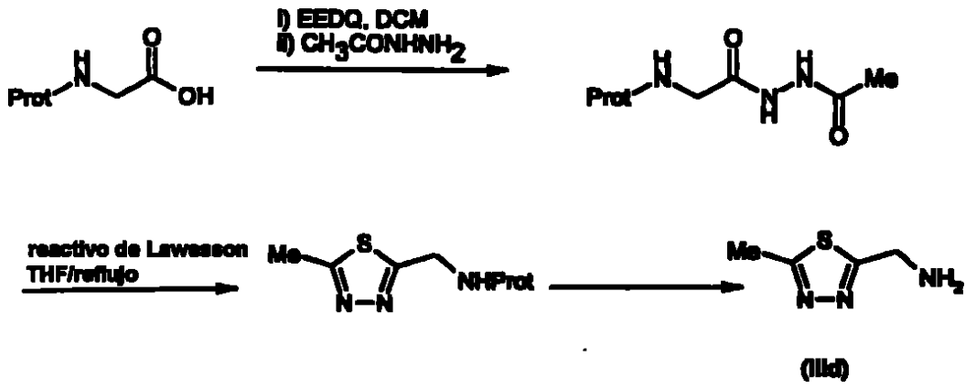
Las condiciones de acoplamiento típicas ácido/amina comprenden tratar XIII y $NHR^{11}R^{12}$ con hexafluorofosfato de benzotriazol-1-iloxitris(pirrolidino)fos-fonio (PYBOP), 1-hidroxibenzotrazol hidratado (HOBT), base de Hünigs, una amina (p.ej. trietilamina), en dimetilformamida a temperatura ambiente durante 2 horas. Alternativamente, XIII y $NHR^{11}R^{12}$ se pueden tratar con hidrocloruro de 1-(3-dimetil-aminopropil)-3-etilcarbodiimida, HOBT, N-metilmorfolina (NMM), en dimetilformamida a temperatura ambiente durante 18 horas.

Las condiciones típicas de reacción para la desprotección cuando $Prot^4$ es terc-butiloxicarbonilo comprenden hacer reaccionar XIV con ácido clorhídrico o ácido trifluoroacético en diclorometano a temperatura ambiente durante 2 a 4 horas.

Los compuestos de fórmula IIId se pueden preparar de acuerdo con el Esquema de reacción 6. El grupo protector es preferiblemente terc-butiloxicarbonilo, que se separa en condiciones clásicas, como se ha descrito previamente.

129

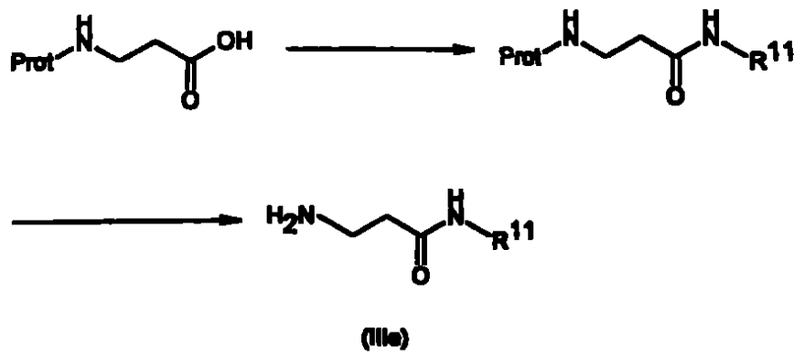
Esquema 6



(IIId)

Los compuestos de fórmula IIIe se preparan según el Esquema de reacción 7 utilizando reacciones clásicas de acoplamiento de ácido/amina, como se ha descrito previamente. El grupo protector es preferiblemente benciloxicarbonilo, que se puede separar en condiciones clásicas, típicamente utilizando paladio sobre carbón vegetal (5-10%) en etanol a temperatura ambiente y 3,5 kg/cm^2 de presión durante 4 horas.

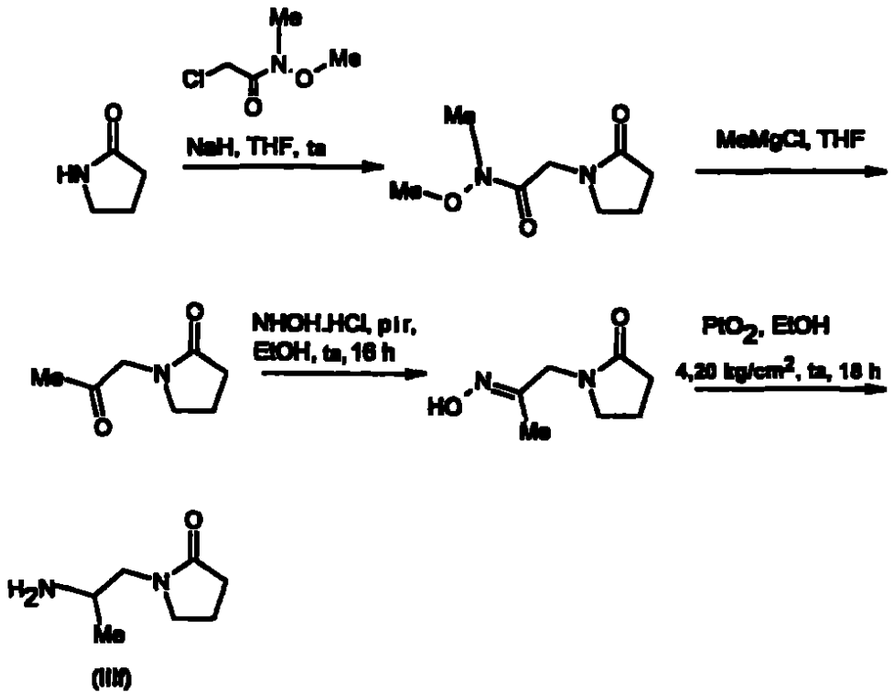
Esquema 7



(IIIe)

Los compuestos de fórmula IIIf se pueden preparar de acuerdo con el Esquema de reacción 8.

Esquema 8

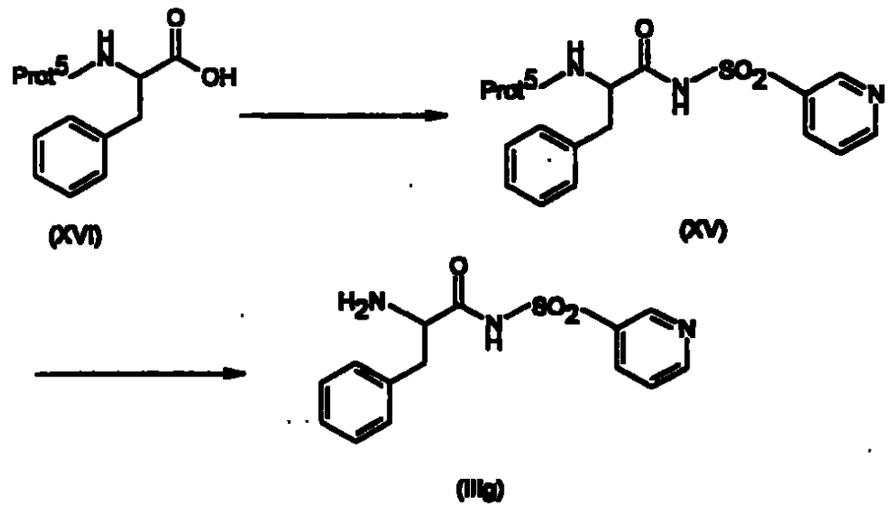


(IIIf)

Los compuestos de fórmula IIIg se pueden preparar en dos etapas de acuerdo con el Esquema de reacción 9. Como una primera etapa, los compuestos de fórmula XV se preparan a partir de compuestos de fórmula XVI utilizando metodología clásica de acoplamiento de ácido/amina, análoga a las condiciones de acoplamiento de ácido/amina descritas para el Esquema de reacción 1. $Prot^5$ representa un grupo fácilmente eliminable apropiado, preferiblemente terc-butiloxicarbonilo. La segunda etapa comprende separar $Prot^5$. Cuando $Prot^5$ es terc-butiloxicarbonilo, entonces las condiciones de reacción preferidas comprenden el tratamiento con ácido clorhídrico en dietil-éter/acetato de etilo a temperatura ambiente durante 18 horas.

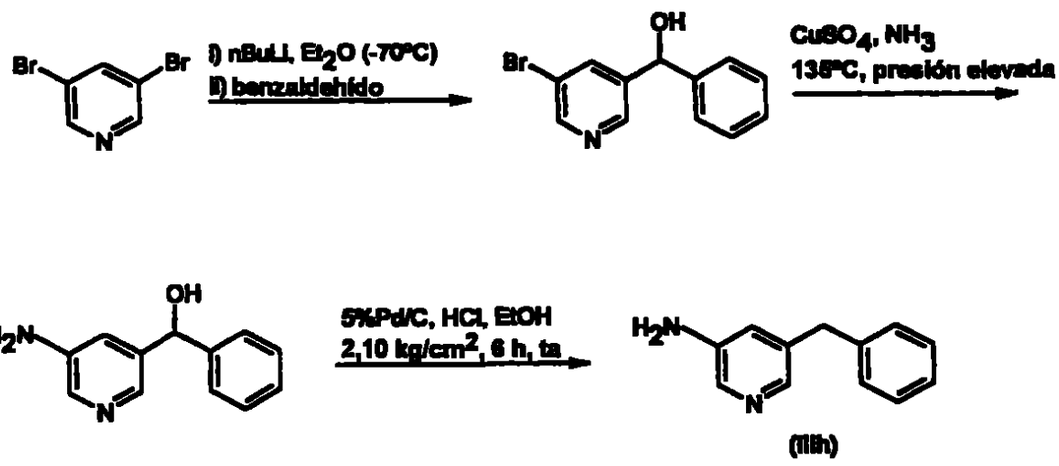
131

Esquema 9



Los compuestos de fórmula IIIh se pueden preparar en tres etapas de acuerdo con el Esquema de reacción 10.

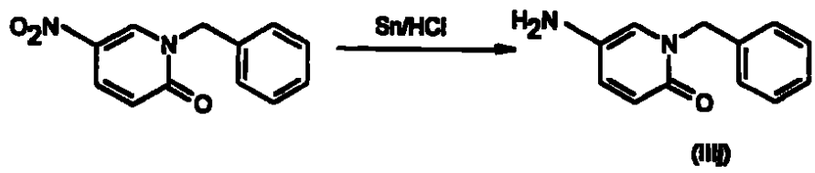
Esquema 10



Los compuestos de fórmula IIIj se pueden preparar por reducción de un grupo nitro de acuerdo con el Esquema de reacción 11.

132

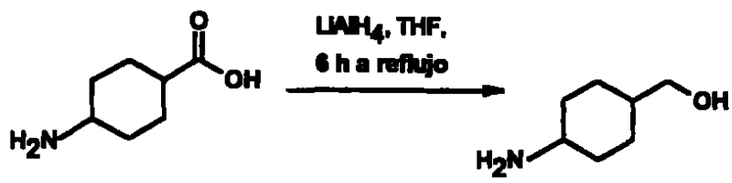
Esquema 11

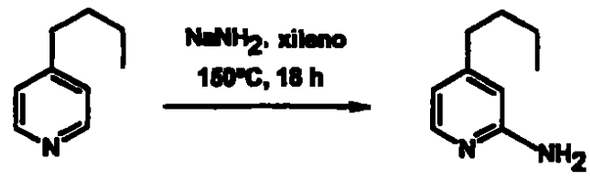


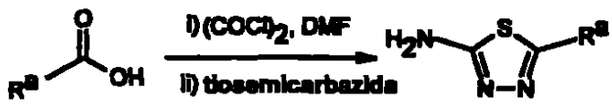
(III)

En el Esquema 12 siguiente se dan métodos adicionales para preparar compuestos de fórmula III, en la que R^a es alquilo C_{1-6} o alcoxi.

Esquema 12





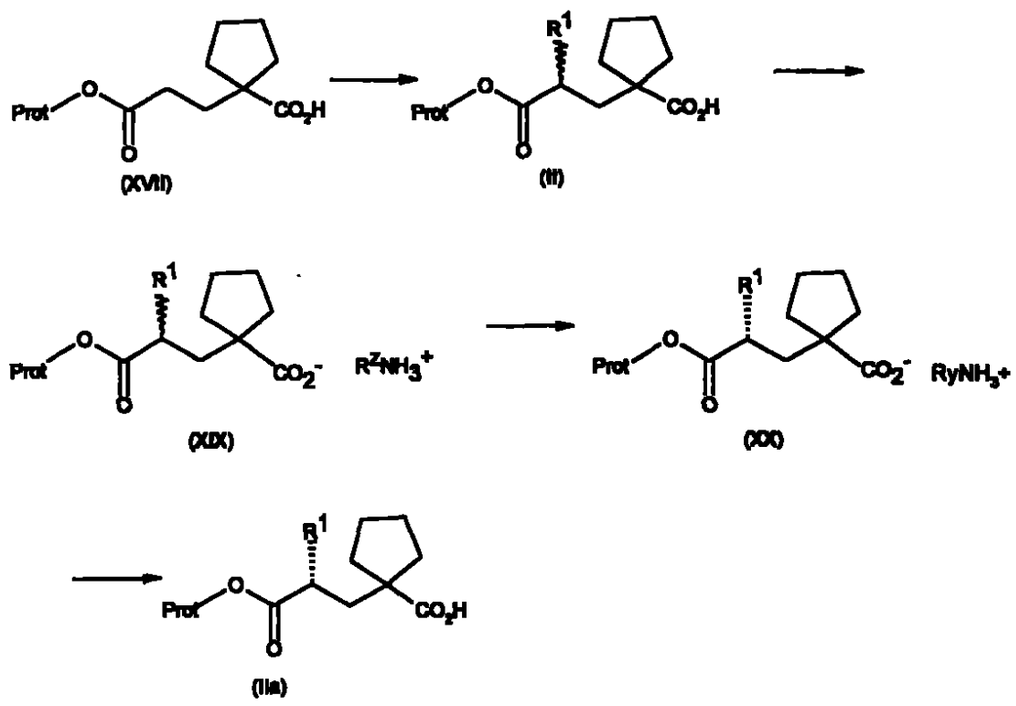


Los compuestos de fórmula general I poseen un centro quiral en el carbono unido a R1. Se pueden obtener enantiómeros individuales de fórmula general I por diversos métodos conocidos por químicos expertos, tales como a partir de un intermedio correspondiente ópticamente puro o vía resolución. Un método preferido de resolución es vía la sal de (+)-pseudoefedrina (véase la preparación 2 de esta menoria). Alternativamente, se pueden preparar compuestos quirales de fórmula I a

partir de compuestos quirales de fórmula II como se discute más adelante.

Ciertos compuestos de fórmula II son conocidos en la técnica (véanse los documentos EP274234-B1 y WO9113054). Otros compuestos de fórmula II se pueden preparar de manera análoga. Los compuestos quirales de fórmula IIa se pueden preparar a partir de compuestos de fórmula XVI como se indica en el Esquema de reacción 13.

Esquema 13



Los compuestos de fórmula II se pueden preparar por tratamiento de compuestos de fórmula XVII con R^1-X^z (donde X^z es halógeno) en condiciones fuertemente básicas, opcionalmente con un aditivo en un disolvente aprótico.

Las condiciones típicas de reacción comprenden, en primer lugar, tratar compuestos de fórmula XVII con al menos un exceso 2:1 de una base fuerte (por ejemplo, diisopropilamiduro de litio, hexametildisilazida de litio, sodio o potasio, una base de alquil-litio, alquil-magnesio o fosfazeno) en un disolvente no prótico (por ejemplo THF, éter dietílico, hexano, heptano o etilbenceno o una mezcla de estos disolventes), opcionalmente con un aditivo (por ejemplo TMEDA, DMPU o HMPA) a

134

temperaturas entre -78°C y temperatura ambiente, y después añadir R^1-R^z (por ejemplo bromuro de alilo, bromuro de propilo o yoduro de metilo) a -78°C y agitar durante una noche mientras se calienta a temperatura ambiente. Un tratamiento adecuado da los compuestos de fórmula II.

Las condiciones preferidas de reacción para preparar compuestos de fórmula II en la que R^1 es alilo, son el tratamiento de compuestos de fórmula XVII (1 equivalente molar) con diisopropilamiduro de litio (2,3 equivalentes) en una mezcla de THF, n-heptano y etilbenceno a -10°C durante 4 horas. Después se añade bromuro de alilo (1,2 equivalentes) a -10°C, se agita la mezcla de reacción a -10°C durante 2 horas y después se calienta a 20°C durante 4 horas y se agita durante 15 horas más a 20°C.

La resolución para dar compuestos de fórmula IIb se puede realizar directamente a partir de compuestos de fórmula II, sin embargo un procedimiento preferido forma una sal de amina de fórmula XIX (es decir R^z-NH_3^+) seguido por recristalización para efectuar la purificación.

Típicamente las sales son las sales de trietilamina, isopropilamina, trietanolamina o ciclohexilamina. Las condiciones típicas de reacción comprenden hacer reaccionar II con la amina requerida en un disolvente adecuado (por ejemplo hexano, heptano o tolueno) a temperaturas elevadas, con enfriamiento para inducir la cristalización a lo largo de 24 horas, seguida por recristalización en el mismo disolvente o en uno diferente, opcionalmente a temperaturas elevadas.

Una sal preferida es la sal de ciclohexilamina. Las condiciones preferidas de reacción comprenden el tratamiento de compuestos de fórmula II (1 equivalente molar) con ciclohexilamina (1 equivalente) a 20°C en heptano, seguido por recristalización en acetato de etilo a 70°C antes de enfriar a 50°C durante 2 horas para inducir cristalización. Después, la mezcla se enfría a 20°C durante 2 horas y se agita durante 0,5 horas.

Los compuestos de fórmula XX (donde $R^yNH_3^+$ es un catión quiral) se pueden preparar a partir de compuestos de fórmula XIX por acidulación en un sistema disolvente adecuado, que opcionalmente contiene agua, utilizando un ácido fuerte, seguido por resolución clásica del ácido carboxílico resultante.

Las condiciones típicas de reacción comprenden el tratamiento de compuestos de fórmula XX en un sistema bifásico de agua y un disolvente orgánico inmiscible (por ejemplo heptano, tolueno, acetato de etilo, éter dietílico o diclorometano) con un

135

ácido fuerte (por ejemplo ácido clohrídrico, ácido sulfúrico, ácido para-toluenosulfónico, ácido trifluoroacético o ácido fosfórico) a temperaturas entre 0°C y 100°C para dar el ácido carboxílico libre, seguido por tratamiento con una amina quiral no racémica como base (por ejemplo α-metilbencilamina, pesudoefedrina, efedrina, norefedrina, un alcaloide de cincona, ésteres de aminoácidos, aminoalcoholes tales como 2-pirrolidinametanol o quinuclidin-3-ol) opcionalmente en un disolvente (por ejemplo un éster, un alcano, un hidrocarburo aromático, un haloalcano, un éter o un alcohol) a temperaturas entre 0°C y 150°C para dar la sal bruta. Después, esta sal se recristaliza una o más veces en el mismo disolvente o en uno diferente a temperaturas entre 0°C y 150°C, para dar la sal quiral.

Una sal preferida es la sal de pseudoefedrina. Las condiciones preferidas de reacción comprenden el tratamiento de una suspensión de un compuesto de fórmula XX en una mezcla de agua/n-heptano con ácido clorhídrico diluido a temperatura ambiente hasta que el pH de la fase acuosa es pH 3, para dar el ácido libre, seguido por tratamiento del ácido carboxílico resultante con (1S,2S)-(+)-pseudoefedrina (1 equivalente) en n-heptano a 80°C, seguido por enfriamiento a 45°C durante 2 horas para inducir cristalización, enfriando después a 20°C durante 2 horas y agitando durante 4 horas. Luego se llevó a cabo una recristalización en n-heptano a 80°C, seguida por enfriamiento a 60°C durante 2 horas para inducir cristalización, enfriando después a 20°C durante 2 horas y agitando durante 1,5 horas.

Los compuestos de fórmula IIa se pueden preparar a partir de compuestos de fórmula XX por acidulación en un sistema disolvente adecuado, opcionalmente que contiene agua, utilizando un ácido fuerte.

Las condiciones típicas de reacción comprenden el tratamiento de compuestos de fórmula XX en un sistema bifásico de agua y un disolvente orgánico inmiscible (por ejemplo heptano, tolueno, acetato de etilo, éter dietílico o diclorometano) y un ácido fuerte (por ejemplo ácido clorhídrico, ácido sulfúrico, ácido para-toluenosulfónico, ácido trifluoroacético o ácido fosfórico) a temperaturas entre 0°C y 100°C para dar compuestos de fórmula IIa.

Las condiciones preferidas de reacción comprenden el tratamiento de una suspensión de un compuesto de fórmula XX en una mezcla de agua/n-heptano con ácido clorhídrico diluido a temperatura ambiente hasta que el pH de la fase acuosa es pH 3 para dar el ácido libre.

Los compuestos de fórmula IIa se pueden preparar por hidrogenación del

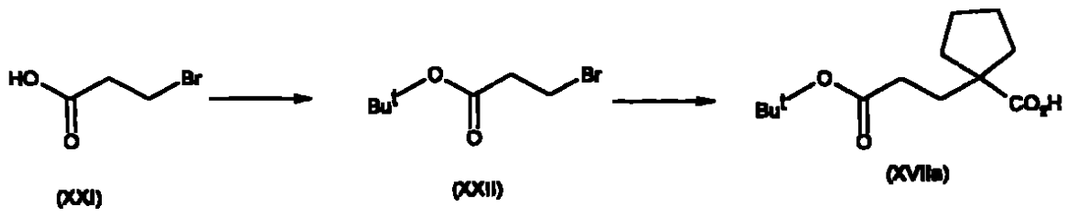
136

correspondiente compuesto en el que R^1 es insaturado. Las condiciones típicas de reacción comprenden agitar en atmósfera de hidrógeno en un disolvente adecuado en presencia de un catalizador (por ejemplo paladio, platino, níquel, iridio, rodio o rutenio opcionalmente adsorbido sobre un soporte adecuado tal como carbono, alúmina, sulfato de bario, carbonato de calcio, o tal como una sal tal como hidróxido de paladio o mezclas de sales tales como H_2PtCl_6 y $SnCl_2.2H_2O$ o tal como un complejo, como el catalizador de Wilkinson, el catalizador de Crabtree, $Co_2(CO)_8$, $RhH(PPh_3)_4$, o $[Co(CN)_5]^{3-}$) a temperaturas entre la temperatura ambiente y 150°C y presiones de hidrógeno entre 2,1 y 10,5 kg/cm^2.

En un procedimiento preferido, los compuestos de fórmula IIa en la cual R^1 es propilo, se pueden preparar por hidrogenación del correspondiente compuesto alílico. Las condiciones preferidas de reacción comprenden agitar una solución etanólica del ácido carboxílico insaturado en atmósfera de hidrógeno con 9% peso/peso de paladio al 5% sobre carbono a temperatura ambiente durante 24 horas.

Los compuestos de fórmula XVIIa, es decir, los compuestos de fórmula XVII en la cual Prot es terc-butilo, se pueden preparar en dos etapas a partir de compuestos de fórmula XXI disponibles en el mercado, de acuerdo con el Esquema de reacción 14.

Esquema 14



(XXI) (XXII) (XVIIa)

Los compuestos de fórmula XXII se pueden preparar a partir de compuestos de fórmula XXI por tratamiento con una fuente de catión ter-butilo o de ter-butóxido, utilizando un catalizador adecuado y/o un agente deshidratante en un disolvente anhidro adecuado, opcionalmente a temperatura elevada, o por activación del ácido carboxílico, seguido por reacción terc-butanol.

Las condiciones típicas de reacción comprenden tratar compuestos de fórmula XXI con cantidades catalíticas de un ácido (por ejemplo ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido nítrico, ácido para-toluenosulfónico o ácido

trifluoroacético) en presencia de isobutileno, terc-butanol, haluros de terc-butilo o éter terc-butílico en un disolvente adecuado (por ejemplo, diclorometano, THF o tolueno) entre -20 y 150°C durante hasta 48 horas.

Condiciones alternativas de reacción comprenden tratar compuestos de fórmula XXI con una combinación de una amina terciaria como base (por ejemplo trietilamina, base de Hunig, piridina o NMM) y un agente deshidratante (por ejemplo diciclohexilcarbodiimida, un cloroformiato de alquilo, diclorofosfato de fenilo, 2-cloro-1,3,5-trinitrobenceno, di-carbonato de 2-piridilo, 1,1'-carbonildiimidazol, (trimetilsilil)-etoxiacetileno, triflato de N,N'-carbonilbis(3-metilimidazolilo) o azodicarboxilato de dietilo) y trifenilfosfina, seguido por adición de terc-butanol, opcionalmente con un catalizador tal como 4-dimetilaminopiridina en un disolvente adecuado (por ejemplo diclorometano, THF o tolueno) entre -20 y 150°C durante hasta 48 horas.

Todavía otras condiciones de reacción comprenden convertir compuestos de fórmula XXI en el cloruro de ácido utilizando cloruro de tionilo, cloruro de oxalilo o reactivo de Ghosez, opcionalmente en presencia de una amina terciaria como base (por ejemplo, trietilamina, base de Hunig, piridina o NMM), seguido por tratamiento con terc-butanol, opcionalmente en presencia de un catalizador tal como 4-dimetilaminopiridina en un disolvente adecuado (por ejemplo diclorometano, THF o tolueno) entre -20 y 150°C durante hasta 48 horas.

Las condiciones preferidas de reacción comprenden tratar compuestos de fórmula XXI con isobutileno (5 equivalentes), ácido sulfúrico concentrado (0,15 equivalentes) y terc-butanol (0,16 equivalentes) en diclorometano agitando a -10 a 25°C durante 24 horas.

Los compuestos de fórmula XVIIa se pueden preparar a partir de compuestos de fórmula XXII por tratamiento con ácido ciclopentanocarboxílico en condiciones fuertemente básicas y en un disolvente aprótico, opcionalmente en presencia de un aditivo.

Las condiciones típicas de reacción comprenden el tratamiento de ácido ciclopentanocarboxílico con un exceso al menos 2:1 de una base fuerte (por ejemplo diisopropilamiduro de litio, hexametildisilazida de litio, sodio o potasio, una base de alquil-litio, alquil-magnesio o fosfazeno) en un disolvente no prótico (por ejemplo THF, éter dietílico, hexano, heptano o etilbenceno o una mezcla de estos), opcionalmente en presencia de un aditivo (por ejemplo TMEDA, DMPU o HMPA) a temperaturas en el intervalo de -78°C a 50°C durante hasta 24 horas, seguido por la adición del

138

compuesto de fórmula XXII y reacción a -20°C durante hasta 24 horas y tratamiento adecuado.

Las condiciones preferidas de reacción comprenden el tratamiento de ácido ciclopentanocarboxílico con diisopropilamiduro de litio (2,15 equivalentes) en una mezcla de THF a -15°C durante 3 horas, seguida por tratamiento con 3-bromopropionato de terc-butilo (1,06 equivalentes) en THF a -15°C durante 15 horas y calentando después a la temperatura ambiente.

Otros compuestos de fórmula II se pueden obtener a partir de fuentes comerciales, conocidas en la técnica anterior, o se pueden preparar a partir de compuestos conocidos en la técnica anterior utilizando métodos conocidos en la técnica anterior o utilizando métodos descritos aquí (véanse las secciones de Ejemplos y Preparaciones).

Se puede preparar fácilmente una sal farmacéuticamente aceptable de un compuesto de fórmula I mezclando soluciones de un compuesto de fórmula I y el ácido o base deseado, como sea apropiado. La sal puede precipitarse en la solución y recogerse por filtración o puede recuperarse por evaporación del disolvente.

Los compuestos de la invención también se pueden combinar con uno o más de los siguientes para el tratamiento de FSD:

1) Una o más prostaglandinas naturales o sintéticas, o sus ésteres. Las prostagladindas adecuadas para su uso aquí incluyen compuestos tales como alprostadil, prostaglandina E_1, prostaglandina E_0, 13-14-dihidroprostaglandina E_1, prostaglandina E_2, eprostinol, prostaglandinas naturales sintéticas y semi-sintéticas y sus derivados, incluidos los descritos en el documento WO-00033825 y/o en la Patente de EE.UU. 6.037.346, expedida el 14 de Marzo de 2000, todos ellos incorporados aquí como referencia, PGE_0, PGE_1, PGA_1, PGB_1, $PGF_1\alpha$, 19-hidroxi-PGA_1, 19-hidroxi-PGB_1, PGE_2, PGB_2, 19-hidroxi-PGA_2, 19-hidroxi-PGB_2, $PGE_3\alpha$, carboprox-trometamina, dinoprost, trometamina, dinoprostona, lipoprost, gemeprost, metenoprost, sulprostuna, tiaprost y moxisilato.

2) Uno o más compuestos antagonistas de los receptores α-adrenégicos, también conocidos como α-adrenoceptores o α-receptores o α-bloqueantes. Compuestos adecuados para uso aquí incluyen: los bloqueantes de receptores α-adrenérgicos descritos en la solicitud PCT WO 99/30697, publicada el 14 de Junio de 1998, cuyas descripciones relacionadas con los

receptores α-adrenérgicos se incorporan aquí como referencia e incluyen bloqueantes selectivos de α_1-adrenoceptores o α_2-adrenoceptores y bloqueantes no selectivos de adrenoceptores, adecuadamente los bloqeuantes adecuados de α_1-adrenoceptores incluyen: fentolamin, mesilato de fentolamin, trazodona, alfuzosin, indoramin, naftopidil, tamsulosin, dapiprazol, fenoxibenzamin, idazoxano, efaraxano, yohimbin, alcaloides rauwolfa, Recordati 15/2739, SNAP 1069, SNAP 5089, RS17053, SL 89.0591, doxazosin, terazosin, abanoquil y prazosin; los α_2-bloquantes del documento US 6.037.346 [14 de Marzo de 2001] dibenamina, tolazolina, trimazosin y dibenarnina; los receptores α-adrenérgicos descritos en las Patentes de EE.UU. 4.188.390; 4.026.894; 3.511.836; 4.315.007; 3.527.761; 3.997.666; 2.503.059; 4.703.063; 3.381.009; 4.252.721 y 2.599.000, cada una de las cuales se incorpora aquí como referencia; los bloqueantes de los α_2-adrenoceptores incluyen: clonidina, papaverina, hidrocloruro de papaverina, opcionalmente en presencia de un agente cariotónico tal como pirxamina.

3) Uno o más compuestos donadores de NO (agonistas de NO). Los compuestos donadores de NO adecuados para uso aquí incluyen nitratos orgánicos, tales como mono-, di- o tri-nitratos o ésteres de nitrato orgánicos, incluidos trinitrato de glicerilo (también conocido como nitroglicerina), 5-mononitrato de isosorbida, dinitrato de isosorbida, tetranitrato de pentaeritritol, tetranitrato de eritrilo, nitroprusiato sódico (SNP), 3-morfolinosidnonimina, molsidomina, S-nitroso-N-acetilpenicilamina (SNAP), S-nitroso-N-glutationa (SNO-GLU). N-hidroxi-L-arginina, nitrato de amilo, linsidomina, hidrocloruro de linsidomina (SIN-1), S-nitroso-N-cisteína, dilatos de diazenio (NONOatos), dinitrato de 1,5-pentano, L-arginina, ginseng, zizphi fructus, molsidomina, Re - 2047, derivados nitroxilados de maxisilita tales como NMI-678-11 y NMI-937 como se describen en la solicitud PCT publicada WO 0012075.

4) Uno o más abridores o moduladores de los canales del potasio. Abridores/moduladores adecuados de los canales del potasio para uso aquí incluyen nicorandil, cromocalim, levecromacalim, lemacalim, pinacidil, cliazóxido, minoxidil, caribdotoxin, gliburida, 4-aminopiridina, $BaCl_2$.

5) Uno o más agentes dopaminérgicos, preferiblemente apomorfina o un agonista selectivo de D2, D3 ó D2/D3 tal como, pramipexol y ropirinol (como

140

se reivindica en el documento WO 0023056) PNU95666 (como se reivindica en el documento WO-0040226).

6) Uno o más agentes vasodilatadores. Agentes vasodilatadores adecuados para uso aquí incluyen nimodepina, pinacidil, ciclandelato, isoxsuprina, cloroprumazina, haloperidol, Rec. 15/2730, trazodona.

7) Uno o más agonistas de tromboxano A2.

8) Uno o más agentes activos sobre el CNS (Sistema Nervioso Central).

9) Uno o más alcaloides ergot. Los alcaloides ergot adecuados están descritos en la Patente de EE.UU. 6.037.346, expedida el 14 de Marzo de 2000, e incluyen acetergamina, brazergolina, bromergurida, cianergolina, delorgotril, disulergina, maleato de ergonovina, tartrato de ergotamina, etisulergina, lergotril, lisergida, mesulergina, metergolina, metergotamina, nicergolina, pergolida, propisergida, protergurida, tergurida.

10) Uno o más compuestos que modulan la acción de los factores natriuréticos, en particular el factor natriurético auricular (también conocido como péptido natriurético auricular), factores natriuréticos de tipo B y tipo C tales como los inhibidores de endopeptidasa neutra.

11) Uno o más compuestos que inhiben la enzima que convierte angiotensina tal como enapril, e inhibidores combinados de enzima que convierte angiotensina y endopeptidasa neutra tal como omapatrilat.

12) Uno o más antagonistas de los receptores de angiotensina tal como losartan.

13) Uno o más substratos para NO-sintasa, tal como L-arginina.

14) Uno o más bloqueantes del canal del calcio, tal como amlodipina.

15) Uno o más antagonistas de los receptores de endotelina e inhibidores de la enzima que convierte endotelina.

16) Uno o más agentes para rebajar el colesterol tales como estatinas (p.ej., atorvastatina/Lipitor^{MC}) y fibratos.

17) Uno o más agentes antiplaquetarios y antitrombóticos, p.ej., tPA, uPA, warfarina, hurdina y otros inhibidores de trombina, heparina, inhibidores del factor activante de tromboplastina.

18) Uno o más agentes sensibilizadores de la insulina, tales como rezulina y agentes hipoglucemiantes tales como glipizida.

19) L-DOPA o carbidopa.

20) Uno o más inhibidores de acetilcolinesterasa, tal como donezipil.

141

21) Uno o más agentes anti-inflamatorios esteroídicos o no esteroídicos.

22) Uno o más moduladores de los receptores de estrógenos y/o agonistas de estrógenos y/o antagonistas de estrógenos, preferiblemente raloxifeno o lasofoxifeno, (-)-cis-6-fenil-5-[4-(2-pirrolidin-1-iletoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol y sus sales farmacéuticamente aceptables para la preparación de lo que se detalla en el documento WO 96/21656.

23) Uno o más moduladores de los receptores de canabinoides.

24) Uno o más de un inhibidor de NPY (neuropéptido Y), más particularmente inhibidor de NPY1 ó NPY5, preferiblemente inhibidor de NPY1, preferiblemente dichos inhibidores de NPY (incluidos NPY Y1 y NPY Y5) que tienen una CI50 menor que 100 nM, más preferiblemente menor que 50 nM. Un ensayo para identificar inhibidores de NPY se presenta en el documento WO-A-98/52890 (véase la página 96, líneas 2 a 28).

25) Uno o más de proteína intestinal vasoactiva (VIP), mimético de VIP, análogo de VIP, más particularmente mediado por uno o más de los subtipos de receptores de VIP VPAC1, VPAC ó PACAP (péptido activante de adenilatociclasa de pituitaria), uno o más de un agonista de un receptor de VIP o un análogo de VIP (p.ej., Ro-125-1553) o un fragmento de VIP, uno o más de un antagonista de un α-adrenoceptor combinado con VIP (p.ej., Invicorp, Aviptadil).

26) Uno o más de un agonista o modulador del receptor de melanocortina o un intensificador de melanocortina, tal como melanotano II, PT-14, PT-141 o compuestos reivindicados en los documentos WO-09964002, WO-00074679, WO-09955679, WO-00105401, WO-00053361, WO-00114879, WO-00113112, WO-09954358.

27) Uno o más de un agonista, antagonista o modulador del receptor de serotonina, más particularmente agonistas, antagonistas o moduladores para los receptores de 5HT1A (incluido VML 670) 5HT2A, 5HT2C, 5HT3 y/o 5HT6, incluidos los descritos en los documentos WO-099902159, WO-00002550 y/o WO-00028993.

28) Uno o más de un agente de sustitución de testosterona (incluido deshidroandrostendiona), testosternona (Tostrelle), dihidrotestosterona o un implante de testosterona.

29) Uno o más de estrógenos, estrógenos y medroxiprogesterona o acetato de

142

medroxiprogesterona (MPA) (es decir como una combinación), o estrógenos y el agente de terapia hormonal sustitutoria metil-testosterona (p.ej., HRT especialmente Premarin, Canestin, Oestrofeminal, Equin, Estrace, Estrofem, Elleste Solo, Estring, Eastraderm, TTS, Eastraderm Matrix, Dermestril, Premfase, Preempro, Prempak, Premique, Estratest, Estratest HS, Tibolona).

30) Uno o más de un modulador de transportadores de noradrenalina, dopamina y/o setotonina, tal como bupropion, GW-320659.

31) Uno o más de un agonista y/o modulador de los receptores purinérgicos.

32) Uno o más de un antagonista del receptor de neuroquinina (NK), incluidos los descritos en el documento WO-09964008.

33) Uno o más de un agonista, antagonista o modulador de los receptores de opioides, preferiblemente agonistas para el receptor ORL-1.

34) Uno o más de un agonista o modulador para los receptores de oxitocina/vasopresina, preferiblemente un agonista o modulador selectivo de oxitocina.

35) Uno o más de un inhibidor de PDE, más particularmente un inhibidor de PDE 2, 3, 4, 5, 7 ú 8, preferiblemente un inhibidor de PED2 ó PDE5 y lo más preferiblemente un inhibidor de PDE5 (véase aquí más adelante), teniendo dichos inhibidores preferiblemente una CI50 frente a la enzima respectiva de menos de 100 nM. Los inhibidores de PDE5 cGMP adecuados para uso de acuerdo con la invención incluyen:

las pirazolo[4,3-d]pirimidin-7-onas descritas en el documento EP-A-0463756; las pirazolo[4,3-d]pirimidin-7-onas descritas en el documento EP-A-0526004; las pirazolo[4,3-d]pirimidin-7-onas descritas en la solicitud de Patente Internacional publicada WO 93/06104; las pirazolo[3,4-d]pirimidin-7-onas isómeras descritas en la solicitud de Patente Internacional publicada WO 93/07149; las quinazolin-4-onas descritas en la solicitud de Patente Internacional publicada WO 93/12095; las pirido[3,2-d]pirimidin-4-onas descritas en la solicitud de Patente Internacional publicada WO 94/05661; las purin-6-onas descritas en la solicitud de Patente Internacional publicada WO 94/00453; las pirazolo[4,3-d]pirimidin-7-onas descritas en la solicitud de Patente Internacional publicada WO 98/49166; las pirazolo[4,3-d]pirimidin-7-onas descritas en la solicitud de Patente Internacional publicada WO

143

99/54333; las pirazolo[4,3-d]pirimidin-4-onas descritas en el documento EP-A-0995751; las pirazolo[4,3-d]pirimidin-7-onas descritas en la solicitud de Patente Internacional publicada WO 00/24745; las pirazolo[4,3-d]pirimidin-4-onas descritas en el documento EP-A-0995750; los compuestos descritos en la solicitud internacional publicada WO 95/19978; los compuestos descritos en la solicitud internacional publicada WO 99/24433 y los compuestos descritos en la solicitud internacional publicada WO 93/07124. Las pirazolo[4,3-d]pirimidin-7-onas descritas en la solicitud internacional publicada WO 01/27112; las pirazolo[4,3-d]pirimidin-7-onas descritas en la solicitud internacional publicada WO 01/27113; los compuestos descritos en el documento EP-A-1092718 y los compuestos descritos en el documento EP-A-1092719.

Inhibidores adicionales adecuados de PDE5 para uso de acuerdo con la presente invención incluyen: 5-[2-etoxi-5-(4-metil-1-piperazinilsulfonil)fenil]-1-metil-3-n-propil-1,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona (sildenafil) también conocida como 1-[[3-(6,7-dihidro-1-metil-7-oxo-3-propil-1H-pirazolo-[4,3-d]pirimidin-5-il)-4-etoxifenil]sulfonil]-4-metilpiperazina (véase el documento EP-A-0463756); 5-(2-etoxi-5-morfolinoacetilfenil)-1-metil-3-n-propil-1,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona (véase el documento EP-A-0526004); 3-etil-5-[5-(4-etilpiperazin-1-ilsulfonil)-2-n-propoxifenil]-2-(piridin-2-il)metil-2,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona (véase el documento WO 98/49166); 3-etil-5-[5-(4-etilpiperazin-1-ilsulfonil)-2-(2-metoxietoxi)piridin-3-il]-2-(piridin-2-il)metil-2,6-dihidro-7H-pirazolo[4,3-d]-pirimidin-7-ona (véase el documento WO 99/54333); (+)-3-etil-5-[5-(4-etilpiperazin-1-ilsulfonil)-2-(2-metoxi-1(R)-metiletoxi)piridin-3-il]-2-metil-2,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona, también conocida como 3-etil-5-{5-[4-etilpiperazin-1-ilsulfonil]-2-([(1R)-2-metoxi-1-metiletil]oxi)piridin-3-il}-2-metil-2,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona (véase el documento WO 99/54333); 5-[2-etoxi-5-(4-etilpiperazin-1-ilsulfonil)piridin-3-il]-3-etil-2-[2-metoxietil]-2,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona, también conocida como 1-{6-etoxi-5-[3-etil-6,7-dihidro-2-(2-metoxietil)-7-oxo-2H-pirazolo[4,3-d]pirimidin-5-il]-piridilsulfonil}-4-etilpiperazina (véase el documento WO 91/27113, Ejemplo 8); 5-[2-*iso*-butoxi-5-(4-etilpiperazin-1-ilsulfonil)piridin-3-

144

il]-3-etil-2-(1-metilpiperidin-4-il)-2,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona (véase el documento WO 91/27113, Ejemplo 15); 5-[2-etoxi-5-(4-etilpiperazin-1-ilsulfonil)piridin-3-il]-3-etil-2-fenil-2,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona (véase el documento WO 01/27113, Ejemplo 66); 5-(5-acetil-2-propoxi-3-piridinil)-3-etil-2-(1-isopropil-3-azetidinil)-2,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona (véase el documento WO 01/27112, Ejemplo 124); 5-(5-acetil-2-butoxi-3-piridinil)-3-etil-2-(1-etil-3-azetidinil)-2,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona (véase el documento WO 01/27112, Ejemplo 132); (6R,12aR)-2,3,6,7,12,12a-hexahidro-2-metil-6-(3,4-metilendioxifenil)pirazino[2',1':6,1]pirido[3,4-b]indol-1,4-diona (IC-351), es decir el compuesto de los Ejemplos 78 a 95 de la solicitud internacional publicada WO 95/19978, así como el compuesto de los Ejemplos 1, 3, 7 y 8; 2-[2-etoxi-5-(4-etilpiperazin-1-il-1-sulfonil)fenil]-5-metil-7-propil-3H-imidazo[5,1-f][1,2,4]triazin-4-ona (vardenafil) también conocida como 1-[[3-(3,4-dihidro-5-metil-4-oxo-7-propilimidazo[5,1-f]-as-triazin-2-il)-4-etoxifenil]sulfonil]-4-etilpiperazina, es decir el compuesto de los Ejemplos 20, 19, 337 y 336 de la solicitud internacional publicada WO 99/24433; y el compuesto del Ejemplo 11 de la solicitud internacional publicada WO 93/07124 (EISAI); y los compuestos 3 y 14 de Rotella D P, *J. Med. Chem.*, 2000, 43, 1257.

Todavía otros inhibidores adecuados de PDE5 incluyen: 4-bromo-5-(piridil-metilamino)-6-[3-(4-clorofenil)propoxi]-3(2H)piridazinona; sal monosódica de ácido 1-[4-[(1,3-benzodioxol-5-ilmetil)amino]-6-cloro-2-quinazolinil]-4-piperidincarboxílico; (+)-cis-5,6a,7,9,9,9a-hexahidro-2-[4-(trifluorometil)fenil-metil-5-metilciclopent-4,5]-imidazo-[2,1-b]purin-4(3H)ona; furazlocilina; cis-2-hexil-5-metil-3,4,5,6a,7,8,9,9a-octahidrociclopent[4,5]imidazo[2,1-b]purin-4-ona; 6-carboxilato de 3-acetil-1-(2-clorobencil)-2-propilindol; 6-carboxilato de 3-acetil-1(2-clorobencil)-2-propilindol; 4-bromo-5-(3-piridil-metilamino)-6-(3-(4-clorofenil)propoxi-3(2H)piridazinona; 1-metil-5-(5-morfolinoacetil-2-n-propoxifenil)-3-n-propil-1,6-dihidro-7H-pirazolo[4,3-d]pirimidin-7-ona; sal monosódica de ácido 1-[4-[(1,3-benzodioxol-5-ilmetil)amino]-6-cloro-2-quinazolinil]-4-piperidincarboxílico; Pharmaprojects No. 4516 (Glaxo Wellcome); Pharmaprojects No. 5051 (Bayer); Pharmaprojects No. 5064 (Kyowa Hakko; véase el documento WO 96/26940); Pharmaprojects No.

145

5069 (Schering Plough); GF-196960 (Glaxo Wellcome); E-8010 y E-4010 (Eisai); Bay-38-3045 y 38-9456 (Bayer) y Sch-51866.

Si se administra una combinación de agentes activos, entonces se pueden administrar de forma simultánea, separada o secuencial.

Los compuestos de la invención se puede administrar a solas, pero en terapia humana generalmente se administrarán en mezcla con un excipiente, diluyente o vehículo farmacéutico adecuado, seleccionado teniendo en cuenta la ruta propuesta de administración y las prácticas farmacéuticas clásicas.

Por ejemplo, los compuestos de la invención se pueden administrar por vía oral, bucal o sublingual en forma de comprimidos, cápsulas (incluidas las cápsulas de gelatina blanda), óvulos, elixires, soluciones o suspensiones, que pueden contener agentes aromatizantes o colorantes, para aplicaciones de aporte inmediato, retardado, modificado, sostenido, doble, de liberación controlada o pulsátil. Los compuestos de la invención también se pueden administrar a través de formas de dosificación que se dispersan o disuelven rápidamente.

Las formas de dosificación de liberación modificada y pulsátil pueden contener excipientes tales como los detallados para las formas de dosificación de liberación inmediata junto con excipientes adicionales que actúan como modificadores de la velocidad de liberación siendo éstos revestidos y/o incluidos en el cuerpo del dispositivo. Los modificadores de la velocidad de liberación incluyen, pero no se limitan exclusivamente a ellos, hidroxipropilmetilcelulosa, metilcelulosa, carboximetil-celulosa sódica, etilcelulosa, acetato de celulosa, polióxido de etileno, goma Xantana, carbómero, copolímero de metacrilato de amonio, aceite de ricino hidrogenado, cera carnauba, cera de parafina, acetato ftalato de celulosa, ftalato de hidroxipropilmetil-celulosa, copolímero de ácido metacrílico y mezclas de ellos. Las formas de dosificación de liberación modificada y pulsátil pueden contener uno o una combinación de excipientes que modifican la velocidad de liberación. Los excipientes que modifican la velocidad de liberación pueden estar presentes tanto dentro de la forma de dosificación, es decir dentro de la matriz, como sobre la forma de dosificación, es decir en la superficie o el revestimiento.

Las formulaciones de dosis que se dispersan o disuelven rápidamente (FDDF) pueden contener los siguientes ingredientes: aspartamo, acesulfamo potásico, ácido cítrico, croscarmelosa sódica, crospovidona, ácido diascórbico, acrilato de etilo, etil-

146

celulosa, gelatina, hidroxipropilmetilcelulosa, estearato de magnesio, manitol, metacrilato de metilo, aroma de menta, polietilenglicol, sílice pirolizada, dióxido de silicio, sal sódica de glicolato de almidón, estearil-fumarato de sodio, sorbitol y xilitol. Los términos dispersar o disolver que se utilizan aquí para describir FDDF son dependientes de la solubilidad de la sustancia farmacológica utilizada, es decir cuando la sustancia farmacológica es insoluble se puede preparar una forma de dosificación que se dispersa rápidamente y cuando la sustancia farmacológica es soluble se puede preparar una forma de dosificación que se disuelve rápidamente.

Las composiciones de la presente invención se pueden administrar por inyección directa. La composición se puede formular para administración parenteral, mucosa, intramuscular, intravenosa, subcutánea, ocular, intraocular o transdérmica. Dependiendo de la necesidad, el agente puede administrarse a una dosis de 0,01 a 30 mg/kg de peso corporal, tal como de 0,1 a 10 mg/kg, más preferiblemente de 0,1 a 1 mg/kg de peso corporal.

El término "administrado" incluye descarga por técnicas virales o no virales. Los mecanismos de descarga viral incluyen, pero sin limitarse a ellos, vectores adenovirales, vectores virales adenoasociados (AAV), vectores virales herpes, vectores retrovirales, vectores lentivirales y vectores baculovirales. Los mecanismos de descarga no viral incluyen transfección mediada por lípidos, liposomas, inmunoliposomas, lipofectina, anfífilos faciales catiónicos (CFA) y combinaciones de ellos. Las rutas para tales mecanismos de descarga incluyen, pero sin limitarse a ellas, las rutas mucosa, nasal, oral, parenteral, gastrointestinal, tópica o sublingual.

Además o alternativamente, las composiciones (o partes componentes de las mismas) de la presente invención pueden administrarse por inyección directa. Además o alternativamente, las composiciones (o partes componentes de las mismas) de la presente invención pueden administrarse tópicamente. Además o alternativamente, las composiciones (o partes componentes de las mismas) de la presente invención pueden administrarse por inhalación. Además o alternativamente, las composiciones (o partes componentes de las mismas) de la presente invención también pueden administrarse por una o más de, una ruta mucosa, por ejemplo, como pulverización o aerosol para inhalación nasal o como solución ingerible tal como por una ruta oral, o por una ruta parenteral en la que la descarga se hace por una forma inyectable, tal como, por ejemplo, por ruta rectal, oftálmica (incluida intravitreal o intracameral), nasal, tópica (incluida bucal o sublingual), intrauterina, vaginal o parenteral (incluida

147

subcutánea, intraperitoneal, intramuscular, intravenosa, intradérmica, intracraneal, intratraqueal y epidural), transdérmica, intraperitoneal, intracraneal, intracerebroventricular, intracerebral, intravaginal, intrauterina o parenteral (p.ej., intravenosa, intraespinal, subcutánea, transdérmica o intramuscular).

Como ejemplo, la composición farmacéutica de la presente invención se puede administrar de acuerdo con una pauta de 1 a 10 veces al día, tal como una o dos veces al día. El nivel específico de la dosis y la frecuencia de dosificación para cualquier paciente particular puede variar y dependerá de diversos factores, entre ellos la actividad del compuesto específico empleado, la estabilidad metabólica y la duración de la acción de ese compuesto, la edad, el peso corporal, la salud general, el sexo, la dieta, el modo y el momento de la administración, la velocidad de excreción, la combinación de fármacos, la gravedad de la enfermedad particular y la terapia que esté experimentando el individuo.

Por lo tanto, el término "administrado" incluye, pero sin limitarse a ella, la descarga por una ruta mucosa, por ejemplo, como pulverización o aerosol para inhalación nasal o como solución ingerible; una ruta parenteral donde la descarga es por una forma inyectable, tal como, por ejemplo, una ruta intravenosa, intramuscular o subcutánea.

Tales comprimidos pueden contener excipientes tales como celulosa microcristalina, lactosa, citrato de sodio, carbonato de calcio, fosfato dibásico de calcio y glicina, disgregantes tales como almidón (preferiblemente almidón de maíz, patata o tapioca), sal sódica de glicolato de almidón, croscarmelosa sódica y ciertos silicatos complejos, y aglutinantes de granulación tales como polivinilpirrolidona, hidroxipropilmetilcelulosa (HPMC), hidroxipropilcelulosa (HPC), sacarosa, gelatina y goma arábiga. Adicionalmente, se pueden incluir agentes lubricantes tales como estearato de magnesio, ácido esteárico, behenato de glicerilo y talco.

También se pueden emplear composiciones sólidas de un tipo similar como cargas en cápsulas de gelatina. Excipientes preferidos a este respecto incluyen lactosa, almidón, una celulosa, azúcares lácteos o polietilenglicoles de alto peso molecular. Para suspensiones y/o elixires acuosos, los compuestos de la invención se pueden combinar con varios agentes endulzantes o aromatizantes, colorantes o tintes, con agentes emulsionantes y/o de suspensión y con diluyentes tales como agua, etanol, propilenglicol y glicerina, y combinaciones de ellos.

148

Los compuestos de la invención también se pueden administrar parenteralmente, por ejemplo, intracavernosa, intravenosa, intraarterial, intraperitoneal, intratecal, intraventricular, intrauretral, intraesternal, intracraneal, intramuscular o subcutáneamente, o se pueden administrar por técnicas de infusión. Para esta clase de administración parenteral, lo mejor es utilizar los compuestos en forma de una solución acuosa estéril que puede contener otras sustancias, por ejemplo suficiente concentración de sal o glucosa para hacer la solución isotónica con la sangre. Las soluciones acuosas deberán ser adecuadamente tamponadas (preferiblemente a un pH de 3 a 9), si es necesario. La preparación de formulaciones parenterales adecuadas en condiciones estériles se lleva a cabo fácilmente por técnicas farmacéuticas clásicas bien conocidas por los expertos en la técnica. Las formulaciones parenterales se pueden formular para descarga inmediata, retardada, modificada, sostenida, liberación controlada de dos clases, o descarga pulsátil.

Los siguientes niveles de dosificación y otros dados aquí son para el tipo medio de ser humano, que tiene un peso en el intervalo de 65 a 70 kg. Los expertos en la técnica serán capaces de determinar los niveles de dosificación que requiere una persona cuyo peso caiga fuera de este intervalo, como es el caso de los niños o los ancianos.

Para administración oral y parenteral, a pacientes humanos, el nivel de dosificación diario de los compuestos de la invención o sales o solvatos de los mismos será habitualmente de 10 a 1.000 mg (en dosis individuales o fraccionadas).

Así, por ejemplo, los comprimidos o cápsulas de los compuestos de la invención o sus sales o solvatos pueden contener de 5 a 1.000 mg, tal como 5 mg a 500 mg de compuesto activo para administración de forma individual o dos o más de una vez, lo que resulte adecuado. En cualquier caso, será el médico el que determine la dosificación real que será la más adecuada para cada paciente en particular y variará con la edad, el peso y la respuesta del paciente particular. Las dosificaciones anteriores son ejemplos del caso medio, por supuesto, puede haber casos individuales que necesiten intervalos de dosificación más altos o más bajos, y también estarán dentro del alcance de esta invención. El experto en la técnica apreciará también que, en el tratamiento de ciertos trastornos (incluidos MED y FSD), los compuestos de la invención se pueden tomar como una dosis individual o en una base de "según se precise" (es decir según se necesite o se desee).

Los compuestos de la invención también se pueden administrar intranasal-

mente o por inhalación y se descargan convenientemente en forma de un inhalador de polvo seco o una presentación de pulverización de aerosol en un envase presurizado, bomba, pulverizador o nebulizador con el uso de un propelente adecuado, p.ej., diclorodifluorometano, triclorotrifluorometano, diclorotetrafluoroetano, un hidrofluoroalcano tal como 1,1,1,2-tetrafluoroetano (HFA 134A [marca comercial] o 1,1,1,2,3,3,3-heptafluoropropano (HFA 227EA [marca comercial]), dióxido de carbono u otro gas adecuado. En el caso de un aerosol presurizado, la unidad de dosificación se puede determinar proporcionando una válvula que libere una cantidad medida. El envase presurizado, la bomba, el pulverizador o el nebulizador pueden contener una solución o suspensión del compuesto activo, p.ej. utilizando una mezcla de etanol y el propelente como disolvente, que puede contener además un lubricante, p.ej. trioleato de sorbitano. Las cápsulas y los cartuchos (hechos, por ejemplo, a partir de gelatina) para uso en un inhalador o insuflador se pueden formular de manera que contengan una mezcla de polvo de un compuesto de la invención y una base de polvo adecuada tal como lactosa o almidón.

Las formulaciones en aerosol o polvo seco se disponen preferiblemente de manera que cada dosis medida o el equivalente a una "pulsación" contiene 1 a 50 mg de un compuesto de la invención para descarga al paciente. La dosis diaria global con un aerosol estará en el intervalo de 1 a 50 mg que se pueden administrar en una sola dosis o más habitualmente en dosis fraccionadas a lo largo de día.

Alternativamente, los compuestos de la invención o sus sales o solvatos se pueden administrar en forma de supositorio o pesario, o se pueden aplicar tópicamente en forma de un gel, hidrogel, loción, solución, crema, pomada o polvo para espolvorear. Los compuestos de la invención o sus sales o solvatos también se pueden administrar dérmicamente. Los compuestos de la invención o sus sales o solvatos también se pueden administrar transdérmicamente, por ejemplo mediante el uso de un parche cutáneo. También se pueden administrar por la ruta ocular, pulmonar o rectal.

Para uso oftámico, los compuestos se pueden formular como suspensiones micronizadas en solución salina estéril isotónica y con su pH ajustado, o preferiblemente como soluciones en solución salina estéril isotónica con pH ajustado, opcionalmente en combinación con un agente conservante tal como cloruro de bencilalconio. Alternativamente, se pueden formular en una pomada tal como vaselina.

Para aplicación tópica a la piel, los compuestos de la invención o sus sales

150

o solvatos se pueden formular como una pomada adecuada que contiene el compuesto activo suspendido o disuelto en, por ejemplo, una mezcla con uno o más de los siguientes: aceite mineral, petrolato líquido, petrolato blanco, propilenglcicl, compuesto de polioxietileno-polioxipropileno, cera emulgente y agua. Alternativamente, se pueden formular como una loción o crema adecuada, suspendidos o disueltos en, por ejemplo, una mezcla de uno o más de los siguientes: aceite mineral, monoestearato de sorbitano, un polietilenglicol, parafina líquida, polisorbato 60, cera de ésteres cetílicos, alcohol cetearílico, 2-octildodecanol, alcohol bencílico y agua.

Los compuestos de la invención también se pueden utilizar en combinación con una ciclodextrina. Se sabe que las ciclodextrinas forman complejos de inclusión y de no inclusión con moléculas de fármacos. La formación de un complejo fármaco-ciclodextrina puede modificar la solubilidad, la velocidad de disolución, la biodisponibilidad y/o la propiedad de estabilidad de una molécula de un fármaco. Los complejos fármaco-ciclodextrina son generalmente útiles para la mayoría de las formas de dosificación y rutas de administración. Como alternativa a la formación directa de complejo con el fármaco, la ciclodextrina se puede utilizar como aditivo auxiliar, por ejemplo como vehículo, diluyente o solubilizante. Las alfa-, beta- y gamma-ciclodextrinas son las más utilizadas corrientemente y se describen ejemplos adecuados de las mismas en los documentos WO-A-91/11172, WO-A-94/02518 y WO-A-98/55148.

En una realización preferida, los compuestos de la invención se descargan sistémicamente (tal como oral, bucal o sublingualmente), más preferiblemente por vía oral. Preferiblemente tal administración sistémica (lo más preferiblemente oral) se utiliza para tratar disfunción sexual en hombres o mujeres, más preferiblemente FSD y más preferiblemente aún FSAD.

Así, en una realización particularmente preferida, se proporciona el uso de los compuestos de la invención en la fabricación de un medicamento descargado sistémicamente (preferiblemente descargado oralmente) para el tratamiento o profilaxis de FSD, más particularmente FSAD.

Una formulación oral preferida utiliza comprimidos de liberación inmediata; o formulaciones en dosis de disolución o dispersión rápida (FDDF).

En una realización preferida adicional, los compuestos de la invención se administran tópicamente, preferiblemente directamente a los genitales femeninos, especialmente la vagina.

Ya que la NEP está presente en todo el cuerpo, es un hecho muy inesperado

151

que los compuestos de la invención pueden administrarse sistémicamente y producir una respuesta terapéutica en los genitales (preferiblemente los genitales femeninos) sin provocar efectos secundarios intolerables (adversos). Así, en los resultados *in vivo* (con conejas) dados aquí más adelante, los compuestos de la invención administrados sistémicamente incrementaron el flujo sanguíneo genital, tras excitación sexual (simulada por estimulación del nervio pélvico) sin afectar adversamente a los parámetros cardiovasculares, tales como la inducción de un estado significativo de hipotensión e hipertensión.

Preferiblemente, los compuestos de la invención se administran para el tratamiento de disfunción sexual (más preferiblemente FSD) en la paciente estimulada sexualmente (por estimulación sexual se entiende estimulación visual auditiva o táctil). La estimulación puede producirse antes, después o durante dicha administración.

Así, los compuestos de la invención intensifican las rutas/mecanismos que son la base de la excitación sexual en los genitales femeninos recuperando o mejorando la respuesta de excitación sexual a la estimulación sexual.

Así, una realización preferida proporciona el uso de un compuesto de la invención en la preparación de un medicamento para el tratamiento o la profilaxis de disfunción sexual (más preferiblemente FSD) en el paciente estimulado.

Para uso veterinario, un compuesto de la invención o una sal veterinariamente aceptable del mismo, o un solvato o profármaco veterinariamente aceptable del mismo, se administra como una formulación adecuadamente aceptable de acuerdo con las prácticas veterinarias normales y el cirujano veterinario determinará el régimen de dosificación y la ruta de administración que será más apropiada para un animal particular.

Los siguientes ejemplos de formulación son simplemente ilustrativos y no pretenden limitar el alcance de la invención. "Ingrediente activo" significa un compuesto de la invención.

152

Formulación 1: Se prepara un comprimido utilizando los siguientes ingredientes:

	peso/mg
Ingrediente activo	250
Celulosa microcristalina	400
Dióxido de silicio, pirolizado	10
Ácido esteárico	5
Total	665

Los componentes se mezclan y prensan para formar comprimidos.

Formulación 2: Se puede preparar una formulación intravenosa como sigue:

Ingrediente activo	100 mg
Solución salina isotónica	1.000 ml

Las formulaciones típicas útiles para administrar los compuestos de la invención tópicamente a los genitales son las siguientes:

Formulación 3: Un aerosol
Ingrediente activo (1,0%) en isopropanol (30%) y agua.

Formulación 4: Una espuma
Ingrediente activo, ácido acético glacial, ácido benzoico, alcohol cetílico, parahidroxibenzoato de metilo, ácido fosfórico, poli(alcohol vinílico), propilenglicol, carboximetilcelulosa sódica, ácido esteárico, dietilestearamida, perfume van Dyke nº 6301, agua purificada e isobutano.

Formulación 5: Un gel
Ingrediente activo, docusato sódico BP, alcohol isopropílico BP, propilenglicol, hidróxido de sodio, carbomero 934P, ácido benzoico y agua purificada.

153

Formulación 6: Un gel

Ingrediente activo, ácido benzoico, alcohol cetílico, lavender, compuesto 13091, metilparaben, propilparaben, propilenglicol, carboximetilcelulosa sódica, laurilsulfato de sodio, ácido esteárico, trietanolamina, ácido acético glacial, aceite de ricino, hidróxido de potasio, ácido sórbico y agua purificada.

Formulación 7: Un pesario

Ingrediente activo, cetomacrogol 1000 BP, ácido cítrico, PEG 1500 y 1000 y agua purificada.

La invención incluye adicionalmente:

(i) Una composición farmacéutica que incluye un compuesto de la invención, junto con un excipiente, diluyente o vehículo farmacéuticamente aceptable.

(ii) Un compuesto de la invención para uso como medicamento.

(iii) Un método para tratar FSD que incluye tratar dicho mamífero con una cantidad eficaz de un compuesto de la invención.

(iv) Una composición farmacéutica para tratar FSDI, que comprende un compuesto de la invención junto con un excipiente, diluyente o vehículo farmacéuticamente aceptable.

(v) Un compuesto de la invención para tratar FSD.

La invención se ilustra por los siguientes Ejemplos no limitantes en los que se utilizan las siguientes abreviaturas y definiciones, que también se utilizan a lo largo de toda la memoria descriptiva:

Arbacel®	agente filtrante
br	ancho
Boc	terc-butoxicarbonilo
CDI	carbonildiimidazol
δ	desplazamiento químico
d	doblete
Δ	calor
DCCI	diciclohexilcarbodiimida
DCM	diclorometano
DMF	N,N-dimetilformamida

154

	DMPU	1,3-dimetil-3,4,5,6-tetrahidro-2(1*H*)-pirimidinona
	DMSO	dimetilsulfóxido
	ES^+	exploración positiva por ionización mediante electropulverización
	ES^-	exploración negativa por ionización mediante electropulverización
	Ej	Ejemplo
	h	horas
	HMPA	hexametilfosforamida
	HOBt	1-hidroxibenzotriazol
	HPLC	cromatografía de líquidos de alta presión
	m/z	pico en espectro de masas
	min	minutos
	MS	espectro de masas
	NMR	resonancia magnética nuclear
	Prec	precursor
	Prep	preparación.
	q	cuartete
	s	singlete
	t	triplete
	Tf	trifluorometanosulfonilo
	TFA	ácido trifluoroacético
	THF	tetrahidrofurano
	TLC	cromatografía en capa fina
	TMEDA	*N,N,N'N'*-tetrametiletilendiamina
	TS^+	exploración positiva por ionización mediante termopulverización
	WSCDI	hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida

Los espectros de resonancia magnética nuclear de protón (NMR) fueron siempre concordantes con las estructuras propuestas. Los desplazamientos químicos característicos (δ) se dan en partes por millón a campo bajo de tetrametilsilano utilizando abreviaturas convencionales para la designación de los picos principales: p.ej., s, singlete; d, doblete; t, triplete; q, cuartete; m, multiplete; br, ancho. Las siguientes abreviaturas han sido utilizadas para los disolventes comunes: $CDCl_3$, deuterocloroformo, DMSO, dimetilsulfóxido. La abreviatura psi significa libras por pulgada al cuadrado y LRMS significa espectrometría de masas de baja resolución.

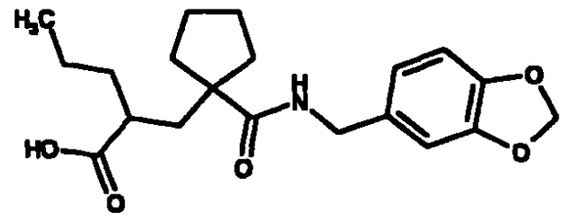
Cuando se ha utilizado la cromatografía en capa fina (TLC) se refiere a TLC sobre gel de sílice utilizando placas de gel de sílice 60 F_{254}, R_f es la distancia recorrida por un compuesto dividida por la distancia recorrida por el frente de disolvente en una placa TLC.

Los modelos de difracción de rayos X en polvo (PXRD) se determinaron utilizando un difractómetro de rayos X en polvo Siemens D5000 equipado con un goniómetro theta-theta, ranuras de divergencia automática de rayos, un monocromador secundario y un contador de centelleo. La muestra era rotada mientras se irradiaba con rayos X producidos por cobre K-alfa1 (longitud de onda = 1,5046 Angstrom) filtrados con un monocromador de grafito (λ = 0,15405 nm) con el tubo de rayos X funcionando a 40 kV/40 mA. Se ilustran los picos principales (en grados 2θ) de los modelos PXRD para la diversas formas sólidas.

Ejemplos

Ejemplo 1

Ácido 2-({1-[(1,3-benzodioxol-5-ilamino)carbonil]ciclopentil}metil)pentanoico

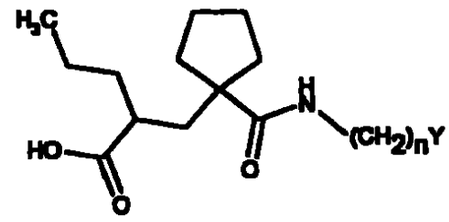


Se añadió ácido trifluoroacético (5 ml) a una solución del éster terc-butílico de la Preparación 34 (130 mg, 0,31 mmol) en diclorometano (5 ml), y la solución se agitó a temperatura ambiente durante 4 horas. La mezcla de reacción se concentró a presión reducida y el residuo se destiló azeotrópicamente con tolueno y diclorometano para proporcionar el compuesto del título como un aceite claro, 112 mg; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0,83 (t, 3H), 1,22-1,40 (m, 3H), 1,50-1,72 (m, 8H), 1,95 (m, 1H), 2,10 (m, 2H), 2,19 (m, 1H), 4,30 (m, 2H), 5,93 (s, 2H), 5,99 (s ancho, 1H), 6,74 (m, 3H); LRMS: m/z 380 (MH^-).

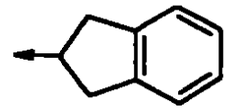
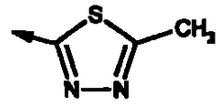
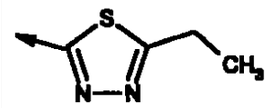
156

Ejemplos 2 a 9

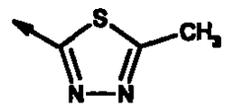
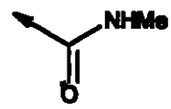
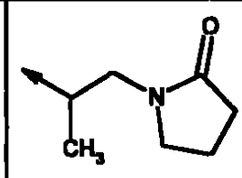
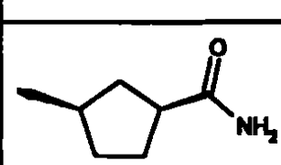
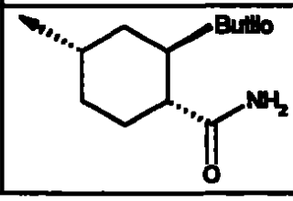
Los compuestos de fórmula Ic, es decir los compuestos de fórmula general I donde R^1 es propilo, fueron preparados a partir del éster terc-butílico correspondiente, siguiendo un procedimiento similar al descrito en el Ejemplo 1.



(Ic)

Ej.	Prec.	n	Y	Datos
2[1]	Prep. 35	0		^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,81 (s, 3H), 1,17-2,04 (m, 14H), 2,27-2,38 (m, 1H), 2,64-2,50 (m, 2H), 3,20-3,31 (m, 2H), 4,60-4,72 (m, 1H), 5,97 (d, 1H), 7,03-7,18 (m, 4H). LRMS: m/z 343,8 (M^+).
3[2,3]	Prep. 36	0		^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,90 (t, 3H), 1,30-1,42 (m, 4H), 1,59-1,81 (m, 7H), 2,18 (m, 1H), 2,30 (m, 1H), 2,42 (m, 1H), 2,55 (m, 1H), 2,61 (s, 3H). LRMS: m/z 324 (MH^-). P.f. 184-186°C Anál. Encontrado: C, 55,50; H, 7,22; N, 12,61. $C_{15}H_{23}N_3O_3S$ requiere C, 55,36; H, 7,14; N, 12,91%.
4[3]	Prep. 37	0		^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,92 (t, 3H), 1,35 (t, 3H), 1,25-1,80 (m, 11H), 2,20-2,50 (m, 4H), 2,95 (q, 2H), 12,10 (s ancho, 1H). LRMS: m/z 339,8 (MH^+). Anál. Encontrado: C, 56,46; H, 7,46; N, 12,36. $C_{16}H_{25}N_3O_3S$ requiere C, 56,62; H, 7,44; N, 12,37%.

157

Ej.	Prec.	n	Y	Datos
5[2]	Prep. 38	1		^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,80 (t, 3H), 1,20-1,70 (m, 11H), 1,90-2,20 (m, 3H), 2,25 (m, 1H), 2,70 (s, 3H), 4,75 (m, 2H), 7,10 (s ancho, 1H). LRMS: m/z 340,6 (MH^+).
6[2]	Prep. 39	2		^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,88 (t, 3H), 1,25-1,40 (m, 3H), 1,41-1,70 (m, 8H), 1,92 (m, 1H), 2,00-2,18 (m, 2H), 2,38 (m, 1H), 2,42 (t, 2H), 2,80 (d, 3H), 3,40-3,60 (m, 2H), 6,50 (s ancho, 1H), 6,74 (s ancho, 1H). LRMS: m/z 313,2 (MH^+).
7	Prep. 40	0		^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,85 (t, 3H), 1,19 (d, 3H), 1,21-1,69 (m, 11H), 1,89-2,10 (m, 5H), 2,30 (m, 1H), 2,41 (m, 2H), 2,95 (m, 1H), 3,35 (m, 1H), 3,63 (m, 2H), 4,20 (m, 1H), 6,58-6,70 (m, 1H). LRMS: m/z 353,1 (MH^+).
8	Prep. 41	0		^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,81 (t, 3H), 1,20-1,30 (m, 3H), 1,41-2,10 (m, 1H), 2,80 (m, 1H), 4,35 (m, 17H), 5,81 (d, 1H), 6,30 (s ancho, 0,5H), 6,43 (s ancho, 0,5H), 7,40 (d ancho, 0,5H), 7,61 (d ancho, 0,5H). LRMS: m/z 339,8 (MH^+).
9	Prep. 32	0		^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,84 (m, 6H), 1,08-2,08 (m, 29H), 4,29 (m, 1H), 5,95 (d, 1H), 6,43 (s, 1H), 7,80 (d, 1H). LRMS: m/z 409,5 (MH^+).

1 = purificado adicionalmente por cromatografía en columna sobre gel de sílice utilizando acetato de etilo:pentano como eluyente.

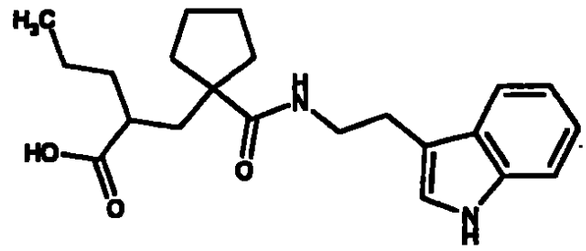
2 = purificado adicionalmente por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol como eluyente.

3 = recristalizado en éter.

158

Ejemplo 10

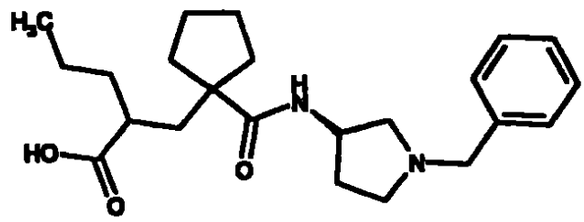
Ácido 2-{[1-({[2-(1*H*-indol-3-il)etil]amino}carbonil)ciclopentil]metil}pentanoico



Se añadió ácido trifluoroacético (2,61 ml, 33,9 mmol) a una solución del éster terc-butílico de la Preparación 44 (482 mg, 1,13 mmol) y anisol (1,23 ml, 11,3 mmol) en diclorometano (4 ml), y la reacción se agitó a temperatura ambiente durante 4 horas. La mezcla se lavó con agua y luego con salmuera, se secó ($MgSO_4$), se concentró a presión reducida y el residuo se destiló azeotrópicamente con tolueno. El aceite pardo residual se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol (95:5) como eluyente, y se volvió a cromatografiar utilizando un gradiente de elución de acetato de etilo:pentano (30:70 a 50:50) para proporcionar el compuesto del título como una espuma clara, 136 mg, 32%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,82 (s, 3H), 1,16-1,77 (m, 12H), 1,78-2,03 (m, 2H), 2,36 (m, 1H), 2,97 (m, 2H), 3,61 (m, 2H), 5,83 (m, 1H), 7,04 (s, 1H), 7,09-7,23 (m, 2H), 7,39 (d, 1H), 7,61 (d, 1H), 8,15 (m, 1H); LRMS: m/z 371,6 (MH^+).

Ejemplo 11

Ácido 2-{[1-({[(3S)-1-bencilpirrolidinil]amino}carbonil)ciclopentil]metil}pentanoico



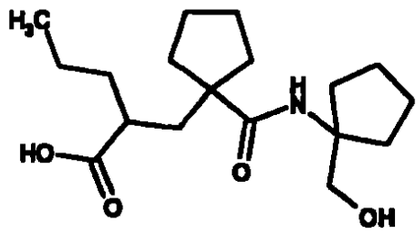
Una solución del éster terc-butílico de la Preparación 45 (70 mg, 0,16 mmol) en ácido trifluoroacético (1 ml) y diclorometano (1 ml) se agitó a temperatura

159

ambiente durante 2 horas. La reacción se concentró a presión reducida y el residuo se destiló azeotrópicamente con diclorometano. El residuo se repartió entre agua (1 ml) y acetato de etilo (5 ml), y el pH de la capa acuosa se ajustó a 6 utilizando una solución de bicarbonato de sodio. Las capas se separaron, la fase orgánica se secó (Na_2SO_4), se evaporó a presión reducida y el residuo se destiló azeotrópicamente con diclorometano para dar el compuesto del título como una espuma de color beige, 45 mg, 73%; ^1H NMR ($CDCl_3$, 400 MHz) δ 0,84 (t, 3H), 1,20-2,95 (m, 19H), 3,52 (m, 1H), 3,75 (m, 1H), 3,95 (m, 1H), 4,25 (m, 1H), 4,5 (m, 1H), 6,96 (s ancho, 1H), 7,39 (m, 5H); LRMS: m/z 387 (MH^+); Anál. Encontrado: C, 61,11; H, 7,69; N, 6,00. $C_{23}H_{34}O_3$;CH_2Cl_2 requiere C, 61,14; H, 7,70; N, 5,94%.

Ejemplo 12

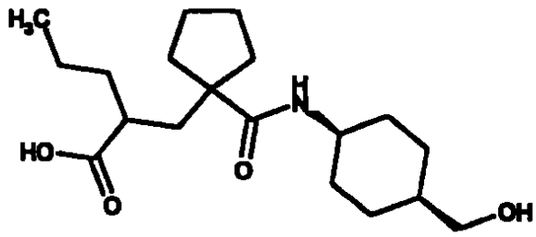
Ácido 2-{[1-({[1-(hidroximetil)ciclopentil]amino}carbonil)ciclopentil]metil}pentanoico



Una solución del éster terc-butílico de la Preparación 33 (38 mg, 0,1 mmol) en ácido trifluoroacético (2 ml) y diclorometano (2 ml) se agitó a temperatura ambiente durante 2 horas. La reacción se concentró a presión reducida y el residuo se destiló azeotrópicamente con tolueno y luego con diclorometano para dar una goma incolora. Ésta se suspendió en una solución de carbonato de potasio (50 mg, 0,3 mmol) en metanol, y la mezcla se agitó durante 2 horas a temperatura ambiente. El metanol se separó a presión reducida, la mezcla acuosa residual se diluyó con agua (20 ml) y se aciduló a pH 2 utilizando ácido clorhídrico 2 N. Esta solución se extrajo con acetato de etilo (2 x 20 ml) y las soluciones orgánicas combinadas se secaron ($MgSO_4$) y se evaporaron a presión reducida para dar un aceite claro, 32 mg, 97%; ^1H NMR ($CDCl_3$, 400 MHz) δ 0,88 (t, 3H), 1,20-1,40 (m, 3H), 1,41-1,90 (m, 17H), 2,01-2,20 (m, 2H), 2,40 (m, 1H), 3,71 (dd, 2H), 5,80 (s ancho, 1H); LRMS: m/z 326,1 (MH^+).

Ejemplo 13

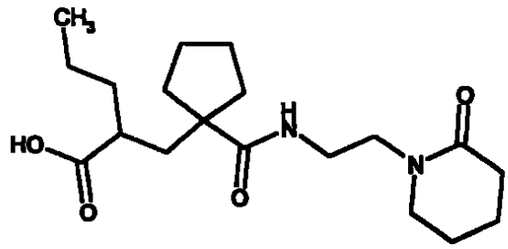
Ácido cis-{[1-({[4-(hidroximetil)ciclohexil]amino}carbonil)ciclopentil]metil}pentanoico



El compuesto del título se obtuvo como una goma incolora con un 68% de rendimiento, a partir del éster terc-butílico de la Preparación 43, siguiendo el procedimiento descrito en el Ejemplo 12, excepto que el producto se purificó adicionalmente por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol (95:5) como eluyente; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,87 (t, 3H), 1,21-1,40 (m, 6H), 11,52-1,70 (m, 15H), 1,92-2,11 (m, 3H), 2,39 (m, 1H), 3,55 (d, 2H), 4,01 (m, 1H), 5,90 (m, 1H); LRMS: m/z 340,3 (MH^+).

Ejemplo 14

Ácido 2-{[1-({[2-(2-oxo-1-piperidinil)etil]amino}carbonil)ciclopentil]metil}pentanoico



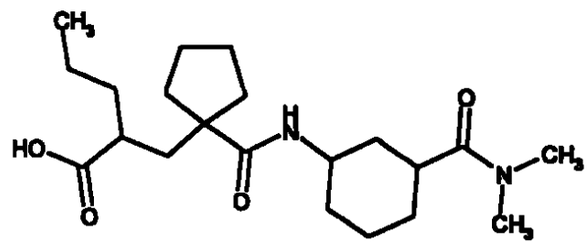
Se pasó una corriente de cloruro de hidrógeno a través de una solución enfriada con hielo del éster terc-butílico de la Preparación 47 (43 mg, 0,105 mmol) en diclorometano (10 ml) durante 20 minutos. Luego la solución se agitó a temperatura ambiente durante 3 horas. La mezcla se concentró a presión reducida y el residuo se destiló azeotrópicamente con diclorometano (3 x), para dar un sólido similar a un vidrio. El producto bruto se purificó por cromatografía en columna sobre gel de sílice

161

utilizando un gradiente de elución de diclorometano:metanol (95:5 a 90:10) para proporcionar el compuesto del título, 6 mg; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,81 (t, 3H), 1,20-1,36 (m, 4H), 1,41-1,69 (m, 7H),1,79 (m, 4H), 1,90-2,10 (m, 3H), 2,30 (m, 1H), 2,38 (t, 2H), 3,30-3,60 (m, 6H), 7,00 (s ancho, 1H); LRMS: m/z $(M-H)^-$.

Ejemplo 15

Ácido 2-({1-[({3-[(dimetilamino)carbonil]ciclohexil}amino)carbonil]ciclopentil}metil)-pentanoico

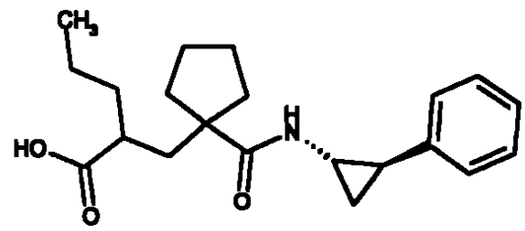


El compuesto del título se obtuvo como un sólido con 85% de rendimiento, a partir del éster terc-butílico de la Preparación 42, siguiendo un método similar al descrito en el Ejemplo 14, excepto que se utilizó diclorometano:metanol:ácido acético (95:3:2) como el eluyente cromatográfico; ^{1}H NMR ($CDCl_3$, 400 MHz) d: 0,89 (t, 3H), 1,09-1,76 (m, 12H), 1,80-2,17 (m, 10H), 2,37 (m, 1H), 2,68 (m, 1H), 2,95 (s, 3H), 3,04 (s, 3H), 3,83 (m, 1H), 6,06 (m, 1H); LRMS: m/z 381 (MH^+); Anál. Encontrado: C, 63,31; H, 9,17; N, 6,53. $C_{21}H_{36}N_2O_4$:H_2O requiere C, 63,29; H, 9,61; N, 7,03%.

162

Ejemplo 16

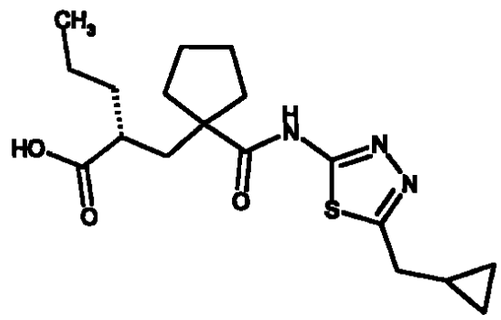
Ácido 2-{[1-({[(1*R*,2*R*)-2-fenilciclopropil]amino}carbonil)ciclopentil]metil}pentanoico



El compuesto del título se obtuvo cuantitativamente como una goma de color naranja a partir del éster terc-butílico de la Preparación 46, siguiendo un procedimiento similar al descrito en el Ejemplo 14; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,90 (t, 3H), 1,12-2,14 (m, 17H), 2,38 (m, 1H), 2,87 (m, 1H), 6,10 (s, 1H), 7,13 (m, 3H), 7,25 (m, 2H); LRMS: m/z 344,3 (MH^+).

Ejemplo 17

Ácido (2*R*)-2-{[1-({[5-(ciclopropilmetil)-1,3,4-tiadiazol-2-il]amino}carbonil)ciclopentil]-metil}pentanoico



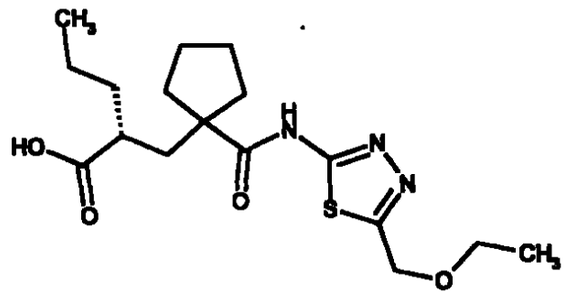
Una solución del éster terc-butílico de la Preparación 50 (63 mg, 0,15 mmol) en ácido trifluoroacético (2 ml) y diclorometano (2 ml), se agitó a temperatura ambiente durante 2 horas. La mezcla se concentró a presión reducida y el residuo se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol (95:5) como eluyente, para dar el compuesto del título como una espuma blanca, 46 mg, 83%; ^{1}H NMR (CD_3OD, 400 MHz) δ: 0,38 (m, 2H), 0,62 (m, 2H), 0,82 (t, 3H), 1,12 (m, 1H), 1,26 (m, 2H), 1,38 (m, 1H), 1,52 (m, 1H), 1,76-1,78 (m,

163

6H), 1,90 (m, 1H), 2,23 (m, 4H), 2,92 (d, 2H); LRMS: m/z 366,0 (MH^+); $[\alpha]_D$ = -7,75° (c = 0,08, metanol).

Ejemplo 18

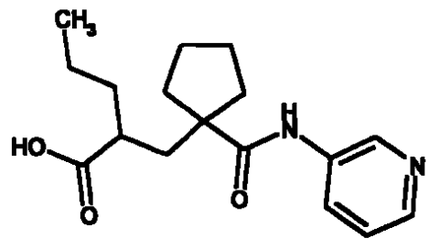
Ácido (2*R*)-2-{[1-({[5-(etoximetil)-1,3,4-tiadiazol-2-il]amino}carbonil)ciclopentil]metil}-pentanoico



El compuesto del título se obtuvo como una espuma blanca con un 62% de rendimiento, a partir del éster terc-butílico de la Preparación 51, siguiendo un procedimiento similar al descrito en el Ejemplo 17; ^1H NMR (CD_3OD, 400 MHz) δ: 0,82 (t, 3H), 1,21-1,40 (m, 7H), 1,50 (m, 1H), 1,60-1,77 (m, 7H), 1,88 (m, 1H), 2,23 (m, 4H), 3,62 (q, 2H); $[\alpha]_D$ = -6,08° (c = 0,25, metanol).

Ejemplo 19

Ácido 2-({1-[(3-piridinilamino)carbonil]ciclopentil}metil)pentanoico

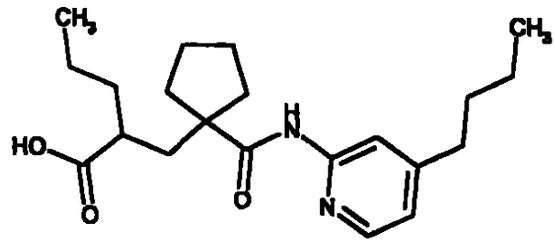


Una mezcla del éster bencílico de la Preparación 52 (130 mg, 0,33 mmol) y paladio al 10% sobre carbón vegetal (20 mg) en etanol acuoso al 95% (3 ml) se hidrogenó a 1,05 kg/cm^2 y temperatura ambiente durante 2 horas. La reacción se

filtró a través de Arbocel®, lavando bien con etanol, y el filtrado se evaporó a presión reducida. La goma residual se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol (95:5) como eluyente para proporcionar el compuesto del título, 103 mg, 83%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,90 (t, 3H), 1,38 (m, 2H), 1,44 (m, 1H), 1,58-1,82 (m, 8H), 2,19 (m, 1H), 2,39 (m, 2H), 2,52 (m, 1H), 6,88 (m, 1H), 7,67 (m, 1H), 7,82 (d, 1H), 8,38 (d, 1H), 9,78 (s, 1H); LRMS: m/z 305 (MH^+).

Ejemplo 20

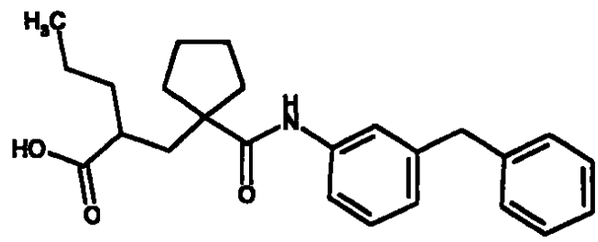
Ácido 2-[(1-{[(4-butil-2-piridinil)amino]carbonil}ciclopentil)metil]pentanoico



El compuesto del título se obtuvo con 92% de rendimiento a partir del éster bencílico de la Preparación 55, siguiendo un procedimiento similar al descrito en el Ejemplo 19; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,90 (m, 6H), 1,28-1,50 (m, 5H), 1,58-1,81 (m, 10H), 2,20 (m, 1H), 2,40 (m, 2H), 2,58 (m, 3H), 6,70 (d, 1H), 7,68 (d, 1H), 8,22 (s, 1H), 9,90 (s ancho, 1H).

Ejemplo 21

Ácido 2-({1-[(3-bencilamino)carbonil]ciclopentil}metil)pentanoico



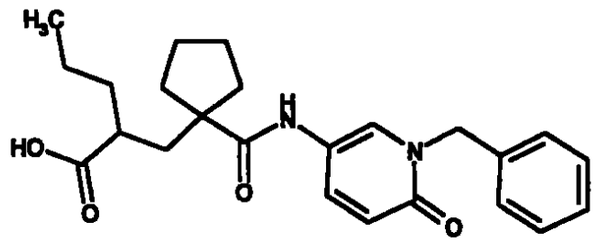
Una mezcla del éster bencílico de la Preparación 53 (1,3 mg, 2,47 mmol) y

165

paladio al 5% sobre carbón vegetal (130 mg) en agua (10 ml) y etanol (40 ml) se hidrogenó a 2,1 kg/cm^2 y temperatura ambiente durante 2 horas. La mezcla de reacción se filtró a través de Arbocel®, el filtrado se concentró a presión reducida y el residuo se trituró con diclorometano. La goma residual se trituró con éter y luego con hexano, y se secó a 50°C, para dar el compuesto del título como un sólido, 0,79 g, 81%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0,95 (t, 3H), 1,24-1,51 (m, 3H), 1,58-1,80 (m, 7H), 1,88 (dd, 1H), 2,15 (m, 2H), 2,24 (m, 1H), 2,48 (m, 1H), 4,00 (s, 2H), 6,98 (d, 1H), 7,24 (m, 6H), 7,40 (m, 3H); Anál. Encontrado: C, 75,48; H, 7,76; N, 3,59. $C_{25}H_{31}NO_3$;0,25H_2O requiere C, 75,44; H, 7,98; N, 3,51%.

Ejemplo 22

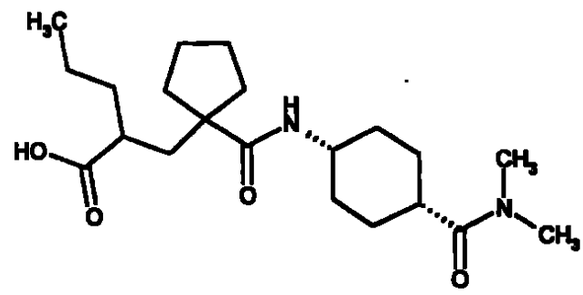
Ácido 2-[(1-{[(1-bencil-6-oxo-1,6-dihidro-3-piridinil)amino]carbonil}ciclopentil)metil]-pentanoico



El compuesto del título se obtuvo como una espuma blanca con 51% de rendimiento a partir del éster bencílico de la Preparación 56, siguiendo un procedimiento similar al descrito en el Ejemplo 21, excepto que el producto se purificó por cromatografía en columna sobre gel de sílice, utilizando acetato de etilo como eluyente; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0,96 (t, 3H), 1,28-1,80 (m, 12H), 2,01 (m, 1H), 2,30-2,52 (m, 2H), 5,02 (dd, 2H), 6,60 (d, 1H), 7,27 (m, 5H), 7,70 (s, 1H), 8,34 (s, 1H); Anál. Encontrado: C, 69,52; H, 7,41; N, 6,51. $C_{24}H_{30}N_2O_4$;0,25H_2O requiere C, 69,45; H, 7,41; N, 6,75.

Ejemplo 23

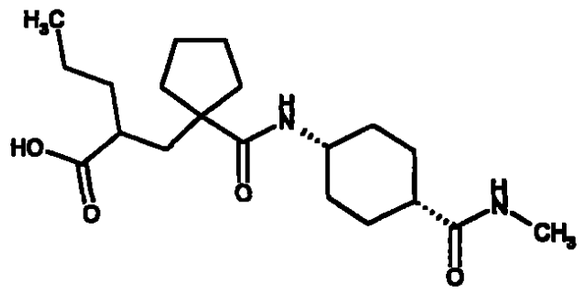
Ácido cis-2-({1-[({4-[(dimetilamino)carbonil]ciclohexil}amino)carbonil]ciclopentil}metil)-pentanoico



Una mezcla del éster bencílico de la Preparación 58 (150 mg, 0,33 mmol) y paladio al 10% sobre carbón vegetal (20 mg) en agua (30 ml) y etanol (3,5 ml) se hidrogenó a 1,05 kg/cm^2 y temperatura ambiente durante 3 días. La mezcla de reacción se filtró a través de Arbocel®, y el filtrado se concentró a presión reducida. La goma residual se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol (95:5) como eluyente para proporcionar el compuesto del título, 85 mg, 65%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,84 (t, 3H), 1,29-1,96 (m, 18H), 2,01-2,23 (m, 4H), 2,37 (m, 1H), 2,62 (m, 1H), 2,96 (s, 3H), 3,03 (s, 3H), 3,96 (m, 1H), 5,98 (m, 1H); LRMS: m/z 381,8 (MH^+); Anál. Encontrado: C, 63,81; H, 9,58; N, 6,99. $C_{21}H_{36}N_2O_4$:0,2CH_2Cl_2 requiere C, 64,06; H, 9,23; N, 7,05%.

Ejemplo 24

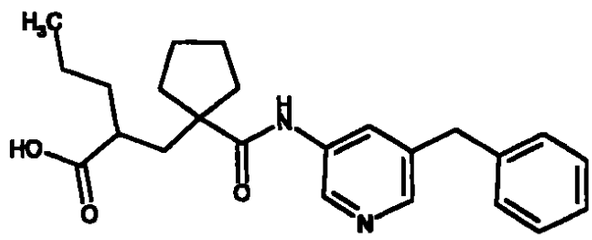
Ácido cis-2-({1-[({4-[(metilamino)carbonil]ciclohexil}amino)carbonil]ciclopentil}metil)-pentanoico



El compuesto del título se obtuvo como un sólido blanco con 34% de rendimiento a partir del éster bencílico de la Preparación 59, siguiendo el procedimiento descrito en el Ejemplo 23; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,90 (t, 3H), 1,26-2,02 (m, 20H), 2,19 (m, 3H), 2,39 (m, 1H), 2,82 (d, 3H), 4,00 (m, 1H), 5,69 (m, 1H), 6,00 (d, 1H); LRMS: m/z 365 ($M-H^-$).

Ejemplo 25

Ácido 2-[(1-{[(5-bencil-3-piridinil)amino]carbonil}ciclopentil)metil]pentanoico

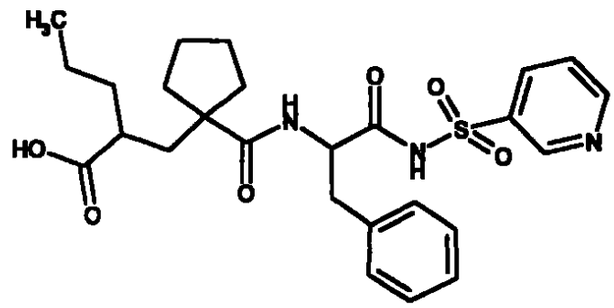


Una mezcla del éster bencílico de la Preparación 54 (850 mg, 1,76 mmol) y paladio al 5% sobre carbón vegetal (100 mg) en etanol acuoso al 20% (30 ml) se hidrogenó a 2,1 kg/cm^2 y temperatura ambiente durante 2 horas. La mezcla se filtró a través de Arbocel®, el filtrado se evaporó a presión reducida y el residuo se destiló azeotrópicamente con diclorometano para dar el compuesto del título como una espuma, 0,63 g; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,92 (t, 3H), 1,30-183 (m, 11H),

2,07 (m, 1H), 2,42 (m, 3H), 3,82 (s, 2H), 7,15-7,38 (5H), 7,80 (s, 1H), 8,48 (s, 1H), 8,59 (s, 1H), 8,62 (s, 1H); Anál. Encontrado: C, 72,29; H, 7,70; N, 6,90. $C_{24}H_{30}N_2O_3$;0,25H_2O requiere C, 72,24; H, 7,70; N, 7,02%.

Ejemplo 26

Ácido 2-({1-[({1-bencil-2-oxo-2-[(3-piridinsulfonil)amino]etil}amino)carbonil]-ciclopentil}metil)pentanoico

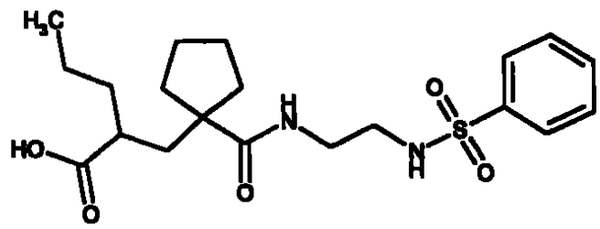


Una mezcla del éster bencílico de la Preparación 57 (918 mg, 1,52 mmol) y paladio al 10% sobre carbón vegetal (90 mg) en agua (10 ml) y etanol (50 ml) se hidrogenó a 3,5 kg/cm^2 y temperatura ambiente durante 4 ½ horas. El análisis por tlc (cromatografía en capa fina) mostró la presencia de material de partida, así que se añadió más catalizador (70 mg), y la mezcla se hidrogenó durante 18 horas más. El análisis por tlc mostró de nuevo la presencia de material de partida, así que se añadió más catalizador (70 mg), y la hidrogenación se continuó durante 6 horas más. La mezcla de reacción se filtró a través de Arbocel®, el filtrado se evaporó a presión reducida y el residuo se destiló azeotrópicamente con diclorometano. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de diclorometano:ácido acético:etanol (99:1:0 a 79,1:0,9:20) para proporcionar el compuesto del título como una espuma blanca, 271 mg, 35%; ^{1}H NMR (DMSOd_6, 300 MHz) δ: 0,75 (m, 3H), 0,96-1,42 (m, 11H), 1,61-1,99 (m, 4H), 2,75-3,02 (m, 2H), 4,45 (m, 1H), 7,20 (m, 6H), 7,62 (m, 1H), 8,24 (m, 1H), 8,83 (s, 1H), 9,01 (s, 1H), 11,98 (s ancho, 1H), 12,70 (s ancho, 1H); IR (pastilla de KBr) 1185, 1195 (m), 1455, 1515, 1640, 1704, 2870, 2930, 2960 (s).

169

Ejemplo 27

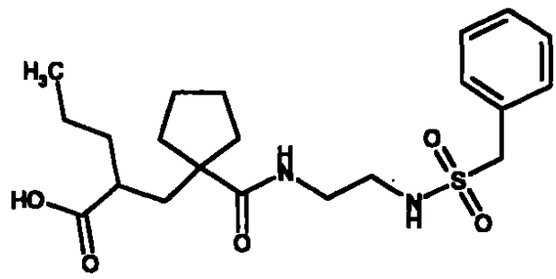
Ácido 2-({1-[({2-[(fenilsulfonil)amino]etil}amino)carbonil]ciclopentil}metil)pentanoico



Una mezcla de la amina de la Preparación 61 (235 mg, 0,72 mmol), cloruro de bencenosulfonilo (127 mg, 0,72 mmol) y trietilamina (150 µl, 1,08 mmol) en diclorometano (6 ml) se agitó a temperatura ambiente durante 2 días. La mezcla se concentró a presión reducida y el residuo se purificó por cromatografía en columna sobre gel de sílice utilizando acetato de etilo:pentano (30:70) como eluyente para dar un aceite claro. Luego, éste se disolvió en ácido trifluoroacético (3 ml) y diclorometano (3 ml) y la solución se agitó a temperatura ambiente durante 6 horas. La mezcla se concentró a presión reducida y el residuo se destiló azeotrópicamente dos veces con tolueno. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando acetato de etilo:pentano (30:70) para proporcionar el compuesto del título como un aceite claro, 204 mg, 69%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,84 (t, 3H), 1,22-1,43 (m, 4H), 1,43-2,18 (m, 10H), 2,36 (m, 1H), 3,11 (m, 2H), 3,20-3,31 (m, 1H), 3,42-3,53 (m, 1H), 6,13-6,24 (m, 1H), 7,42-7,59 (m, 3H), 7,84 (m, 2H); LRMS: m/z 411,8 (MH^+); Anál. Encontrado: C, 57,26; H, 7,40; N, 6,61. $C_{20}H_{30}N_2O_5S$ requiere C, 57,18; H, 7,22; N, 6,62%.

Ejemplo 28

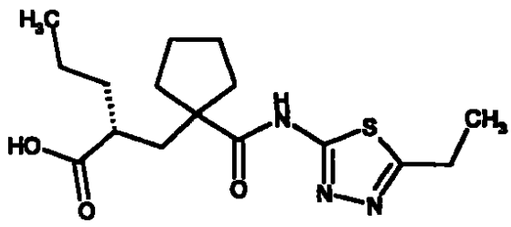
Ácido 2-[{1-[({2-[(bencilsulfonil)amino]etil}amino)carbonil]ciclopentil}metil]pentanoico



El compuesto del título se obtuvo como un aceite claro con 97% de rendimiento, a partir de la amina de la Preparación 61, siguiendo el procedimiento descrito en el Ejemplo 27; ^{1}H NMR ($CDCl_3$, 300 MHz) δ:0,87 (t, 3H), 1,19-1,72 (m, 11H), 1,80-1,96 (m, 1H), 2,00-2,16 (m, 2H), 2,27-2,38 (m, 1H), 2,92-3,21 (m, 3H), 3,23-3,39 (m, 1H), 4,25 (s, 2H), 5,80-6,06 (m, 1H), 6,38 (m, 1H), 7,29-7,43 (m, 5H); LRMS: m/z 425,8 (MH^+).

Ejemplo 29

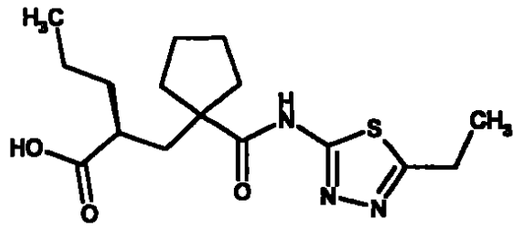
Ácido (-)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]pentanoico



y

Ejemplo 30

Ácido (+)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]pentanoico



El ácido del Ejemplo 4 (824 mg) se purificó adicionalmente por HPLC utilizando una columna AD y utilizando hexano:isopropanol:ácido trifluoroacético (85:15:0,2) como eluyente para dar el compuesto del Ejemplo 29 como una espuma blanca, 400 mg, 99,5% ee (exceso enantiomérico), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,90 (t, 3H), 1,36 (m, 6H), 1,50-1,80 (m, 9H), 2,19 (m, 1H), 2,30 (m, 1H), 2,44 (m, 1H), 2,60 (m, 1H), 2,98 (q, 2H), 12,10-12,30 (s ancho, 1H); LRMS: m/z 338 (MH^-), $[\alpha]_D$ = -9,0° (c = 0,1, metanol), y el compuesto del título del Ejemplo 30 como una espuma blanca, 386 mg, 99% ee, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,90 (t, 3H), 1,38 (m, 6H), 1,50-1,79 (m, 9H), 2,19 (m, 1H), 2,30 (m, 1H), 2,44 (m, 1H), 2,60 (m, 1H), 2,98 (q, 2H), 12,10-12,27 (s ancho, 1H); LRMS: m/z 338 (MH^-); y $[\alpha]_D$ = +3,8° (c = 0,1, metanol).

Alternativamente, el Ejemplo 29 se puede preparar como sigue:

A una solución del producto de la Preparación 51a (574 g, 1,45 mol) en diclorometano (2,87 l) se añadió ácido trifluoroacético (1,15 l) a lo largo de un período de 50 minutos con enfriamiento a 10°C. Una vez terminada la adición, la reacción se dejó calentar a temperatura ambiente con agitación en atmósfera de nitrógeno durante 24 horas. Luego se añadió agua desionizada (2,6 l). Luego la mezcla de reacción se lavó con agua desionizada (3 x 2,6 l). La capa de diclorometano se concentró a un volumen de aproximadamente 1 l para dar el compuesto del título bruto (439 g, 1,29 mol, 96% de rendimiento) como una solución en diclorometano. Se obtuvo una muestra purificada del compuesto del título utilizando el siguiente procedimiento. A una solución en diclorometano (2,34 l) del producto bruto, que se había filtrado para separar cualquier contaminación en partículas, se añadió acetato de isopropilo (1,38 l). La mezcla resultante se destiló a presión atmosférica mientras se reemplazaba

simultáneamente con acetato de isopropilo hasta que la temperatura de la solución alcanzó 87°C. El calentamiento se detuvo y la solución se dejó enfriar a temperatura ambiente con agitación durante 14 horas para dar una solución de color pardo turbio. A continuación, se incrementó la velocidad de agitación y comenzó la cristalización. Luego, la suspensión se dejó granular durante 12 horas a temperatura ambiente. Luego la suspensión resultante se enfrió a 0°C durante 3,5 horas y el sólido se recogió por filtración. A continuación, la torta del filtro se lavó con acetato de isopropilo (2 x 185 ml, después 2 x 90 ml) y el material sólido se secó a vacío a 40-45°C durante 18 horas para dar el compuesto del título (602 g, 0,18 mol, 70% de rendimiento) como un sólido cristalino de color crema; p.f.: 130-136°C; LRMS (APCI negativa): m/z $[M-H]^-$ 338; ^1H NMR ($CDCl_3$, 300 MHz) δ 0,92 (t, 3H), 1,27-1,52 (m, 7h), 1,52-1,89 (m, 8H), 2,11-2,27 (m, 1H), 2,27-2,37 (m, 1H), 2,42-2,55 (m, 1H), 2,65 (dd, 2H), 3,00 (q, 2H), 12,25 (s ancho, 1H).

El Ejemplo 29 se puede purificar como sigue:

El producto del título del Ejemplo 29 se disolvió en metanol. A esta solución se añadió metóxido de sodio (1 equivalente) en metanol (1 ml/g del Ejemplo 29) y la mezcla se agitó a temperatura ambiente durante 20 minutos. El disolvente se separó a vacío y el residuo se destiló azeotrópicamente con acetato de etilo para dar un residuo de color pardo. Se añadió acetato de etilo y la solución se filtró para dar un sólido de color pardo que se lavó con éter terc-butilmetílico para dar la sal de sodio bruta del Ejemplo 29. Este producto bruto (35 g) se repartió entre agua (200 ml) y acetato de etilo (350 ml). Se añadió ácido clorhídrico concentrado (~7 ml) hasta que el pH de la capa acuosa fue pH 2. La fase acuosa se lavó con acetato de etilo (2 x 100 ml). Las capas combinadas se secaron utilizando sulfato de magnesio. El disolvente se separó a vacío para dar un sólido de color pardo claro. (31 g). Se añadieron acetato de etilo (64 ml, 2 ml/g) y éter diisopropílico (155 ml, 5 ml/g) y la mezcla se calentó a 68°C hasta que se obtuvo una solución clara (~30 min). Al enfriar a temperatura ambiente, ocurrió la cristalización del ácido libre. Después de 30 minutos de agitación a temperatura ambiente, se recogió el producto por filtración y se lavó con éter diisopropílico. El producto se secó en una estufa a vacío a 50°C durante una noche (20,2 g, 61% de recuperación a partir de la sal de sodio); p.f. 135°C (determinado utilizando un instrumento Perkin-Elmer DSC7 y una velocidad de calentamiento de 20°C/minuto).

Los picos principales (de 2 a 40 grados 2θ) en el modelo PXRD son los siguientes: El PXRD se realizó sin referencia a una fuente interna (p.ej., polvo de silicio). Las posiciones de los picos están sujetas, por tanto, a un posible descentramiento respecto del cero instrumental, y a errores en la altura de la muestra.

Ángulo °2-Theta	Intensidad %	Ángulo °2-Theta	Intensidad %	Ángulo °2-Theta	Intensidad %
6,701	59,8	20,121	36,1	28,539	17,3
8,908	11,2	20,425	7,1	30,092	56,0
9,305	8,6	21,104	8,9	31,236	8,8
9,590	4,5	21,326	5,7	32,010	12,8
10,039	25,3	21,733	100,0	32,810	8,0
10,206	59,3	22,150	18,7	33,106	6,6
10,578	11,3	22,816	10,2	33,720	8,0
10,984	12,2	23,327	46,2	34,033	9,2
12,075	10,3	23,681	6,4	34,388	12,4
13,054	5,5	23,960	9,5	34,646	8,5
14,256	8,2	24,248	14,0	34,821	8,2
15,695	45,2	24,722	9,4	35,806	7,3
16,316	17,1	25,505	15,5	36,272	13,5
16,418	8,5	25,779	10,5	36,633	12,1
16,698	24,1	26,267	7,0	37,500	7,8
17,129	16,5	26,724	20,4	37,862	11,7
17,605	20,9	26,890	70,4	38,281	6,5
18,575	23,6	27,663	7,2	39,975	11,8
19,296	14,0	28,158	16,8		

Sales del Ejemplo 29

Sales de sodio

a) **Forma de bajo punto de fusión**

El producto del título del Ejemplo 29 se disolvió en metanol (80 mg en 2,5 ml-33 ml/g). Se añadió hidróxido de sodio (0,024 ml como solución

10 N). Al evaporar el metanol, se produjo la cristalización de la sal de sodio, que se utilizó sin más purificación. La sal se secó a alto vacío durante 5 horas para dar 85 mg, rendimiento cuantitativo; p.f. 214 grados C (determinado utilizando un instrumento TA DSC2910 a un ritmo de calentamiento de 10°C/minuto).

Los picos principales (de 2 a 40 grados 2θ) en el modelo PXRD son los siguientes: El PXRD se realizó sin referencia a una fuente interna (p.ej., polvo de silicio). Las posiciones de los picos están sujetas, por tanto, a un posible descentramiento respecto del cero instrumental, y a errores en la altura de la muestra.

Ángulo °2-Theta	Intensidad %	Ángulo °2-Theta	Intensidad %	Ángulo °2-Theta	Intensidad %
7,748	92,8	15,814	14,0	25,595	15,4
8,380	17,8	16,496	43,9	26,753	18,4
8,950	14,3	17,349	40,9	28,085	20,4
9,515	8,9	17,687	100,0	28,883	19,9
10,043	11,7	18,229	35,3	29,737	19,7
11,000	13,2	18,736	40,9	30,227	21,6
11,829	17,5	19,754	37,5	31,204	28,1
12,258	17,3	20,258	50,3	31,729	20,6
13,087	19,0	20,498	93,1	34,825	19,7
13,464	22,3	21,719	47,4	35,394	24,2
13,808	13,4	22,499	35,5	36,018	22,9
14,840	14,3	23,310	29,9	37,722	24,5
15,197	27,1	24,111	19,5	39,304	20,6
15,489	21,4	25,165	19,1		

b) <u>Forma de alto punto de fusión</u>

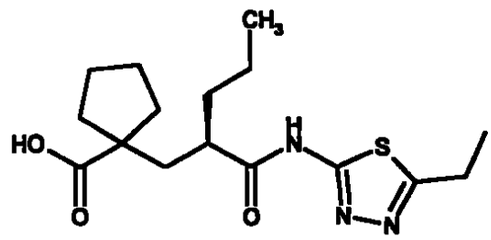
El producto del título del Ejemplo 29 se disolvió en metanol (450 mg en 15 ml-33 ml/g). Se añadió hidróxido de sodio (1,33 ml como solución 1 N). El metanol se evaporó utilizando un rotavapor a vacío para dar una goma. La goma se destiló azeotrópicamente una vez con alcohol isopropílico (8 ml)

para separar el metanol residual y agua antes de añadir una porción adicional de alcohol isopropílico (15 ml). La mezcla se calentó hasta que se obtuvo una mezcla homogénea. Al enfriar, se produjo la cristalización de la sal de sodio. La mezcla se mantuvo a temperatura ambiente durante 10 minutos. La sal se separó por filtración, se lavó con alcohol isopropílico y se secó en una estufa a vacío a 60°C durante 30 min; se recuperaron 200 mg de la sal de sodio; p.f. 252 grados C (determinado utilizando un instrumento TA DSC2910 a un ritmo de calentamiento de 10°C/minuto).

Los picos principales (de 2 a 40 grados 2θ) en el modelo PXRD son los siguientes: El PXRD se realizó sin referencia a una fuente interna (p.ej., polvo de silicio). Las posiciones de los picos están sujetas, por tanto, a un posible descentramiento respecto del cero instrumental, y a errores en la altura de la muestra.

Ángulo °2-Theta	Intensidad %	Ángulo °2-Theta	Intensidad %	Ángulo °2-Theta	Intensidad %
3,419	0,8	20,682	1,5	30,090	1,1
6,674	100,0	20,758	1,7	30,735	0,9
7,394	0,4	20,841	1,4	31,360	1,4
8,200	1,7	21,058	1,0	32,025	3,5
11,465	0,4	21,462	0,5	32,742	0,7
13,010	1,5	21,935	2,0	33,072	0,7
13,337	0,8	22,965	0,6	33,723	1,2
16,414	0,7	23,585	1,1	34,261	0,7
17,288	2,8	24,868	1,4	35,946	1,8
18,165	0,9	25,387	1,1	36,839	0,7
18,987	1,4	25,733	1,8	37,484	0,7
19,330	0,5	26,124	0,9	38,519	1,4
19,687	0,8	26,826	0,9	38,801	1,3
20,069	1,8	28,719	1,8		

El compuesto del título del Ejemplo 29 metaboliza para formar ácido (2*R*)-1-(2-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}pentil)ciclopentanocarboxílico.

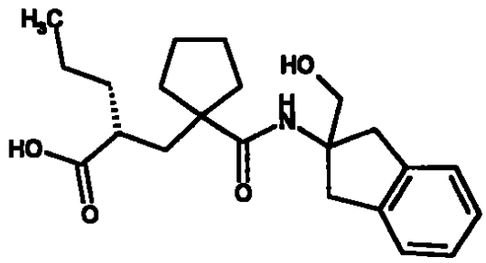


Este compuesto se preparó como sigue:

El producto de la Preparación 102 (430 mg, 1 mmol) se recogió en etanol (5 ml) y metanol (1 ml) y se hidrogenó a una presión de hidrógeno de 1,05 kg/cm^2 a temperatura ambiente durante 2 h. Luego la mezcla se filtró a través de un tapón de Arbocel® y se evaporó para dar un aceite amarillo. Este aceite se purificó por cromatografía en columna utilizando en primer lugaf 19:1, después 9:1 DCM:MeOH como eluyente para proporcionar el compuesto del título como un aceite claro (120 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) 0,88 (t, 3H), 1,20-188 (m, 13H), 1,90-2,03 (m, 1H), 2,24-2,38 (m, 1H), 2,43-2,72 (m, 2H), 2,95 (q, 2H); LRMS m/z 340,2 (M+H).

Ejemplo 31

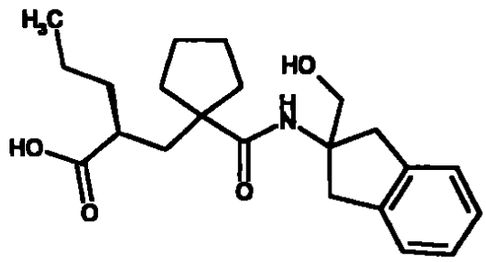
Ácido (+)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)ciclopentil]-metil}pentanoico



y

Ejemplo 32

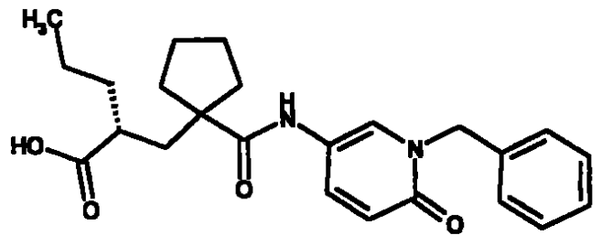
Ácido (-)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)ciclopentil]-metil}pentanoico



El ácido 2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}pentanoico (documento WO 9110644) se purificó adicionalmente por HPLC utilizando una columna AD y hexano:isopropanol:ácido trifluoroacético (90:10:0,1) como eluyente, para dar el compuesto del título del Ejemplo 31, 99% ee, $[\alpha]_D$ = +10,4° (c = 0,067, etanol) y el compuesto del título del Ejemplo 32, 99% ee, $[\alpha]_D$ = -10,9° (c = 0,046, etanol).

Ejemplo 33

Ácido (2*R*)-2-[(1-{[(1-bencil-6-oxo-1,6-dihidro-3-piridinil)amino]carbonil}ciclopentil)-metil]pentanoico

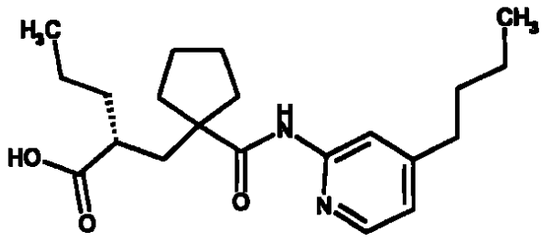


Se añadieron hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (191 mg, 1,0 mmol), hidrato de 1-hidroxibenzotriazol (135 mg, 1,0 mmol), N-metilmorfolina (165 µl, 1,5 mmol) y finalmente la amina de la Preparación 28 (150 mg, 0,69 mmol) a una solución del ácido de la Preparación 2 (284 mg, 1,0 mmol) en N,N-dimetilformamida (8 ml), y la reacción se agitó a 90°C durante 18 horas. La solución enfriada se

diluyó con acetato de etilo (90 ml), se lavó con agua (4 x 50 ml) y salmuera (50 ml), luego se secó ($MgSO_4$) y se evaporó a presión reducida. El producto bruto se purificó por cromatografía sobre gel de sílice, utilizando acetato de etilo:pentano (30:70) para dar un aceite amarillo, 191 mg. Este intermedio se disolvió en diclorometano (3 ml) y ácido trifluoroacético (3 ml) y la solución se agitó a temperatura ambiente durante 5 horas. La mezcla se concentró a presión reducida y el residuo se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol (95:5) como eluyente para dar el compuesto del título como una espuma, 77 mg, ^1H NMR ($CDCl_3$, 300 MHz) δ: 0,86 (t, 3H), 1,20-1,76 (m, 12H), 1,93-2,02 (m, 1H), 2,20-2,46 (m, 3H), 4,95 (d, 1H), 5,04 (d, 1H), 6,61 (d, 1H), 7,21 (m, 1H), 7,50 (s, 1H), 8,23 (s, 1H); LRMS: m/z 411,6 $(MH)^+$; $[\alpha]_D$ = -3,8° (c = 0,052, etanol).

Ejemplo 34

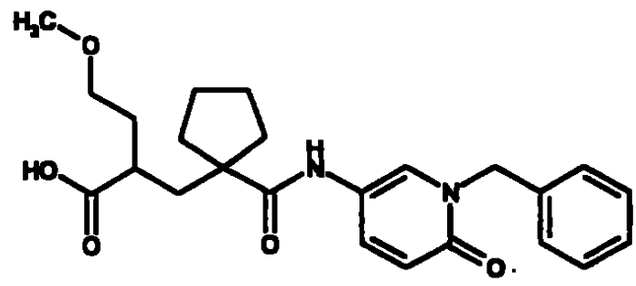
Ácido (2*R*)-2-[(1-{[(4-butil-2-piridinil)amino]carbonil}ciclopentil)metil]pentanoico



El compuesto del título se obtuvo con 43% de rendimiento a partir del ácido de la Preparación 2 y la amina de la Preparación 30, siguiendo un procedimiento similar al descrito en el Ejemplo 33, ^1H NMR ($CDCl_3$, 400 MHz) δ: 0,80-1,00 (m, 6H), 1,22-1,84 (m, 18H), 2,03-2,56 (m, 3H), 2,77 (m, 1H), 7,14 (d, 1H), 8,08 (d, 1H), 8,23 (s, 1H), 11,71 (s ancho, 1H); LRMS: m/z 361,7 $(MH)^+$, $[\alpha]_D$ = -1,4° (c = 0,14, etanol).

Ejemplo 35

Ácido 2-[(1-{[(1-bencil-6-oxo-1,6-dihidro-3-piridinil)amino]carbonil}ciclopentil)metil]-4-metoxibutanoico

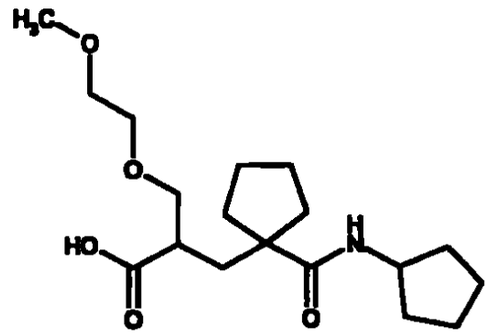


Una mezcla del éster bencílico de la Preparación 62 (850 mg, 1,64 mmol) y paladio al 5% sobre carbón vegetal (250 mg) en 40% de etanol acuoso (21 ml), se hidrogenó a 2,1 kg/cm^2 y temperatura ambiente durante 30 minutos. La mezcla de reacción se filtró a través de Hyflo®, y el filtrado se evaporó a presión reducida. La espuma residual se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol (97:3) como eluyente para dar el compuesto del título como una espuma blanca, 550 mg, 79%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1,24-2,17 (m, 12H), 2,18-2,31 (m, 1H), 3,07 (s, 3H), 3,21 (t, 2H), 5,08 (s, 2H), 6,63 (d, 1H), 7,23-7,41 (m, 5H), 7,72 (d, 1H), 8,24 (s, 1H). Anál. Encontrado: C, 67,46; H, 7,18; N, 6,24. $C_{24}H_{30}N_2O_5$ requiere C, 67,58; H, 7,09; N, 6,57%.

180

Ejemplo 36

Ácido 3-{1-[(ciclopentilamino)carbonil]ciclopentil}-2-[(2-metoxietoxi)metil]propanoico

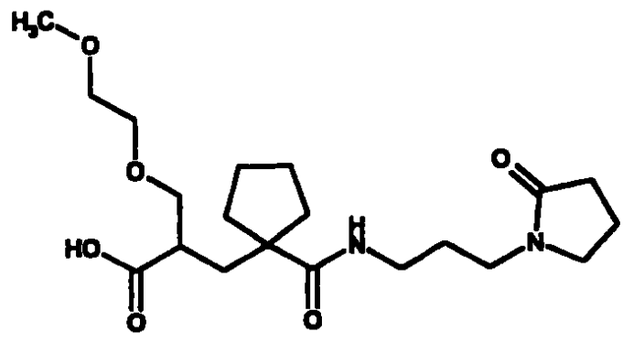


Una solución del éster terc-butílico de la Preparación 64 (320 mg, 0,80 mmol) en ácido trifluoroacético (2 ml) y diclorometano (2 ml) se agitó a temperatura ambiente durante 8 horas. La mezcla se concentró a presión reducida y el residuo se destiló azeotrópicamente con tolueno dos veces. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol (95:5) para dar el compuesto del título como un aceite claro, 171 mg, 62%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,29-140 (m, 2H), 1,42-1,69 (m, 10H), 1,75 (dd, 1H), 1,87-2,03 (m, 5H), 2,64 (m, 1H), 3,34 (s, 3H), 3,43-3,52 (m, 3H), 3,57 (m, 2H), 3,61 (m, 1H), 4,08-4,20 (m, 1H), 5,89 (d, 1H); LRMS: m/z 340 (MH^-).

181

Ejemplo 37

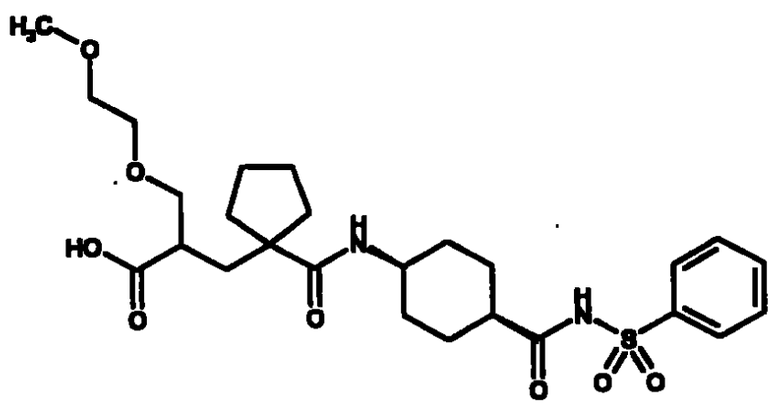
Ácido 3-(2-metoxietoxi)-2-{[1-({[3-(2-oxo-1-pirrolidinil)propil]amino}carbonil)-ciclopentil]metil}propanoico



El compuesto del título se obtuvo como un aceite claro con 57% de rendimiento a partir del éster terc-butílico de la Preparación 65, siguiendo el procedimiento descrito en el Ejemplo 36; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1,56-1,78 (m, 8H), 1,94-2,17 (m, 6H), 2,44 (m, 2H), 2,68-2,76 (m, 1H), 3,10-3,21 (m, 1H), 3,22-3,31 (m, 1H), 3,37 (s, 3H), 3,40 (m, 2H), 3,44-3,56 (m, 5H), 3,60 (m, 2H), 3,68 (m, 1H), 6,91-7,01 (m, 1H); LRMS: m/z 398,7 (M^+).

Ejemplo 38

Ácido cis-3-(2-metoxietoxi)-2-{(1-{[(4-{[(fenilsulfonil)amino]carbonil}ciclohexil)amino]-carbonil}ciclopentil)metil]propanoico



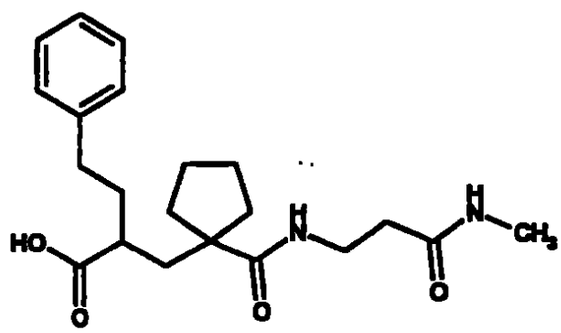
Una solución del éster terc-butílico de la Preparación 66 (446 mg, 0,75

182

mmol) en diclorometano (5 ml) y ácido trifluoroacético (5 ml) se agitó a temperatura ambiente durante 18 horas. La mezcla de reacción se concentró a presión reducida y el residuo se destiló azeotrópicamente con diclorometano, luego con tolueno y finalmente con éter, para proporcionar el compuesto del título como una espuma blanca, 385 mg, 95%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,48-2,17 (m, 18H), 2,40 (s, 1H), 2,66 (s, 1H), 3,37 (s, 3H), 3,50-3,70 (m, 6H), 3,94 (s, 1H), 6,10 (d, 1H), 6,59 (s, 1H), 7,55 (t, 2H), 7,61 (m, 1H), 8,02 (d, 2H), 9,11 (s, 1H); Anál. Encontrado: C, 54,88; H, 6,90; N, 5,04. $C_{26}H_{38}N_2O_8S;1,7_{H2}O$ requiere C, 57,97; H, 7,11; N, 5,20%.

Ejemplo 39

Ácido 2-{[1-({[3-(metilamino)-3-oxopropil]amino}carbonil)ciclopentil]metil}-4-fenil-butanoico

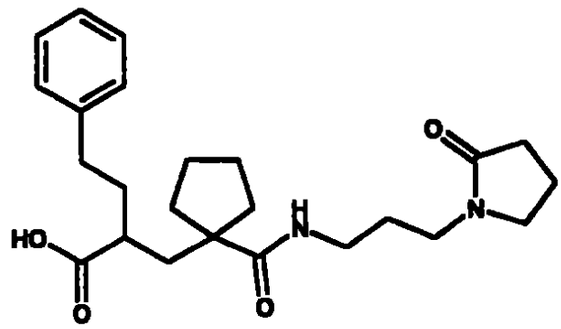


Una mezcla del éster bencílico de la Preparación 68 (160 mg, 0,34 mmol) y paladio al 10% sobre carbón vegetal (100 mg) en etanol (30 ml) se hidrógeno a temperatura ambiente y 4,2 kg/cm^2 durante 18 horas. La mezcla se filtró a través de Arbocel® y el filtrado se concentró a presión reducida y se destiló azeotrópicamente con diclorometano. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de diclorometano:metanol:ácido acético (95:5:0 a 95:5:0,5) para proporcionar el compuesto del título como una espuma blanca, 100 mg, 79%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,40-1,70 (m, 8H), 1,95 (m, 3H), 2,10 (m, 1H), 2,35 (d, 3H), 2,59 (m, 2H), 2,75 (t, 3H), 3,42 (m, 2H), 6,25 (s ancho, 1H), 6,70 (s ancho, 1H), 7,13-7,25 (m, 5H); y LRMS: m/z 375,0 (MH^+).

183

Ejemplo 40

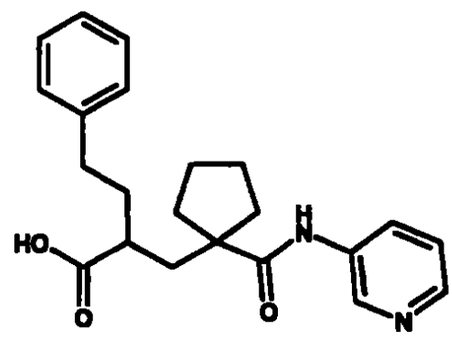
Ácido 2-{[1-({[3-(2-oxo-1-pirrolidinil)propil]amino}carbonil)ciclopentil]metil}-4-fenil-butanoico



Una mezcla del éster bencílico de la Preparación 67 (780 mg, 1,55 mmol) y paladio al 10% sobre carbón vegetal (100 mg) en etanol:agua (90:10 en volumen) (30 ml), se hidrogenó a temperatura ambiente una presión de H_2 de 4,2 kg/cm^2 durante 1,5 horas. El catalizador se separó por filtración y el filtrado se evaporó a presión reducida para proporcionar el compuesto del título como una espuma blanca, 473 mg, 74%; ^{1}H NMR ($CDCl_3$, 300 MHz) d: 1,26-1,77 (m, 10H), 1,78-2,46 (m, 11H), 2,49-2,70 (m, 2H), 2,95-3,36 (m, 4H), 6,92-7,38 (m, 5H); Anál. Encontrado: C, 64,05; H, 7,73; N, 6,22. $C_{24}H_{34}N_2O_4$:0,75H_2O requiere C, 65,88; H, 7,83; N, 6,40%.

Ejemplo 41

Ácido 4-fenil-2-({1-[(3-piridinilamino)carbonil]ciclopentil}metil)butanoico

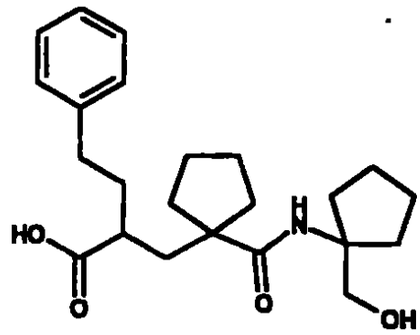


Una mezcla del éster bencílico de la Preparación 71 (700 mg, 1,53 mmol) y paladio al 5% sobre carbón vegetal (70 mg) en etanol:agua (90:10 en volumen, 50

ml) se hidrogenó a temperatura ambiente a una presión de H_2 de 2,1 kg/cm^2 durante 5 horas. El catalizador se filtró a través de Arbocel®, lavando bien con etanol, y el filtrado se evaporó a presión reducida. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol (95:5) como eluyente para proporcionar el compuesto del título como una espuma blanca, 510 mg, 91%; p.f. 80-85 °C (se aplasta dando una goma); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1,40-2,78 (m, 15H), 6,93-7,39 (m, 5H), 7,93 (m, 1H), 8,59 (d, 1H), 9,17 (d, 1H), 9,41 (s, 1H). Anál. Encontrado: C, 70,83; H, 7,10; N, 7,64. $C_{22}H_{26}N_2O_3$;0,3H_2O requiere C, 70,94; H, 7,22; N, 7,52%.

Ejemplo 42

Ácido 2-{[1-({[1-(hidroximetil)ciclopentil]amino}carbonil)ciclopentil]metil}-4-fenilbutanoico

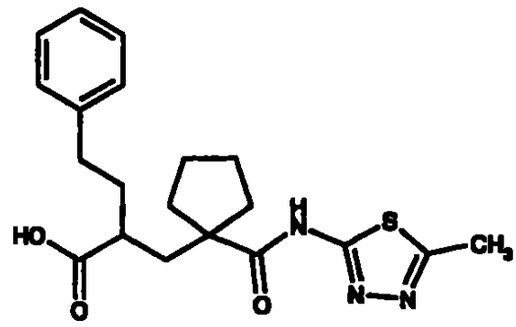


Una mezcla del éster bencílico de la Preparación 69 (118 mg, 0,25 mmol) y paladio al 10% sobre carbón vegetal (100 mg) en etanol (20 ml) se hidrogenó a temperatura ambiente y 4,2 kg/cm^2 durante 18 horas. La mezcla se filtró a través de Arbocel®, el filtrado se concentró a presión reducida y se destiló azeotrópicamente con diclorometano para dar el compuesto del título como una goma incolora, 95 mg, 98%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1,41-1,80 (m, 17H), 1,90 (m, 1H), 1,92-2,20 (m, 3H), 2,40 (m, 1H), 2,60 (m, 2H), 3,60 (d, 1H), 3,71 (d, 1H), 5,80 (s ancho, 1H), 7,15-7,30 (m, 5H); LRMS: m/z 388,1 (MH^+).

185

Ejemplo 43

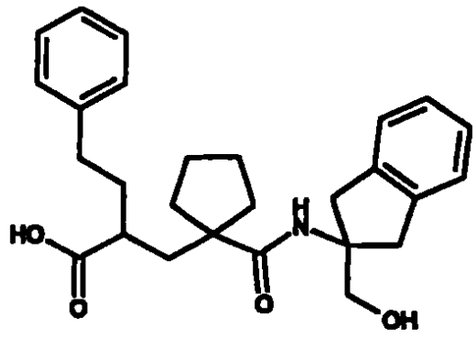
Ácido 2-[(1-{[(5-metil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]-4-fenil-butanoico



Una mezcla del éster bencílico de la Preparación 70 (187 mg, 0,39 mmol) y paladio al 10% sobre carbón vegetal (80 mg) en etanol (20 ml) se hidrogenó a 4,2 kg/cm^2 durante 18 horas. El análisis por tlc mostró la presencia de material de partida, así que se añadió más paladio al 10% sobre carbón vegetal (100 mg), y la reacción continuó durante 5 horas más. El análisis por tlc mostró de nuevo material de partida, así que se añadió más catalizador (100 mg), y la hidrogenación se continuó durante 18 horas. La mezcla se filtró a través de Arbocel® y el filtrado se concentró a presión reducida y se destiló azeotrópicamente con diclorometano. El producto bruto se purificó por cromatografía sobre gel de sílice utilizando una columna Biotage® y diclorometano:metanol (95:5) como eluyente para proporcionar el compuesto del título como un aceite claro, 80 mg, 53%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1,51-1,89 (m, 9H), 2,03 (m, 1H), 2,20 (m, 1H), 2,40 (m, 2H), 2,60 (m, 5H), 7,15-7,30 (m, 5H); LRMS: m/z 387,3 (MH^+).

Ejemplo 44

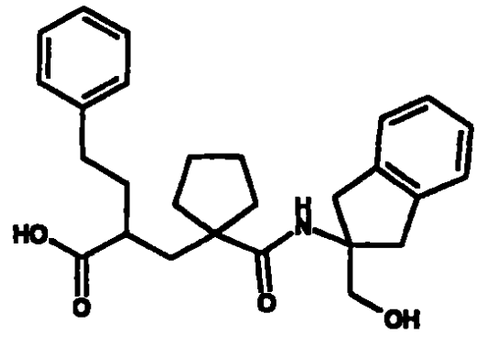
Ácido (*R*)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)ciclopentil]-metil}-4-fenilbutanoico



y

Ejemplo 45

Ácido (*S*)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)ciclopentil]-metil}-4-fenilbutanoico

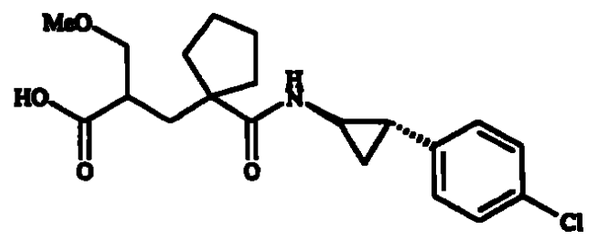


El ácido (+)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}-4-fenilbutanoico (documento WO 9110644) se puede purificar por procedimientos convencionales de HPLC utilizando una columna AD y hexano:isopropanol:ácido trifluoroacético (70:30:0,2) como eluyente, para dar el compuesto del título del Ejemplo 44, 99,5% ee; $[\alpha]_D$ = +9,1° (c = 1,76 en etanol); y el compuesto del título del Ejemplo 45, 99,5% ee; $[\alpha]_D$ = -10,5° (c = 2,2 en etanol).

187

Ejemplo 46

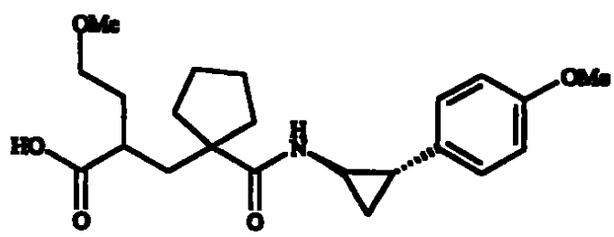
Ácido *trans*-3-[1-({[2-4-(clorofenil)ciclopropil]amino}carbonil)ciclopentil]-2-(metoximetil)propanoico



El producto de la Preparación 72 (160 mg, 0,39 mmol) se recogió en 50 ml de DCM y se enfrió a 0°C. Luego se burbujeó gas cloruro de hidrógeno a través de la solución durante 15 min y después se dejó agitando a temperatura ambiente durante 16 h. La mezcla de reacción se concentró a vacío y luego se purificó por cromatografía en columna utilizando MeOH:DCM 5:95 como eluyente para proporcionar el ácido como una película incolora. (18 mg, 12%); R_f 5:95 (DCM:MeOH) 0,2; ^1H NMR (400 MHz, $CDCl_3$) 1,04-1,18 (m, 2H), 1,20-1,36 (m, 2H), 1,36-1,70(m, 7H), 1,83-2,08 (m, 4H), 2,57-2,66 (m, 1H), 2,73-2,83 (m, 1H), 3,27 (s, 3H, OMe), 3,32-3,41 (m, 1H), 3,48 (app. dd, 1H, CHOMe), 6,21 (s, NH), 7.03 (d, 2H, Ar), 7,18 (d, 2H, Ar); LRMS: m/z 378; HRMS Encontrado MH+ 380. 1622. Calculado MH+ 380. 1623.

Ejemplo 47

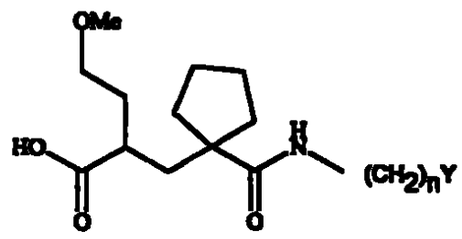
Ácido *trans*-3-[1-({[2-(4-metoxifenil)ciclopropil]amino}carbonil)ciclopentil]-2-(metoxietil)propanoico



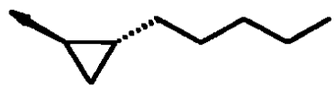
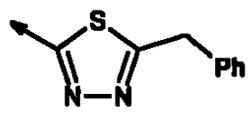
El producto de la Preparación 81 (113 mg, 0,25 mmol) se recogió en una

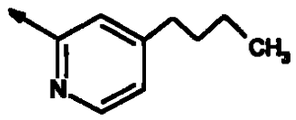
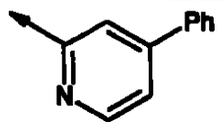
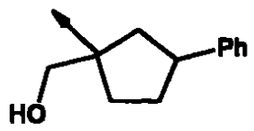
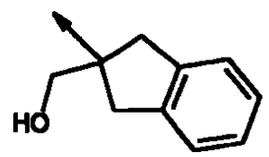
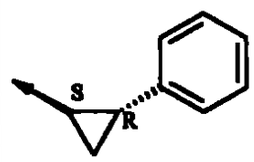
solución 4 M de cloruro de hidrógeno en dioxano (10 ml) y se agitó durante 3 h. Se concentró a vacío y se purificó por cromatografía en columna utilizando MeOH:DCM (5:95) como eluyente para proporcionar el ácido como una película incolora (45mg, 44%); R_f (DCM:MeOH 95:5) 0,2; LRMS: m/z M-H, 388; ^{1}H NMR (400 MHz, $CDCl_3$) 1,01-1,22 (m, 2H), 1,40-2,22 (m, 15H), 2,42-2,57 (m, 1H), 2,73-2,82 (m, 1H), 3,23 (s, 3H, OMe), 3,27-3,44 (m, 2H), 3,72 (s, 3H, OMe), 6,12 (s, 1H, NH), 6,78 (d, 2H, Ar), 7,06 (d, 2H, Ar).

Los compuestos de fórmula Id, es decir los compuestos de fórmula I donde R^1 es metoxietilo, fueron preparados a partir de: a) el éster terc-butílico correspondiente, siguiendo un procedimiento similar al descrito en el Ejemplo 47, o bien b) el éster bencílico indicado siguiendo un procedimiento similar al descrito en el Ejemplo 42.

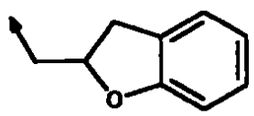


(Id)

Ej.	Prec.	-$(CH_2)_nY$	Datos
48	Prep. 89		^{1}H NMR (400 MHz, $CDCl_3$) δ: 0,50-0,63 (m, 3H), 0,82 (t, 3H, Me), 0,77-08,4 (m, 1H), 1,01-1,18 (m, 1H), 1,20-1,78 (m,), 1,82-2,08 (m, 2H), 3,27 (s, 3H, OMe), 3,33-3,41 (m, 2H), 5,92 (s, 1H, NH). LRMS: m/z 352 (M-H)
49	Prep. 95		^{1}H NMR (400 MHz, $CDCl_3$) δ 0,62-2,57 (m, 13H), 3,07 (s, 3H, OMe), 2,96-3,44 (m, 2H), 4,09 (s, 2H), 7,20 (s ancho, 5H). HRMS: Encontrado m/z 418,1796. $C_{21}H_{27}H_3O_4S$ requiere m/z 418,1795.

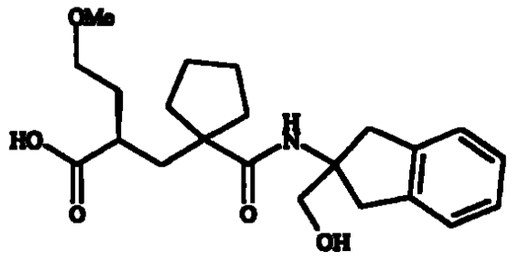
Ej.	Prec.	-$(CH_2)_nY$	Datos
50	Prep. 91		^{1}H NMR (400 MHz, $CDCl_3$) δ 0,92 (t, 3H), Me), 1,24-2,60 (m, 19H), 3,25 (s, 3H, OMe), 3,39 (t, 2H, CH_2OMe), 6,68-6,71 (m, 1H, Ar), 7,63-7,70 (m, 1H, Ar), 8,21 (s, 1H, Ar), 9,77 (s ancho, NH). LRMS: m/z 376 (M^+).
51	Prep. 92		^{1}H NMR (400 MHz, $CDCl_3$) δ 1,24-2,34 (m, 10H), 2,37-2,54 (m, 1H), 2,54-2,73 (m, 2H), 3,33 (s, 3H, OMe), 3,38-4,49 (m, 2H), 7,00 (d, 1H, Ar), 7,38-7,56 (m, 3H), 7,59-7,69 (m, 2H), 7,80 (t, 1H, Ar), 8,66 (s, 1H, Ar), 9,77-9,93 (m, NH). LRSM: m/z 395 (M-H).
52	Prep. 88		^{1}H NMR (400 MHz, $CDCl_3$) δ 0,84 (t ancho, 3H), 1,20-2,20 (m, 19H), 2,24-2,58 (m, 2H), 3,07-3,33 (m, 1H), 3,60-3,96 (m, 2H), 5,82-5,98 (m, 1H), 7,14-7,36 (m, 5H). LRMS: m/z M-H 400.
53	Prep. 93		^{1}H NMR (400 MHz, $CDCl_3$) δ: 1,43-1,76 (m, 7H), 1,80-2,24 (m, 4H), 2,57-2,68 (m, 2H), 3,06 (d, 1H), 3,12 (d, 1H), 3,27 (d, 1H), 3,32 (s, 3H), 3,36-3,48 (m, 2H), 3,80 (d, 1H), 3,87 (d, 1H), 6,04 (s, 1H), 7,16-7,22 (m, 4H).
54	Prep. 81a		^{1}H NMR (400 MHz, $CDCl_3$) δ: 1,02-1,26 (m, 2H), 1,37-1,84 (m, 7H), 1,85-2,16 (m, 4H), 2,62 (s ancho, 1H), 2,80-2,93 (m, 1H), 3,29 (s, 3H, Me), 3,22-3,58 (m, 2H), 6,21 (s ancho, 1H), 7,03-7,34 (m, 5H). LRMS m/z M+H 346 HRMS m/z M+H Encontrado 346,2011. $C_{20}H_{27}NO_4$ requiere 346,2013.

190

Ej.	Prec.	-$(CH_2)_nY$	Datos
55	Prep. 96		^{1}HNMR ($CDCl_3$, 400 MHz) δ: 1,45-1,67 (m, 8H), 1,85-2,00 (m, 4H), 2,05 (m, 1H), 2,46 (s ancho, 1H), 2,90 (dd, 1H), 3,30 (s, 3H), 3,35 (m, 2H), 3,40 (m, 2H), 3,70 (m ancho, 1H), 4,90 (s ancho, 1H), 6,25 (s ancho, 1H), 6,75 (d, 1H), 6,81 (t, 1H), 7,10 (d, 1H),7,15 (t, 1H); LRMS m/z M-H 374; HRMS m/z M+H Encontrado 376,2118. $C_{20}H_{27}NO_4$ requiere 376,2123.

Ejemplo 56

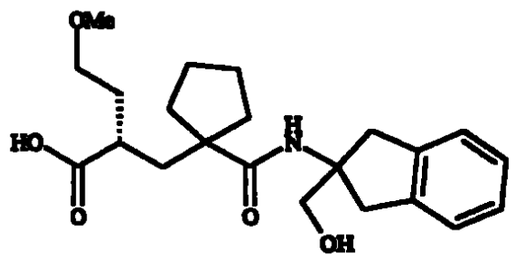
Ácido (*R*)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)ciclopentil]-metil}-4-metoxibutanoico



y

Ejemplo 57

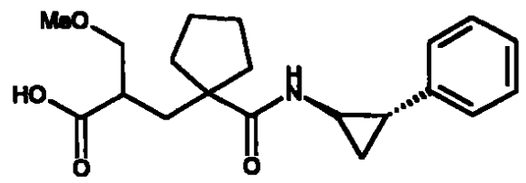
Ácido (*S*)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)ciclopentil]-metil}-4-metoxibutanoico



El producto del Ejemplo 53 se purificó por HPLC utilizando una columna Chiracel OD (250*20 mm) a temperatura ambiente utilizando una mezcla de 70% de hexano que contiene 0,3% de TFA y 0,2% de DEA y 30% de IPA que contiene 0,3% de TFA y 0,2% de DEA a un caudal de 10 ml/min. El Ejemplo 55 es el enantiómero R que eluye el primero después de 6 minutos (α_D 11,00, c=1 mg/ml en EtOH). El Ejemplo 56 es el enantiómero S que eluye el segundo después de 7 minutos (α_D -8,62 c 1,07 mg/ml en EtOH).

Ejemplo 58

Ácido 3-metoxi-2-{[(1-({[(trans)-2-fenilciclopropil]amino}carbonil)ciclopentil]metil}-propanoico



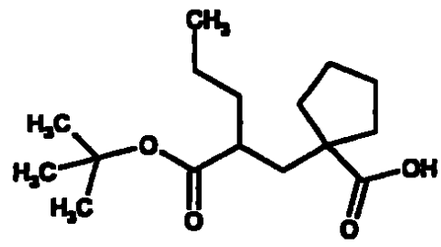
El compuesto del título se preparó de acuerdo con el procedimiento del Ejemplo 47 a partir del producto del título de la Preparación 82; ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 1,2 (m, 2H), 1,5 (m, 3H), 1,6 (bs, 3H), 1,8 (d, 1H), 2,0 (m, 4H), 2,6 (bs, 1H), 2,9 (bs, 1H), 3,3 (s, 3H), 3,4 (t, 1H), 3,5 (t, 1H), 6,4 (s, 1H), 7,1 (m, 3H), 7,3 (t, 2H); LRMS 344 (M-H).

Las siguientes Preparaciones describen la preparación de ciertos intermedios utilizados en los Ejemplos precedentes.

Preparaciones

Preparación 1

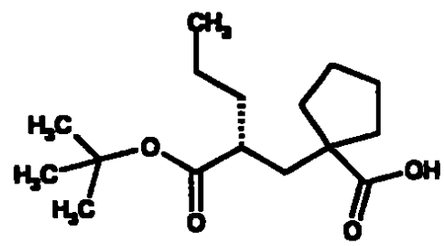
Ácido 1-[2-(terc-butoxicarbonil)-4-pentil]ciclopentanocarboxílico



Una mezcla del ácido 1-[2-(terc-butoxicarbonil)-4-pentenil]ciclopentanocarboxílico (documento EP 274234) (23 g, 81,5 mmol) y paladio al 10% sobre carbón vegetal (2 g) en etanol seco (200 ml) se hidrogenó a 2,1 kg/cm^2 y temperatura ambiente durante 18 horas. La mezcla de reacción se filtró a través de Arbocel®, y el filtrado se evaporó a presión reducida para dar un aceite amarillo. El producto bruto se purificó por cromatografía en columna sobre gel de sílice, utilizando acetato de etilo:pentano (40:60) como eluyente, para proporcionar el producto deseado como un aceite claro, 21 g, 91%; ^{1}H NMR ($CDCl_3$, 0,86 (t, 3H), 1,22-1,58 (m, 15H), 1,64 (m, 4H), 1,78 (dd, 1H), 2,00-2,18 (m, 3H), 2,24 (m, 1H); LRMS: m/z 283 $(M-H)^-$.

Preparación 2

Ácido 1-[(2*R*)-2-(terc-butoxicarbonil)-4-pentil]ciclopentanocarboxílico



Una mezcla del ácido (R)-1-[2-(terc-butoxicarbonil)-4-pentenil]ciclopentanocarboxílico (documento WO 9113054) (10 g, 35,4 mmol) y paladio al 10% sobre carbón vegetal (600 mg) en etanol seco (25 ml) se hidrogenó a 1 atmósfera y temperatura ambiente durante 18 horas. La mezcla de reacción se filtró a través de

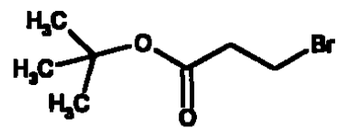
Arbocel®, y el filtrado se evaporó a presión reducida para dar el compuesto del título como un aceite amarillo, 9,6 g, 95%; ^{1}H NMR ($CDCl_3$, 0,86 (t, 3H), 1,22-1,58 (m, 15H), 1,64 (m, 4H), 1,78 (dd, 1H), 2,00-2,18 (m, 3H), 2,24 (m, 1H); $[\alpha]_D$ = -3,3° (c = 0,09, etanol).

Alternativamente, el producto del título de la Preparación 2 se puede preparar como sigue:

Una solución del producto de la etapa f) siguiente (1,12 kg, 4,0 mol) en etanol (6,5 l) se dividió en dos porciones iguales que se sometieron a las siguientes condiciones de reacción. A una solución del producto de la etapa f) siguiente (561 g, 2,0 mol) en etanol (3,25 l) se añadió el catalizador de hidrogenación (50,5 g de Pd al 5% sobre carbono - 50% de humedad) y el recipiente de reacción se presurizó con gas hidrógeno (2,1 kg/cm^2). La reacción se agitó durante 18 horas a temperatura ambiente antes de recombinar los dos lotes y separar el catalizador por filtración. La torta del filtro se lavó con etanol (2 x 450 ml) y los filtrados combinados se concentraron después a vacío. La suspensión resultante se filtró y se añadió n-heptano (1 l). El disolvente se separó por destilación a vació y se añadió n-heptano (1 l). El disolvente se separó por destilación a vacío para dar el compuesto del título (1,1 kg, 386 mol, 97% de rendimiento) como un aceite amarillo que se utilizó directamente en la etapa siguiente; LRMS (APCI negativo): m/z $[M-H]^-$ 283; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,79-0,98 (t, 3H), 1,24-1,39 (m, 3H), 1,42-1.50 (s, 9H), 1,50-1,61 (m, 3H), 1,61-1,74 (m, 4H), 1,74-1,84 (m, 1H), 2,02-2,23 (m, 3H), 2,23-2,35 (m, 1H).

Preparación de materiales de partida

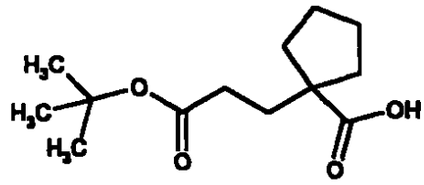
a) **3-Bromopropionato de terc-butilo**



A una solución de ácido 3-bromopropiónico (6,0 kg, 39,2 mol) en dicloro-metano (60 l) a 0°C se añadieron terc-butanol (0,6 l) y ácido sulfúrico concentrado (0,33 l). La solución resultante se enfrió a -15°C y se burbujeó

isobutileno (11 kg, 196 mol) a través de él. Luego la reacción se agitó durante 3 horas a -5°C antes de calentar a 20°C durante 4 horas y luego se agitó a esta temperatura durante 15 horas. La reacción se detuvo por adición cuidadosa en una solución acuosa saturada de bicarbonato sódico (0,6 M, 72 l, 43,2 mol). Después las capas se separaron y la capa orgánica se lavó con una solución acuosa saturada de bicarbonato sódico (2 x 48 l) seguida por agua desionizada (48 l). Este ciclo de lavado se repitió y el pH de la capa acuosa se midió y se observó que era superior a pH 7. Se añadió carbonato de potasio (90 g, 1,5% p/p) a la capa orgánica antes de concentrar la solución a un volumen de 9 l por destilación a presión atmosférica. Se añadió tetrahidrofurano (40 ml) y el resto del diclorometano se separó por destilación a presión atmosférica para dar una solución (12 l) del compuesto del título (5,27 kg, 25,2 mol, 64% de rendimiento) en tetrahidrofurano que se utilizó directamente en la etapa siguiente; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1,45 (s, 9H), 2,80 (t, 2H), 3,53 (t, 2H); LRMS (EI): m/z [MH+] 209 (^{79}Br).

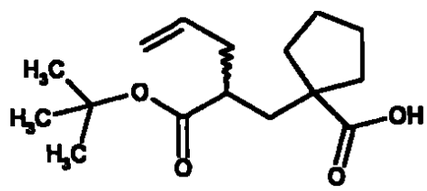
b) <u>Ácido 1-(3-terc-butoxi-3-oxopropil)ciclopentanocarboxílico</u>



A una solución de diisopropilamiduro de litio disponible en el mercado (20,2 kg de una solución 2 M en tetrahidrofurano/n-heptano/etilbenceno, 51,0 mol) a -15°C se añadió una solución de ácido ciclopentanocarboxílico (2,7 kg, 23,7 mol) en tetrahidrofurano acuoso (8,1 l) cuidadosamente a lo largo de un período de 1 hora con agitación en una atmósfera de nitrógeno. La solución resultante se agitó a 0°C durante 3 horas, tiempo durante el cual se formó un precipitado. Luego esta suspensión se añadió a una solución del producto de la etapa a) anterior (5,24 kg, 25,1 mol) en tetrahidrofurano (52 l) a -15°C a lo largo de un período de 1,25 horas. Una vez terminada la adición, la reacción se agitó a 0°C durante 1 hora y luego se calentó a 20°C a lo largo de 4 horas y se dejó agitar a esta temperatura

durante 13,5 horas. Luego la mezcla de reacción se enfrió a -15°C y a esto se añadió n-heptano (27 l) y ácido clorhídrico acuoso 5 M (23,7 l, 118,5 mol) cuidadosamente con agitación. Luego las capas se separaron y la fase acuosa se extrajo con n-heptano (13,5 l). Las fases orgánicas reunidas se extrajeron con solución acuosa de bicarbonato de sodio al 5% (3 x 30 l) y luego con solución acuosa de carbonato de potasio al 10% (3 x 30 l). Los extractos acuosos se separaron y se analizó el producto en cuanto a su contenido. Los tres extractos acuosos de carbonato de potasio se combinaron y se añadió n-heptano (27 l) antes de que el pH de esta mezcla fuera ajustado a pH 7-8 utilizando ácido clorhídrico acuoso 5 M (10,5 l) con agitación. Luego las capas se separaron y se añadió más n-heptano (40,5 l). Luego el pH de la mezcla se ajustó adicionalmente a pH 3 utilizando ácido clorhídrico acuoso 5 M (19,5 l). Luego las capas se separaron y la fase orgánica se lavó con agua desionizada (2 x 27 l). La fase orgánica se secó azeotrópicamente por destilación a vacío y el volumen de disolvente se redujo a aproximadamente 4 l. Luego la solución se enfrió a 0°C con agitación para permitir que ocurriera la cristalización. Se continuó la agitación a 0°C durante 5 horas, tiempo tras el cual el producto se recogió por filtración. El sólido resultante se secó a vacío a 50°C durante 22,5 horas para dar el compuesto del título (1,17 kg, 4,8 mol, 20% de rendimiento) como un sólido cristalino blanco; p.f.: 89-92°C; LRMS (APCI negativo): m/z $[M-H]^-$ 241; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1,47 (s, 9H), 1,50-1,60 (m, 2H), 1,60-18,2 (m, 4H), 1,69-2,0 (m, 2H), 2,08-2,23 (m, 2H), 2,23-2,34 (m, 2H).

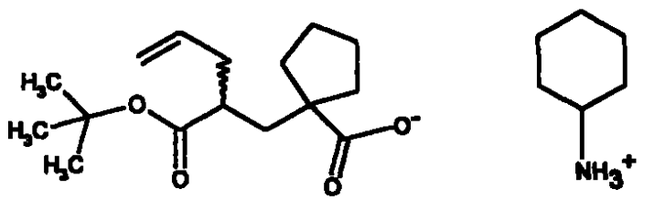
c) <u>Ácido 1-[2-(terc-butoxicarbonil)-4-pentenil]ciclopentanocarboxílico</u>



A una solución de diisopropilamiduro de litio disponible en el mercado (7,63 kg de una solución 2 M en tetrahidrofurano/n-heptano/etilbenceno, 19,3 mol) en tetrahidrofurano anhidro (18,3 l) a -10°C se añadió

una solución del producto de la etapa b) anterior (2,0 kg, 8,25 mol) en tetrahidrofurano anhidro (10 l) con agitación a lo largo de un período de 4 horas mientras que se mantenía la temperatura de reacción a -10°C. A la solución resultante se añadió una solución de bromuro de alilo (1,2 kg, 9,9 mol) en tetrahidrofurano (10 l) a lo largo de un período de 2 horas antes de calentar la reacción a 20°C a lo largo de un período de 4 horas. Después de agitar a esta temperatura durante 9,5 horas, la reacción se detuvo por adición de agua (40 l) y se separaron las dos fases. Luego la fase orgánica se extrajo sucesivamente con agua (20 l) y solución acuosa de hidróxido de potasio 0,3 M (12 l). Luego a las fases orgánicas reunidas se añadieron n-heptano (20 l) y ácido clorhídrico acuoso 5 M (7,5 l) hasta que el pH de la capa acuosa fue pH 2. Luego las capas se separaron y las fases acuosas se extrajeron con n-heptano (20 l). Luego las fases orgánicas reunidas se lavaron con salmuera saturada (2 x 8,0 l) y se concentraron a vacío para dar el producto bruto (2,22 kg, 7,86 mmol, 95% de rendimiento) como una solución en n-heptano (12,5 kg de peso total de solución) que se utilizó directamente en la etapa siguiente; LRMS (EI): $[M^+-C_4H_8]$ 226, $[M^+-HO^tBu]$ 208, [208-CO] 180; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1,45 (s, 9H), 1,48-1-60 (m, 2H), 1,60-1,76 (m, 4H), 1,80 (dd, 1H), 2,03-2,27 (m, 4H), 2,27-2,45 (m, 2H), 5,06 (dd, 2H), 5,75 (dd, 1H).

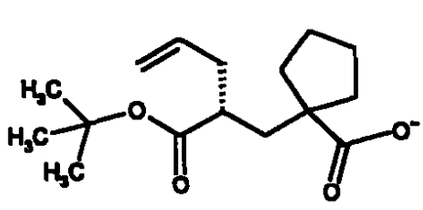
d) <u>1-[2-(terc-Butoxicarbonil)-4-pentenil]ciclopentanocarboxilato de ciclohexanaminio</u>

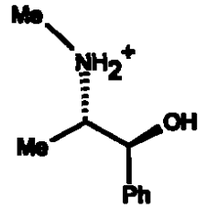


A una solución del producto bruto de la etapa c) anterior (3,83 kg, 13,6 mol) en n-heptano (23 l) se añadió ciclohexilamina (1,35 kg, 13,8 mol) en n-heptano (7,0 l) a lo largo de un período de 0,5 horas. Los conductos de adición se lavaron con n-heptano (0,7 l, 0,2 ml/g) y esto se añadió a la

mezcla de reacción. La suspensión resultante se granuló a 20°C durante 2 horas y luego el sólido se recogió por filtración. La torta del filtro se lavó con n-heptano (2 x 1,9 l) y se secó a vacío a 50°C durante 23 horas. El sólido blanco resultante (4,42 kg, 11,6 mol, 85% de rendimiento) se disolvió en acetato de etilo (24 l, 5,4 ml/g) y se calentó a 70°C para formar una solución clara. Luego la solución resultante se enfrió a 50°C y se sembró con compuesto auténtico (1 g). Luego la suspensión se enfrió de 50°C a 20°C a lo largo de un período de 4 horas. La suspensión se granuló a 20°C durante 0,5 horas y el sólido se recogió por filtración. La torta del filtro se lavó con acetato de etilo (2 x 1,8 l) y el sólido se secó a vacío a 45°C durante 14,5 horas para dar el compuesto del título (3,76 kg, 9,85 mol, 85% de recuperación) como un sólido cristalino blanco; p.f.: 129,5-131,0°C; Anál. Encontrado: C, 69,28; H, 10,31; N, 3,60. $C_{22}H_{39}NO_4$ requiere C, 69,25; H, 10,30; N, 3,67%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1,05-1,35 (m, 6H), 1,35-1,53 (m, 10H), 1,53-1,69 (m, 5H), 1,69-1,89 (m, 3H), 1,89-2,02 (m, 3H), 2,02-2,18 (m, 2H), 2,18-2,31 (m, 2H), 2,31-2,44 (m, 1H), 2,71-2,94 (m, 1H), 5,03 (dd, 2H), 5,73 (ddt, 1H), 6,4 (s ancho, 3H).

e) <u>1-[(2*R*)-2-(terc-Butoxicarbonil)-4-pentenil]ciclopentanocarboxilato de (1*S*,2*S*)-1-hidroxi-1-*N*-metil-1-fenil-2-propanaminio</u>



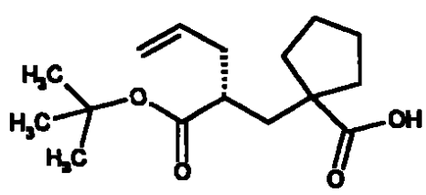


A una mezcla de agua (22,6 l) y n-heptano (22,6 l) se añadió el producto de la etapa d) anterior (3,76 kg, 9,85 mol) con agitación. Se añadió ácido clorhídrico acuoso 5 M (2,2 l, 11,0 mol) hasta que el pH de la fase acuosa fue pH 3. Las capas se separaron y la fase acuosa se extrajo adicionalmente con n-heptano (2 x 22,6 l). Luego los extractos orgánicos reunidos se lavaron con salmuera saturada (3,8 l, 10 ml/g) y después se concentraron a vacío hasta un volumen de 20,3 l de volumen total de

198

solución. A esta solución se añadió (1*S*,2*S*)-(+)-pseudoefedrina (1,63 kg, 9,86 mol, 1,0 equiv.) con agitación y la suspensión se calentó a 80°C, ocurriendo seguidamente la disolución completa. La solución resultante se mantuvo a esta temperatura durante 20 minutos antes de enfriar a 45°C. Luego se añadió una muestra de cristales de siembra (0,2 g) y la suspensión se enfrió a 20°C a lo largo de un período de 2 horas. La suspensión resultante se granuló durante un período de 4 horas y luego el sólido se recogió por filtración. Luego la torta del filtro se lavó con n-heptano (3 x 0,5 l) y se secó a vacío a 40-45°C durante 23 horas para dar un sólido blanco (2,15 kg, 4,8 mol, 49% de rendimiento). Una suspensión de este material (2,15 kg, 4,8 mol) en n-heptano (10,8 l) se calentó a 80°C para dar una solución clara. Después de mantener esta temperatura durante 10 minutos, la solución se enfrió a 60°C y se añadió una muestra de cristales de siembra (1 g). Luego la suspensión resultante se enfrió a 20°C a lo largo de un período de 2 horas y después se granuló durante 1,5 horas a esta temperatura. Luego el sólido se recogió por filtración, se lavó con n-heptano (2 x 0,59 l) y se secó a vacío a 50°C durante 17,5 horas para dar el compuesto del título (1,80 kg, 4,0 mol, 84% de recuperación) como un sólido cristalino blanco; p.f.: 109-110°C; Anál. Encontrado: C, 69,48; H, 9,25; N, 3,17. $C_{26}H_{41}NO_5$ requiere C, 69,77; H, 9,23; N, 3,17%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1,05 (d, 3H), 1,34-1,55 (m, 2H), 1,44 (s, 9H), 1,55-1,73 (m, 4H), 1,82-2,03 (m, 2H), 2,03-2,21 (m, 2H), 2,21-2,35 (m, 2H), 2,35-2,41 (m, 1H), 2,60 (s, 3H), 3,03 (pent., 1H), 4,52 (d, 1H), 5,03 (dd, 2H), 5,76 (ddt, 1H), 7,21-7,45 (m, 5H).

f) <u>Ácido 1-[(2*R*)-2-(terc-butoxicarbonil)-4-pentenil]ciclopentanocarboxílico</u>

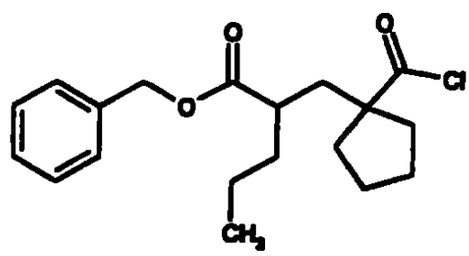


A una mezcla de agua desionizada (10,8 l) y n-heptano (10,8 l) se añadieron el producto de la etapa e) anterior (1,80 kg, 4,0 mol) y ácido

clorhídrico acuoso 5 M (1,3 l, 6,5 mol) hasta que el pH de la capa acuosa fue pH 3. Luego las capas se separaron y la capa acuosa se extrajo con n-heptano (2 x 10,8 l). Las capas orgánicas reunidas se lavaron con salmuera (1,8 l) y luego se concentraron por destilación a presión atmosférica a un volumen de 6,4 l. Luego se añadió etanol (18,0 l) y la solución se concentró de nuevo por destilación a presión atmosférica para dar el compuesto del título (1,14 kg, 4,0 mol, 100% de recuperación) como una solución en etanol (6,4 l de volumen total de solución) que se utilizó directamente en la etapa siguiente (véase lo anterior); LRMS (EI): $[M^+-C_4H_8]$ 226, $[M^+-HO^tBu]$ 208, [208-CO] 180; ^1H NMR (300 MHz, $CDCl_3$) δ: 1,45 (s, 9H), 1,48-1,60 (m, 2H), 1,60-1,76 (m, 4H), 1,80 (dd, 1H), 2,03-2,27 (m, 4H), 2,27-2,45 (m, 2H), 5,06 (dd, 2H), 5,75 (ddt, 1H).

Preparación 3

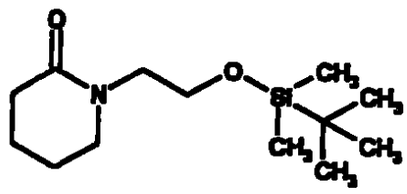
2-{[1-(Clorocarbonil)ciclopentil]metil}pentanoato de bencilo



Se añadió cloruro de oxalilo (1,15 ml, 13,2 mmol) a una solución enfriada con hielo de ácido 1-{2-[(benciloxi)carbonil]pentil}ciclopentanocarboxílico (documento EP 274234) (2,0 g, 6,3 mmol) en diclorometano seco (20 ml), y la solución se agitó a temperatura ambiente durante 2 horas. La mezcla de reacción se concentró a presión reducida y el residuo se destiló azeotrópicamente con diclorometano (3 x), para dar el compuesto del título como un aceite de color dorado, 2,1 g; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0,88 (t, 3H), 1,28 (m, 2H), 1,43 (m, 2H), 1,63 (m, 6H), 2,00 (m, 1H), 2,08-2,35 (m, 3H), 2,44 (m, 1H), 5,15 (s, 2H), 7,28 (m, 5H).

Preparación 4

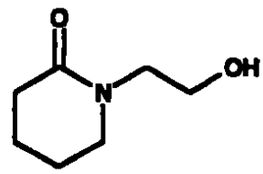
1-(2-{[terc-Butil(dimetil)silil]oxi}etil)-2-piperidinona



Se añadió poco a poco hidruro de sodio (807 mg, dispersión al 60% en aceite mineral, 20,18 mmol) a una solución de d-valerolactama (2,0 g, 20,2 mmol) en tetrahidrofurano (100 ml) y en atmósfera de nitrógeno. Se añadió poco a poco (2-bromoetoxi)(terc-butil)dimetilsilano (4,33 ml, 20,2 mmol), y la reacción se calentó a 70°C durante 18 horas. Se añadió agua (50 ml) a la reacción enfriada, la mezcla se concentró a vacío para separar el tetrahidrofurano, y se extrajo con acetato de etilo (200 ml). La solución orgánica se secó ($MgSO_4$) y se evaporó a presión reducida para dar un aceite amarillo. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando una gradiente de elución de diclorometano:metanol (98:2 a 97:3) para dar el compuesto del título, 3,25 g; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0,00 (s, 6H), 0,83 (s, 9H), 1,75 (m, 4H), 2,35 (m, 2H), 3,39 (m, 4H), 3,75 (t, 2H); LRMS: m/z 257,9 (M^+).

Preparación 5

1-(2-Hidroxietil)-2-piperidinona

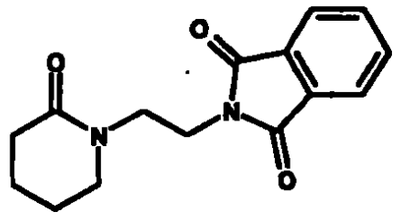


Se añadió fluoruro de tetra-n-butilamonio (14 ml, solución 1 M en tetrahidrofurano, 14 mmol) a una solución de la lactama de la Preparación 4 (3,3 g, 12, 8 mmol) en tetrahidrofurano (50 ml), y la reacción se agitó a temperatura ambiente durante 2 horas. La mezcla se concentró a presión reducida, el residuo se destiló azeotrópicamente con diclorometano y se purificó por cromatografía en columna sobre gel de

sílice utilizando un gradiente de elución de diclorometano:metanol (97:3 a 95:5) para dar el compuesto del título como un aceite; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,80 (m, 4H), 2,40 (t, 2H), 3,38 (t, 2H), 3,42 (t, 1H), 3,56 (t, 2H), 3,80 (t, 2H).

Preparación 6

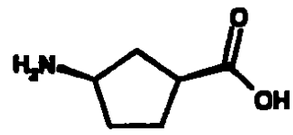
2-[2-(2-Oxo-1-piperidinil)-1*H*-isoindol-1,3-(2*H*)-diona



Se añadió ftalimida (952 mg, 6,47 mmol) a una solución del alcohol de la Preparación 5 (842 mg, 5,88 mmol) en tetrahidrofurano (30 ml), y la mezcla se sonicó hasta que se obtuvo una solución. Se añadieron trifenilfosfina sobre un soporte polímero (2,5 g, 7,5 mmol) y azodicarboxilato de dietilo (1,15 ml, 7,31 mmol), y la reacción se agitó a temperatura ambiente durante 18 horas. La mezcla se filtró a través de Arbocel®, el filtrado se concentró a presión reducida y el residuo se destiló azeotrópicamente con diclorometano. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de acetato de etilo:pentano (70:30 a 100:0) para dar el compuesto del título como una espuma blanca, 1,6 g (que contiene algunas impurezas); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,60-1,80 (m, 4H), 2,17 (m, 2H), 3,30 (m, 2H), 3,60 (m, 2H), 3,83 (m, 2H), 7,62 (m, 2H), 7,79 (m, 2H); LRMS: m/z 273,2 (MH^+).

Preparación 7

Ácido (1*S*,3*R*)-3-aminociclopentanocarboxílico

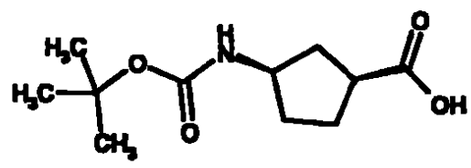


Se añadió óxido de platino (1 g) a una solución de ácido (1*R*,4*S*)-4-amino-

ciclopent-2-enocarboxílico (5,3 g, 41,7 mmol) en agua (70 ml), y la mezcla se hidrogenó a 3,15 kg/cm^2 y temperatura ambiente durante 18 horas. La mezcla se filtró a través de Arbocel®, el filtrado se evaporó a presión reducida y el residuo se destiló azeotrópicamente con tolueno para proporcionar el compuesto del título como un sólido blanquecino; ^{1}H NMR (D_2=, 400 MHz) δ: 1,70-1,92 (m, 3H), 2,00 (m, 2H), 2,18 (m, 1H), 2,77 (m, 1H), 3,68 (m, 1H); LRMS: m/z 129,8 (MH^+).

Preparación 8

Ácido (1*S*,3*R*)-3-[(terc-butoxicarbonil)amino]ciclopentanocarboxílico

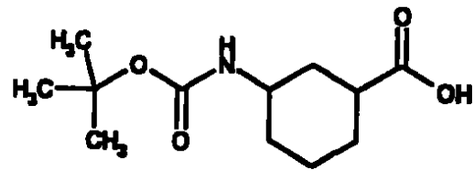


Se añadió dicarbonato de di-terc-butilo (10 g, 45,8 mmol) a una solución enfriada con hielo del aminoácido de la Preparación 7 (5,4 g, 41,8 mmol) en dioxano (42,5 ml) y solución de hidróxido de sodio (42,5 ml, 1 N, 42,5 mmol), y la reacción se agitó a temperatura ambiente durante 18 horas. La mezcla de reacción se concentró a presión reducida para separar el dioxano, luego se aciduló a pH 2 utilizando ácido clorhídrico 2 N. La solución acuosa se extrajo con acetato de etilo (5 x 100 ml), los extractos orgánicos reunidos se secaron ($MgSO_4$) y se evaporaron a presión reducida para dar un sólido blanco. Éste se trituró con hexano para dar el compuesto deseado como un sólido cristalino, 8,0 g, 83%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,41 (s, 9H), 1,58-2,06 (m, 5H), 2,21 (m, 1H), 2,84 (m, 1H), 4,01 (m, 1H), 4,84 (m, 1H); LRMS: m/z 228 $(M-H)^-$.

203

Preparación 9

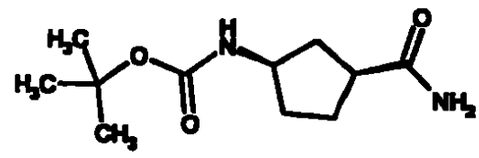
Ácido 3-[(terc-butoxicarbonil)amino]ciclohexanocarboxílico



El compuesto del título se obtuvo como un sólido blanco con 81% de rendimiento, a partir del ácido 3-aminociclohexanocarboxílico, siguiendo el procedimiento descrito en la Preparación 8; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,04 (m, 1H), 1,19-1,50 (m, 13H), 1,83 (m, 1H), 1,97 (m, 2H), 2,24 (m, 1H), 2,40 (m, 1H), 3,44 (s ancho, 1H), 4,42 (s ancho, 1H).

Preparación 10

(1*R*,3*S*)-3-(Aminocarbonil)ciclopentilcarbamato de terc-butilo

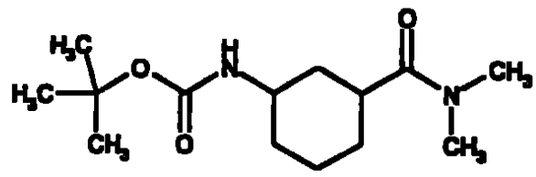


Se añadieron secuencialmente hexafluorofosfato de benzotriazol-1-iloxitris-(pirrolidino)fosfonio (3,4 g, 6,54 mmol), hidrato de 1-hidroxibenzotriazol (883 mg, 6,54 mmol), cloruro de amonio (467 mg, 8,72 mmol) y N-etildiisopropilamina (3,04 ml, 17,5 mmol) a una solución del ácido de la Preparación 8 (1,0 g, 4,37 mmol) en N,N-dimetilformamida (16 ml), y la reacción se agitó a temperatura ambiente durante 2 horas. La mezcla se diluyó con acetato de etilo (100 ml), se lavó con agua (3 x) y salmuera, luego se secó ($MgSO_4$) y se evaporó a presión reducida. La goma residual se purificó por cromatografía sobre gel de sílice utilizando una columna Biotage® y un gradiente de elución de diclorometano:metanol (98:2 a 95:5). El producto se trituró con éter para proporcionar el compuesto del título como un sólido blanco, 438 mg, 44%; ^{1}H NMR ($DMSOd_6$, 400 MHz) δ: 1,34 (s, 9H), 1,40 (m, 2H), 1,64 (m, 3H), 1,90 (m, 1H), 2,55 (m, 1H), 3,70 (m, 1H), 6,70 (s ancho, 1H), 6,80 (d, 1H), 7,22 (s

ancho, 1H).

Preparación 11

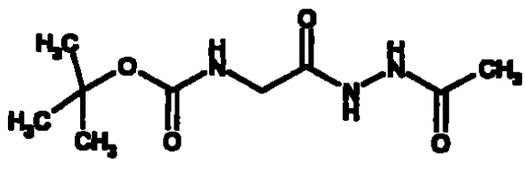
3-[(Dimetilamino)carbonil]ciclohexilcarbamato de terc-butilo



Se añadieron hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (1,19 g, 6,19 mmol), hidrato de 1-hidroxibenzotriazol (840 mg, 6,19 mmol), N-metilmorfolina (1,1 ml, 10,1 mmol) y finalmente dimetilamina etanólica al 33% (1,5 ml) a una solución del ácido de la Preparación 9 (1,37 g, 5,6 mmol) en N,N-dimetilformamida (30 ml), y la reacción se agitó a temperatura ambiente durante 18 horas. La mezcla se concentró a presión reducida, el residuo se diluyó con acetato de etilo y se lavó con agua (2 x). La mezcla se secó ($MgSO_4$) y se evaporó a presión reducida. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de metanol:diclorometano (5:95 a 10:90) para dar el compuesto del título, 998 mg, 66%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1,12 (m, 1H), 1,40 (m, 11H), 1,70 (m, 2H), 1,85 (m, 1H), 2,00 (m, 2H), 2,62 (m, 1H), 2,96 (s, 3H), 3,05 (s, 3H), 3,50 (m, 1H), 4,50 (m, 1H).

Preparación 12

2-(2-Acetilhidrazino)-2-oxoetilcarbamato de terc-butilo

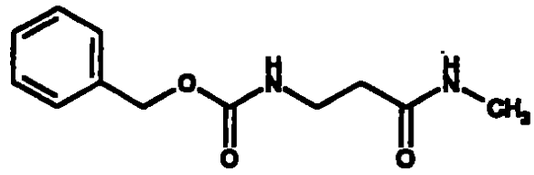


Se añadió 2-etoxi-1-etoxicarbonil-1,2-dihidroquinolina (7,06 g, 28,5 mmol) a una solución de N-terc-butoxicarbonilglicina (5,0 g, 28,6 mmol) en diclorometano (75 ml), y la solución se agitó durante 15 minutos. Se añadió hidrazida acética (2,6

g, 35,1 mmol) y la reacción se agitó a temperatura ambiente durante 18 horas. El precipitado resultante se separó por filtración y se secó a vacío, para proporcionar un sólido cristalino blanco, 2,42 g. El filtrado se concentró a presión reducida, se diluyó con éter y el precipitado resultante se filtró y se secó a vacío, para proporcionar el producto adicional como un sólido blanco, 4,4 g, 67% en total; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,41 (s, 9H), 2,02 (s, 3H), 3,87 (d, 2H), 5,22 (s ancho, 1H), 8,27 (s ancho, 1H), 8,84 (s ancho, 1H); LRMS: m/z 249,2 (MNH_4^+); Anál. Encontrado: C, 46,41; H, 7,36; N, 17,98. $C_9H_{17}N_3O_4$ requiere C, 46,66; H, 7,41; N, 18,13%.

Preparación 13

3-(Metilamino)-3-oxopropilcarbamato de bencilo

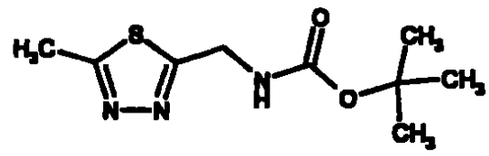


Una mezcla de N-[(benciloxi)carbonil]-β-alanina (10 g, 44,8 mmol), hidrocloruro de metilamina (3,33 g, 49,28 mmol), hidrato de 1-hidroxibenzotriazol (6,05 g, 44,8 mmol), hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (10,3 g, 53,76 mmol) y N-metilmorfolina (11,33 ml, 103 mmol) en diclorometano (200 ml) se agitó a temperatura ambiente durante 18 horas. El precipitado resultante se separó por filtración para dar el producto deseado como una espuma incolora, y el filtrado se evaporó a presión reducida. El residuo se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de acetato de etilo:hexano (90:10 a 100:0) para dar el producto adicional, 7,96 g, 75% en total; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2,42 (t, 2H), 2,80 (s, 3H), 3,50 (m, 2H), 5,21 (s, 2H), 5,59 (s ancho, 1H), 5,63 (s ancho, 1H), 7,36 (m, 5H); Anál. Encontrado: C, 60,68, H, 7,00; N, 11,95. $C_{12}H_{16}N_2O_3$ requiere C, 61,00; H, 6,83; N, 11,86%.

206

Preparación 14

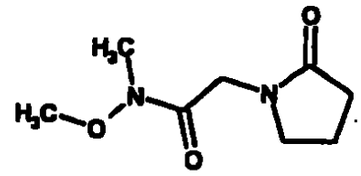
(5-Metil-1,3,4-tiadiazol-2-il)metilcarbamato de terc-butilo



Se añadió reactivo de Lawesson (960 mg, 2,38 mmol) a una solución de la hidrazida de la Preparación 12 (500 mg, 2,16 mmol) en tetrahidrofurano (40 ml), y la reacción se calentó a reflujo durante 3 horas y luego se agitó a temperatura ambiente durante 18 horas. La mezcla se evaporó a presión reducida y el residuo se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de acetato de etilo:pentano (70:30 a 80:20) para dar un aceite. Éste se disolvió en acetato de etilo (100 ml), se añadió carbón vegetal (2 g), la mezcla se agitó durante 10 minutos y luego se filtró. El filtrado se concentró a presión reducida y el residuo se destiló azeotrópicamente con diclorometano para proporcionar el compuesto del título como un sólido cristalino, 441 mg, 89%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,45 (s, 9H), 2,77 (s, 3H), 4,66 (d, 2H), 5,22 (s ancho, 1H); LRMS: m/z 230,1 (MH^+).

Preparación 15

***N*-Metoxi-*N*-metil-2-(2-oxo-1-pirrolidinil)acetamida**

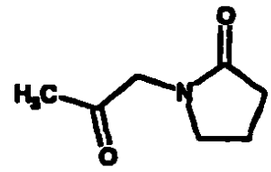


Se añadió 2-cloro-N-metoxi-N-metilacetamida (3,2 g, 23,3 mmol) a una suspensión de 2-pirrolidinona (2,0 g, 23,5 mmol) e hidruro de sodio (940 mg, dispersión al 60% en aceite mineral, 23,5 mmol) en tetrahidrofurano (60 ml), y la reacción se agitó a temperatura ambiente durante 48 horas. La mezcla se inactivó con agua (150 ml) y se extrajo con acetato de etilo (200 ml) y diclorometano (200 ml). Los extractos orgánicos reunidos se secaron ($MgSO_4$) y se evaporaron a presión

reducida. El residuo se trituró con hexano y luego con éter para proporcionar el compuesto del título como cristales blancos, 1,8 g, 41%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2,02 (m, 2H), 2,40 (t, 2H), 3,17 (s, 3H), 3,48 (t, 2H), 3,72 (s, 3H), 4,19 (s, 2H); LRMS: m/z 186,9 (MH^+).

Preparación 16

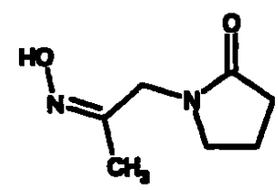
1-(2-Oxopropil)-2-pirrolidinona



Se añadió cloruro de metilmagnesio (2,7 ml, 3 M en tetrahidrofurano, 8,1 mmol) a una solución enfriada (-20°C) de la amida de la Preparación 15 (1,5 g, 8,1 mmol) en tetrahidrofurano (50 ml), y la reacción se dejó calentar a temperatura ambiente y luego se agitó durante una hora. La mezcla se inactivó por adición de una solución acuosa de cloruro de amonio y luego se extrajo con acetato de etilo (3 x 50 ml). Las soluciones orgánicas combinadas se secaron ($MgSO_4$) y se evaporaron a presión reducida para dar el compuesto del título como un aceite, 645 mg, 56%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2,07 (m, 2H), 2,17 (s, 3H), 2,42 (t, 2H), 3,42 (t, 2H), 4,10 (s, 2H).

Preparación 17

1-[2-(Hidroxilimino)propil]-2-pirrolidinona

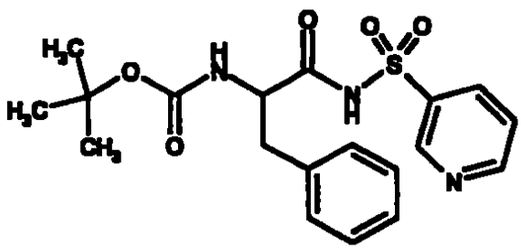


Se añadieron hidrocloruro de hidroxilamina (316 mg, 4,55 mmol) y luego piridina (370 µl, 4,58 mmol) a una solución de la amida de la Preparación 16 (643 mg, 4,55 mmol) en etanol (30 ml), y la reacción se agitó a temperatura ambiente durante

18 horas. La mezcla se evaporó a presión reducida y el residuo se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de diclorometano:metanol (97:3 a 90:10). El producto se trituró con éter para dar el compuesto del título como un sólido blanco, 375 mg, 53%; ^{1}Hmr (DMSOd_6, 400 MHz) δ: 1,60 (s, 3H), 1,87 (m, 2H), 2,20 (t, 2H), 3,19 (t, 2H), 3,78 (s, 2H), 10,77 (s, 1H); LRMS: m/z 157,4 (MH^+).

Preparación 18

1-Bencil-2-oxo-2-[(3-piridinsulfonil)amino]etilcarbamato de terc-butilo

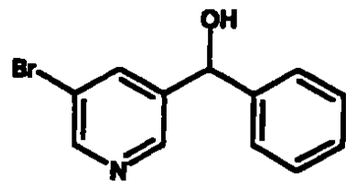


Se añadieron hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (939 mg, 4,9 mmol), hidrato de 1-hidroxibenzotriazol (562 mg, 4,15 mmol) y N-metilmorfolina (952 mg, 9,42 mmol) a una solución enfriada con hielo de N-terc-butoxicarbonil-L-fenilalanina (1,0 g, 3,77 mmol) en diclorometano (20 ml), y la mezcla se agitó durante 15 minutos. Se añadió 3-piridinsulfonamida (Mon. für Chemie; 7; 77; 1938) (596 mg, 3,77 mmol) y la reacción se agitó a temperatura ambiente durante 24 horas. La mezcla se evaporó a presión reducida y el residuo se repartió entre acetato de etilo (50 ml) y agua (50 ml) y se separaron las capas. La capa acuosa se extrajo bien con acetato de etilo y luego con diclorometano, los extractos orgánicos reunidos se secaron ($MgSO_4$) y se evaporaron a presión reducida. El producto bruto se purificó dos veces por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de acetato de etilo:etanol (100:0 a 90:10) para dar el producto deseado como una espuma blanca, 1,01 g, 66%; ^{1}H NMR (DMSOd_6, 300 MHz) δ: 1,30 (s, 9H), 2,77 (m, 1H), 2,97 (m, 1H), 3,84 (m, 1H), 5,95 (s ancho, 1H), 6,96 (m, 2H), 7,08 (m, 3H), 7,42 (m, 1H), 8,05 (d, 1H), 8,60 (d, 1H), 8,84 (m, 1H); $[\alpha]_D$ = -10° (solución en metanol al 0,1%).

209

Preparación 19

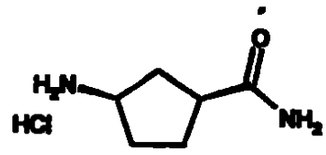
5-(Bromo-3-piridinil)(fenil)metanol



Se añadió gota a gota n-butil-litio (17 ml, 2,5 M en hexano, 42,5 mmol) a una solución enfriada (-78°C) de 3,5-dibromopiridina (10 g, 42,2 mmol) en éter (200 ml) para mantener una temperatura interna por debajo de -70°C. Luego la mezcla se agitó durante 15 minutos y se añadió gota a gota una solución de benzaldehído (4,5 g, 42,5 mmol) en éter (20 ml), manteniendo de nuevo la temperatura por debajo de -70°C. La mezcla se agitó durante 15 minutos, luego se dejó calentar a la temperatura ambiente a lo largo de una hora. La reacción se inactivó por adición de una solución de cloruro de amonio 0,9 M (200 ml), se separaron las capas y la fase acuosa se extrajo con éter. Los extractos orgánicos reunidos se secaron ($MgSO_4$) y se evaporaron a presión reducida. El aceite amarillo residual se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de diclorometano:éter (95:5 a 80:20) para dar el compuesto del título como un aceite amarillo, 7,6 g, 68%; ^1H NMR (D_2O, 300 MHz) δ: 5,80 (s, 1H), 7,37 (m, 5H), 7,90 (s, 1H), 8,40 (s, 1H), 8,44 (s, 1H).

Preparación 20

Hidrocloruro de (1*S*,3*R*)-3-aminociclopentanocarboxamida

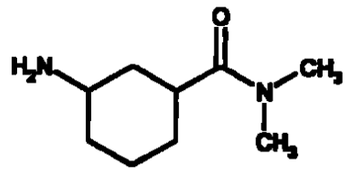


Se burbujeó gas cloruro de hidrógeno a través de una solución enfriada con hielo de la amida de la Preparación 10 (438 mg, 1,92 mmol) en diclorometano (50 ml) durante 10 minutos, y la suspensión resultante se agitó a temperatura ambiente durante 2 horas. La mezcla se purgó con nitrógeno y luego se evaporó a presión

reducida. El residuo se trituró con éter para proporcionar el compuesto del título como un sólido; ^{1}H NMR (D_2O, 400 MHz) δ: 1,63-1,82 (m, 3H), 1,92-2,07 (m, 2H), 2,19 (m, 1H), 2,82 (m, 1H), 3,62 (m, 1H).

Preparación 21

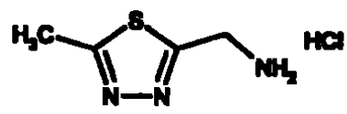
3-Amino-N,N-dimetilciclohexanocarboxamida



Una solución de la amida de la Preparación 11 (997 mg, 3,69 mmol) en ácido trifluoroacético (8 ml) y diclorometano (8 ml) se agitó a temperatura ambiente durante 4 horas. La mezcla se concentró a presión reducida y el residuo se repartió entre diclorometano (25 ml) y una solución de bicarbonato de sodio (25 ml). El pH se ajustó a 9 utilizando una solución de hidróxido de sodio, se separaron las capas y la fase acuosa se evaporó a presión reducida. El sólido resultante se trituró con acetato de etilo caliente, la suspensión se filtró y el filtrado se concentró a presión reducida. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol:amoníaco 0,88 (84:14:2) para proporcionar el compuesto del título como un aceite incoloro, 346 mg, 55%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1,08 (m, 1H), 1,25-1,54 (m, 6H), 1,72 (m, 1H), 1,86 (m, 2H), 2,53-2,75 (m, 2H), 2,96 (s, 3H), 3,03 (s, 3H).

Preparación 22

Hidrocloruro de (5-metil-1,3,4-tiadiazol-2-il)metilamina

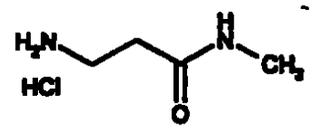


Se burbujeó gas cloruro de hidrógeno a través de una solución enfriada con hielo del tiadiazol de la Preparación 14 (425 mg, 1,85 mmol) en diclorometano (50 ml)

durante 15 minutos, y la reacción se agitó a temperatura ambiente durante 1 hora. La mezcla se purgó con nitrógeno y luego se evaporó a presión reducida para proporcionar el compuesto del título como un sólido blanco; ^{1}H NMR (DMSOd_6, 400 MHz) δ: 2,75 (s, 3H), 4,48 (m, 2H), 8,80 (s ancho, 3H).

Preparación 23

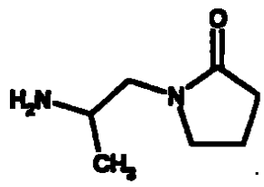
Hidrocloruro de 3-amino-N-metilpropanamida



Una mezcla del carbamato de bencilo de la Preparación 13 (7,92 g, 33,5 mmol) y paladio al 5% sobre carbón vegetal (800 mg) en etanol (300 ml) se hidrogenó a 3,5 kg/cm^2 y temperatura ambiente durante 4 horas. La mezcla de reacción se filtró a través de Arbocel®, lavando a fondo con etanol, y se añadió ácido clorhídrico 1 N (36,9 ml, 36,9 mmol) al filtrado combinado. Esta solución se evaporó a presión reducida y el residuo se destiló azeotrópicamente con diclorometano para proporcionar el compuesto del título como una espuma incolora, 4,66 g; ^{1}H NMR (DMSOd_6, 300 MHz) δ: 2,46 (t, 2H), 2,60 (s, 3H), 2,95 (m, 2H), 7,98-8,16 (m, 2H).

Preparación 24

1-(2-Aminopropil)-2-pirrolidinona

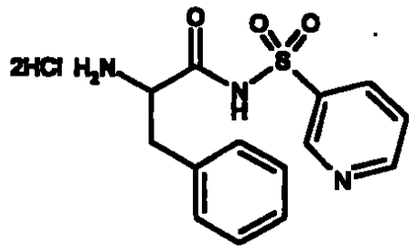


Una mezcla de la oxima de la Preparación 17 (375 mg, 2,40 mmol) y óxido de platino (300 mg) en etanol (20 ml) se hidrogenó a 4,2 kg/cm^2 y temperatura ambiente durante 18 horas. El análisis por tlc mostró la presencia de material de partida, así que se añadió óxido de platino adicional (100 mg) y la reacción se continuó durante 4 horas más. La mezcla se filtró a través de Arbocel® y el filtrado se

evaporó a presión reducida. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de diclorometano:metanol:amoníaco 0,88 (95:5:0,5 a 90:10:1) para dar el compuesto del título como un aceite claro, 170 mg, 50%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,02 (d, 3H), 1,36 (s ancho, 2H), 2,00 (m, 2H), 2,38 (t, 2H), 3,00-3,16 (m, 2H), 3,21 (m, 1H), 3,35-3,45 (m, 2H); LRMS: m/z 143 (MH^+).

Preparación 25

Dihidrocloruro de N-(2-amino-3-fenilpropanoil)-3-piridinsulfonamida

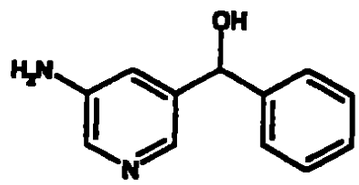


Se añadió ácido clorhídrico etéreo saturado (40 ml) a una solución enfriada con hielo de la sulfonamida de la Preparación 18 (959 mg, 2,37 mmol) en acetato de etilo (30 ml) y éter (10 ml), y la solución se agitó a temperatura ambiente durante 18 horas. La mezcla de reacción se concentró a presión reducida y el residuo se destiló azeotrópicamente con diclorometano (3 x) para proporcionar el compuesto del título como un sólido blanco, 959 mg; ^{1}H NMR ($DMSOd_6$, 300 MHz) δ: 3,23-3,50 (m, 1H), 3,70-3,98 (m, 1H), 4,13 (m, 1H), 7,05 (m, 2H), 7,20 (m, 3H), 7,78 (m, 1H), 8,36 (d, 1H), 8,44 (s ancho, 2H), 8,95 (d, 1H), 9,02 (s, 1H); $[\alpha]_D$ = +138° (solución en metanol al 0,5%).

213 26

Preparación 26

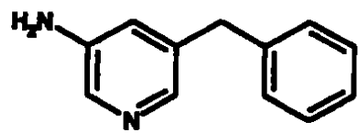
(5-Amino-3-piridinil)(fenil)metanol



Una mezcla del bromuro de la Preparación 19 (2,0 g, 7,60 mmol) y sulfato de cobre (II) pentahidratado (350 mg, 1,40 mmol) en amoníaco 0,88 (18 ml) se calentó a 135°C en un recipiente cerrado durante 24 horas. Se añadió una solución de hidróxido de sodio (1 N, 10 ml) a la solución enfriada, y luego la mezcla se extrajo con éter (6 x). Los extractos orgánicos reunidos se secaron ($MgSO_4$) y se concentraron a presión reducida hasta dejar un bajo volumen. El precipitado resultante se filtró, se lavó con éter y se secó para dar el compuesto del título como un sólido, 1,25 g, 83%; p.f. 92-94°C; ^1H NMR ($DMSOd_6$, 300 MHz) δ: 5,22 /s, 2H), 5,59 (d, 1H), 5,86 (d, 1H), 6,83 (s, 1H), 7,20 (m, 1H), 7,34 (m, 4H), 7,78 (m, 2H).

Preparación 27

5-Bencil-3-piridinilamina



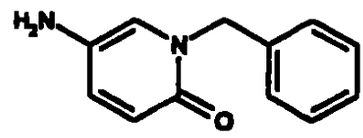
Una mezcla del alcohol de la Preparación 26 (700 mg, 3,5 mmol) y paladio al 5% sobre carbón vegetal (70 mg) en ácido clorhídrico (5 ml, 1 N) y etanol (20 ml) se hidrogenó a 2,1 kg/cm^2 y temperatura ambiente durante 6 horas. La mezcla se filtró a través de Arbocel® y el filtrado se concentró a presión reducida. El residuo se alcalinizó utilizando una solución acuosa de bicarbonato de sodio, se extrajo con diclorometano (3 x), y los extractos orgánicos reunidos se secaron ($MgSO_4$) y se evaporaron a presión reducida. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol:amoníaco 0,88 (92:8:0,4) como eluyente, para dar el compuesto del título como un sólido, 500 mg,

214

78%; p.f. 107-109°C ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3,61 (s ancho, 2H), 3,94 (s, 2H), 6,78 (s, 1H), 7,24 (m, 5H), 7,98 (s, 2H).

Preparación 28

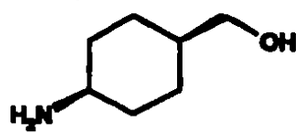
5-Amino-1-bencil-2-(1*H*)-piridinona



Una mezcla de 1-bencil-5-nitro-1H-piridin-2-ona (Justus Liebigs Ann. Chem. 484; 1930; 52) (1,0 g, 4,35 mmol) y estaño granulado (3,5 g, 29,5 mmol) en ácido clorhídrico concentrado (14 ml) se calentó a 90°C durante 1,5 horas. La solución enfriada se diluyó con agua, se neutralizó utilizando una solución de carbonato de sodio y se extrajo con acetato de etilo (250 ml en total). Los extractos orgánicos reunidos se filtraron, se secaron ($MgSO_4$) y se evaporaron a presión reducida para dar el compuesto del título como un sólido verde pálido (que se volvió azul con el tiempo), 440 mg, 51%; ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 4,12-4,47 (s ancho, 2H), 5,00 (s, 2H), 6,31 (d, 1H), 6,86 (s, 1H), 7,07 (m, 1H), 7,14-7,42 (m, 5H).

Preparación 29

Cis-(4-aminociclohexil)metanol

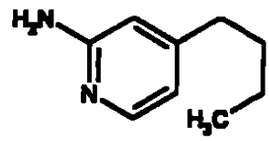


Se añadió gota a gota hidruro de litio y aluminio (14 ml, solución en tetrahidrofurano 1 M, 14 mmol) a una solución enfriada con hielo de ácido cis-4-aminociclohexanocarboxílico (1,33 g, 9,29 mmol) en tetrahidrofurano (50 ml), y una vez que se completó la adición, la reacción se calentó a reflujo durante 6 horas. La suspensión resultante se enfrió a 5°C, y se añadieron secuencialmente agua (0,6 ml), solución acuosa de hidróxido de sodio (1,1 ml, 2 M) y luego agua (0,6 ml). La suspensión resultante se filtró y el filtrado se evaporó a presión reducida para dar un

aceite, que se utilizó sin más purificación; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1,40-1,80 (m, 12H), 3,00 (m, 1H), 3,55 (d, 2H); LRMS: m/z 130,2 (MH^+).

Preparación 30

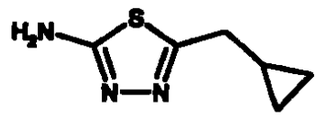
2-Amino-4-butilpiridina



Una mezcla de 4-butilpiridina (5,0 g, 37,0 mmol) y amiduro de sodio al 95% (1,7 g, 40,7 mmol) en xileno (10 ml) se calentó a 150°C durante 18 horas. La mezcla enfriada se diluyó con éter (100 ml) y se extrajo con ácido clorhídrico 2 N (dos veces). Los extractos acuosos se alcalinizaron utilizando una solución de hidróxido de sodio, y se extrajeron de nuevo con éter. Estos extractos orgánicos reunidos se secaron ($MgSO_4$) y se evaporaron a presión reducida. El aceite residual se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol:amoníaco 0,88 (97:3:0,15) como eluyente, para proporcionar el compuesto del título como un sólido cristalino, 2,1 g, 38%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,96 (t, 3H), 1,38 (m, 2H), 1,60 (m, 2H), 2,52 (t, 2H), 4,38 (s ancho, 2H), 6,38 (s 1H), 6,55 (d, 1H), 7,98 (d, 1H); Anál. Calculado: C, 72,01; H, 9,47; N, 18,53. $C_9H_{14}N_2$ requiere C, 71,96; H, 9,39; H, 18,65%.

Preparación 31

5-(Ciclopropilmetil)-1,3,4-tiadiazol-2-amina

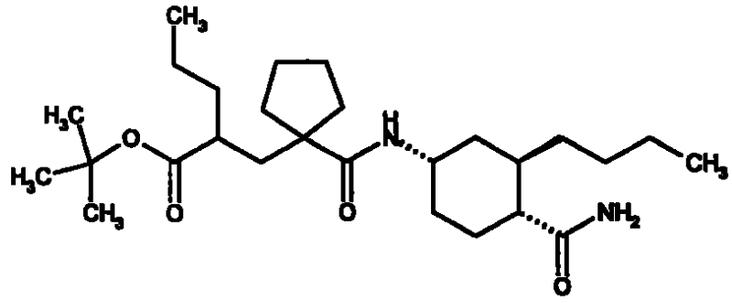


Se añadieron cloruro de oxalilo (3,13 ml, 35,9 mmol) y N,N-dimetilformamida (1 gota) a una solución de ácido ciclopropilacético (3 g, 29,9 mmol) en diclorometano (30 ml), y la reacción se agitó a temperatura ambiente durante 18 horas. La mezcla se concentró a presión reducida y se destiló azeotrópicamente con diclorometano para

dar un aceite de color pardo. Una mezcla de este cloruro de ácido intermedio (887 mg, 7,48 mmol) y tiosemicarbazida (455 mg, 4,99 mmol) se calentó a 70°C durante 18 horas y luego se enfrió. Se añadió agua, la mezcla se alcalinizó a pH 9 utilizando una solución acuosa de hidróxido de sodio al 50%, y el precipitado resultante se filtró y se secó para dar un sólido de color crema, 410 mg, 53%; ^{1}H NMR (CD_3OD, 400 MHz) δ: 0,28 (m, 2H), 0,60 (m, 2H), 1,02 (m, 1H), 2,77 (d, 2H); LRMS: m/z 155,2 (MH^+).

Preparación 32

(1*R*,2*R*,4*S*)-4-[({1-[2-(terc-Butoxicarbonil)pentil]ciclopentil}carbonil)amino]-2-butilciclohexanocarboxamida

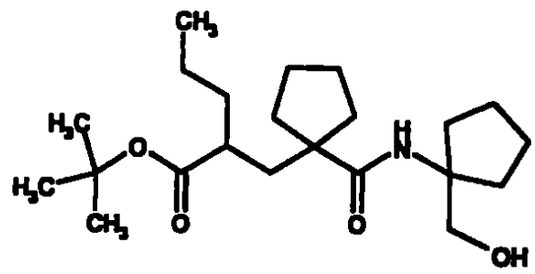


El producto de la Preparación 49 (65 mg, 0,14 mmol) se recogió en DCM (2 ml) y se añadió 1,1'-carbonildiimidazol (23 mg, 0,15 mmol) en una porción. Se añadió solución de amonio acuoso 0,88 (0,5 ml) y la mezcla se agitó durante 16 h. Los materiales volátiles se separaron a presión reducida, y la suspensión resultante se trató con solución saturada de $NaHCO_3$ acuoso (5 ml). Los materiales orgánicos se extrajeron con EtOAc (2 x), se secaron ($MgSO_4$) y se evaporaron para dar un aceite, que se purificó por cromatografía en columna utilizando 19:1 (DCM:MeOH) como eluyente para proporcionar el producto del título (43 mg); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,81 (t, 6H), 1,02-1,28 (m, 37H),2,22 (m, 1H), 4,13 (m, 1H), 5,42-5,97 (m, 3H).

217 220

Preparación 33

2-{[1-({[1-(Hidroximetil)ciclopentil]amino}carbonil)ciclopentil]metil}pentanoato de terc-butilo

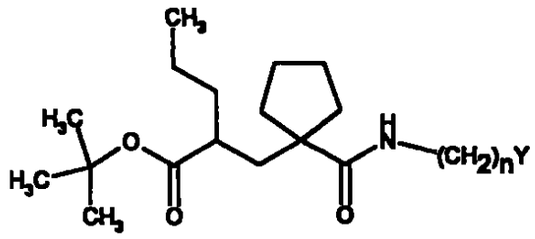


Se añadieron hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (41 mg, 0,21 mmol), hidrato de 1-hidroxibenzotriazol (27 mg, 0,2 mmol), N-metilmorfolina (35 µl, 0,31 mmol) y finalmente 1-amino-1-ciclopentanometanol (25 mg, 0,22 mmol) a una solución del ácido de la Preparación 1 (150 mg, 0,53 mmol) en N,N-dimetilformamida (3 ml) y la reacción se agitó a 90°C durante 18 horas. La solución enfriada se diluyó con acetato de etilo (90 ml), se lavó con agua (3 x 25 ml) y salmuera (25ml), luego se secó ($MgSO_4$) y se evaporó a presión reducida. El producto bruto se purificó por cromatografía sobre gel de sílice utilizando acetato de etilo:pentano (30:70) como eluyente para proporcionar el compuesto del título, 38 mg, 57%; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0,88 (t, 3H), 1,29 (m, 3H), 1,41-1,78 (m, 26H), 1,78-1,98 (m, 4H), 2,04 (m, 1H), 2,26 (m, 1H), 3,59 (dd, 1H), 3,70 (dd, 1H), 4,80 (t, 1H), 5,81 (s, 1H); LRMS: m/z 380 (MH^-).

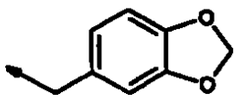
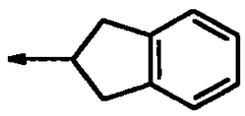
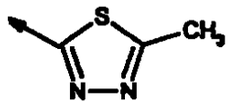
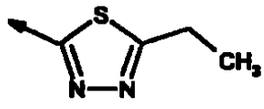
Preparaciones 34 a 43

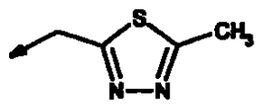
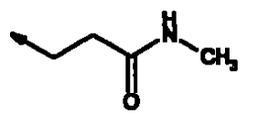
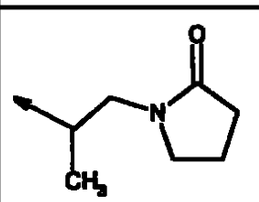
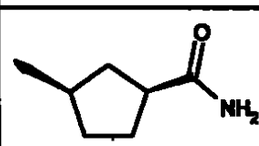
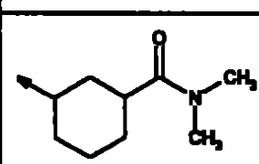
Los compuestos de fórmula IVc, es decir los compuestos de fórmula general IVc en donde **Prot** es terc-butilo y R^1 es propilo, se prepararon a partir del producto del título de la Preparación 1 y la amina indicada, siguiendo un procedimiento similar al descrito en la Preparación 33.

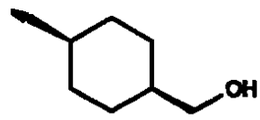
218



(IVc)

Prep.	-$(CH_2)_nY$	Amina precursora	Datos
34		piperonilamina (vendido por Aldrich Chemical Co.)	^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,85 (t, 3H), 1,26 (m, 4H), 1,42 (s, 9H), 1,46 (m, 2H), 1,59-1,75 (m, 5H), 1,95 (m, 2H), 2,06 (m, 1H), 2,22 (m, 1H), 4,26 (dd, 1H), 4,39 (dd, 1H), 5,95 (m, 3H), 6,78 (m, 3H).LRMS: m/z 418,3 (MH^+).
35[1]		hidrocloruro de 2-aminoindano (vendido por Aldrich Chemical Co.)	^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,87 (t, 3H), 1,25 (m, 3H), 4H), 1,42 (m, 12H), 1,56-1,70 (m, 4H), 1,90 (m, 2H), 2,02 (m, 1H), 2,22 (m, 1H), 2,80 (m, 2H), 3,35 (m, 2H), 4,76 (m, 1H), 5,86 (d, 1H), 7,19 (m, 4H). LRMS: m/z 400,3 (MH^+).
36[2]		2-amino-5-metil-1,3,4-tiadiazol (vendido por Lancaster)	^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,82 (t, 3H), 1,20-1,85 (m, 20H), 2,18 (m, 4H), 2,67 (s, 3H), 9,80 (s ancho, 1H). LRMS: m/z 382,3 (MH^+).
37[2]		2-amino-5-etil-1,3,4-tiadiazol (vendido por Lancaster)	^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,82 (t, 3H), 1,20-1,80 (m, 22H), 1,84 (m, 1H), 2,20 (m, 4H), 3,04 (q, 2H), 9,10 (s ancho, 1H). LRMS: m/z 396,2 (MH^+).

Prep.	$-(CH_2)_nY$	Amina precursora	Datos
38		Preparación 22	^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,84 (t, 3H), 1,20-1,38 (m, 4H), 1,42 (s, 9H), 1,44-1,76 (m, 7H), 1,95-2,12 (m, 3H), 2,20 (m, 1H), 2,76 (s, 3H), 4,74 (dd, 1H), 4,82 (dd, 1H), 6,54 (s ancho, 1H). LRMS: m/z 396,2 (MH^+).
39[1,2]		Preparación 23	^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,88 (t, 3H), 1,21-1,38 (m, 3H), 1,40-1,70 (m, 17H), 1,88-2,04 (m, 3H), 2,20 (m, 1H), 2,39 (t, 2H), 2,80 (d, 3H), 3,53 (m, 2H), 6,13 (s ancho, 1H), 6,40 (m, 1H). LRMS: m/z 369,5 (MH^+).
40[2]		Preparación 24	^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,82 (m, 3H), 1,16 (2xd, 3H), 1,20-1,72 (m, 21H), 1,83 (m, 1H), 1,98 (m, 3H), 2,17 (m, 1H), 2,38 (m, 2H), 1,96 (m, 1H), 3,34 (m, 1H), 3,54-3,62 (m, 2H), 4,15-4,20 (m, 1H), 6,21-6,35 (2xd ancho, 1H). LRMS: m/z 409,3 (MH^+).
41[2]		Preparación 20	^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,82 (t, 3H), 1,19-1,38 (m, 4H), 1,42 (m, 12H), 1,60 (m, 3H), 1,74-2,02 (m, 10H), 2,18 (m, 1H), 2,78 (m, 1H), 4,38 (m, 1H), 5,32 (s ancho, 1H), 5,57 (s ancho, 1H), 7,28 (s ancho, 1H). LRMS: m/z 395 (MH^+).
42[2]		Preparación 21	^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,86 (t, 3H), 1,18-1,78 (m, 25H), 1,84-2,03 (m, 6H), 2,22 (m, 1H), 2,68 (m, 1H), 2,96 (s, 3H), 3,03 (s, 3H), 3,84 (m, 1H), 5,78 (m, 1H). LRMS: m/z 437,7 (MH^+).

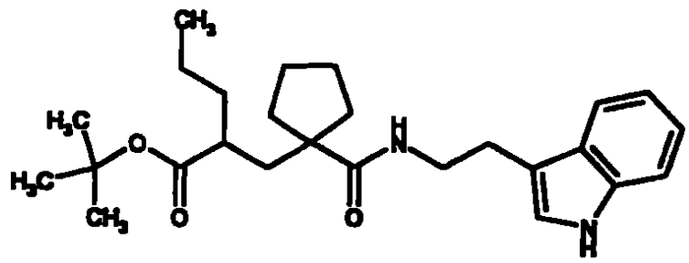
Prep.	-$(CH_2)_nY$	Amina precursora	Datos
43[2]		Preparación 29	^1H NMR ($CDCl_3$, 300 MHz) δ: 0,85 (t, 3H), 1,20-1,79 (m, 30H), 1,90 (m, 2H), 2,05 (m, 1H), 2,24 (m, 1H), 3,56 (m, 2H), 4,04 (m, 1H), 5,82 (d ancho, 1H). LRMS: m/z 396,4 (MH^+).

1 = reacción efectuada a temperatura ambiente

2 = se utilizó metanol:diclorometano como eluyente de la columna

Preparación 44

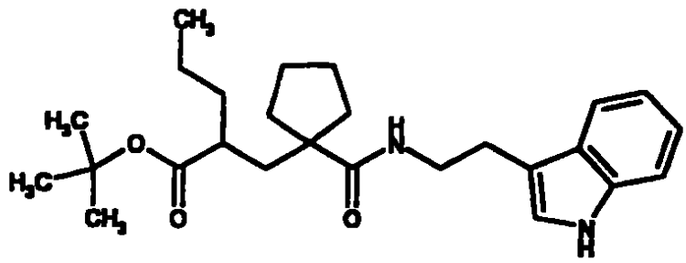
2-{[1-({[2-(1*H*-indol-3-il)etil]amino}carbonil)ciclopentil]metil}pentanoato de terc-butilo



El compuesto del título se obtuvo como un aceite amarillo pálido con un 80% de rendimiento a partir del ácido de la Preparación 1 y triptamina, siguiendo un procedimiento similar al descrito en la Preparación 33, excepto que la reacción se efectuó en diclorometano a temperatura ambiente; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0,86 (t, 3H), 1,26 (m, 3H), 1,42 (m, 11H), 1,50-1,69 (m, 6H), 1,83 (m, 1H), 1,90-2,05 (m, 2H), 2,22 (m, 1H), 2,99 (t, 3H), 3,60 (m, 2H), 5,78 (m, 1H), 7,06 (s, 1H), 7,14 (m, 1H), 7,20 (m, 1H), 7,38 (d, 1H), 7,63 (d, 1H), 8,02 (s ancho, 1H); LRMS: 427,5 (MH^+).

Preparación 45

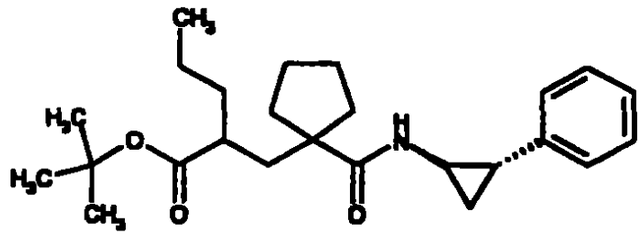
2-[(1-{[(3*S*)-1-Bencilpirrolidinil]amino}ciclopentil)metil]pentanoato de terc-butilo



El compuesto del título se obtuvo cuantitativamente a partir del ácido de la Preparación 1 y (3S)-1-bencil-3-aminopirrolidina, siguiendo un procedimiento similar al descrito en la Preparación 44; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,84 (t, 3H), 1,10-1,76 (m, 21H), 1,90-2,05 (m, 3H), 2,20-2,38 (m, 3H), 2,58 (m, 2H), 2,84 (m, 1H), 3,62 (s, 2H), 4,45 (m, 1H), 6,02 (m, 1H), 7,33 (m, 5H).

Preparación 46

2-{[1-{{[(1*S*,2*S*)-2-fenilciclopropil]amino}carbonil)ciclopentil]metil}pentanoato de terc-butilo



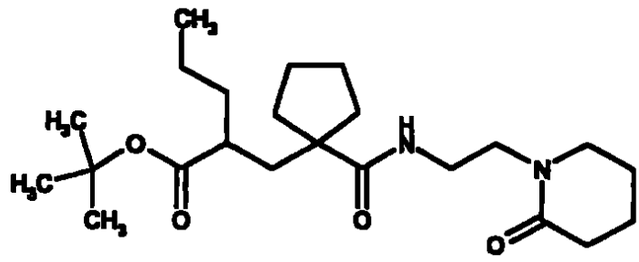
Se añadieron hidrocloruro de 1,(3-dimetilaminopropil)-3-etilcarbodiimida (81 mg, 0,42 mmol), N-metilmorfolina (0,15 ml, 1,06 mmol) e hidrocloruro de tranilcipromina (60 mg, 0,35 mmol) a una solución del ácido de la Preparación 1 (100 mg, 0,35 mmol) en diclorometano (10 ml), y la reacción se agitó a temperatura ambiente durante 18 horas. La mezcla de reacción se evaporó a presión reducida y el residuo se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de diclorometano:metanol (98:2 a 95:5) para proporcionar el compuesto del título como un aceite amarillo, 85 mg, 55%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,88 (t, 3H), 1,16 (m, 1H), 1,20-1,58 (m, 16H), 1,63 (m, 5H), 1,90-2,14 (m, 4H), 2,23 (m,

222

1H), 2,90 (m, 1H), 6,00 (m, 1H), 7,19 (m, 3H), 7,24 (m, 2H); LRMS: m/z 400 (MH^+).

Preparación 47

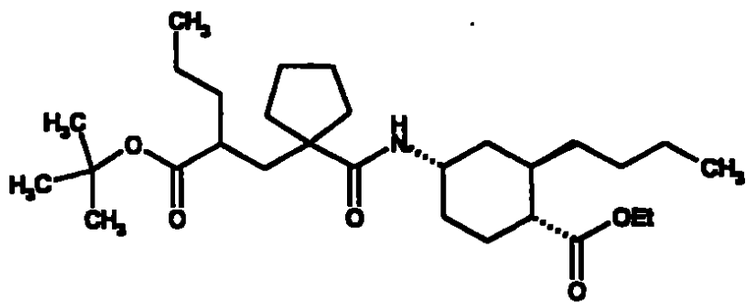
2-{[1-({[2-(2-Oxo-1-piperidinil)etil]amino}carbonil)ciclopentil]metil}pentanoato de terc-butilo



Se añadió monohidrato de hidrazina (34 µl, 0,70 mmol) a una solución del compuesto de la Preparación 6 (171 mg, 0,63 mmol) en etanol (10 ml), y la reacción se calentó a reflujo durante 5 horas. La mezcla enfriada se filtró, el filtrado se concentró a presión reducida, el residuo se suspendió en diclorometano y la suspensión se filtró de nuevo. El filtrado resultante se concentró a presión reducida y el residuo se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:metanol:amoníaco 0,88 (90:10:1) como eluyente para dar la amina, 16 mg. El ácido de la Preparación 1 (32 mg, 0,11 mmol), hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (25 mg, 0,13 mmol), hidrato de 1-hidroxibenzotriazol (17 mg, 0,13 mmol) y N-metilmorfolina (25 µl, 0,23 mmol) se añadieron a una solución de esta amina en N,N-dimetilformamida (2 ml), y la reacción se agitó a temperatura ambiente durante 18 horas. La mezcla se repartió entre acetato de etilo y agua, y se separaron las capas. La fase orgánica se lavó con agua (2 x), se secó ($MgSO_4$) y se evaporó a presión reducida. El aceite residual se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de diclorometano:metanol (98,5:1,5 a 95:5) para proporcionar el compuesto del título como un aceite, 43 mg, 17%; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0,82 (t, 3H), 1,22 (m, 3H), 1,38-1,65 (m, 17H), 1,58 (m, 4H), 1,95 (m, 3H), 2,17 (m, 1H), 2,37 (m, 2H), 3,30 (m, 2H), 3,38 (m, 2H), 3,50 (m, 2H), 6,76 (m, 1H); LRMS: m/z 409,2 (MH^+).

Preparación 48

(1*R*,2*R*,4*S*)-4-[({1-[2-(terc-Butoxicarbonil)pentil]ciclopentil}carbonil)amino]-2-butil-ciclohexanocarboxilato de etilo

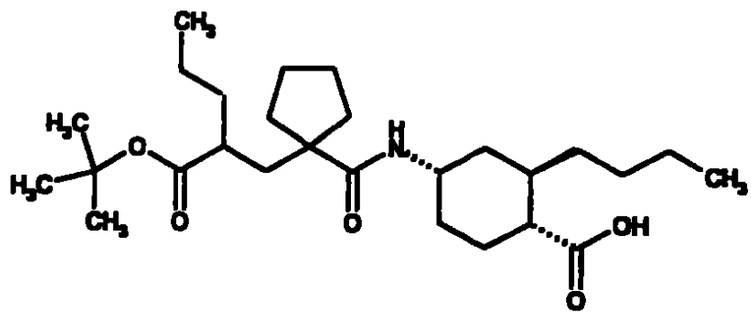


Una mezcla del ácido de la Preparación 1 (109 mg, 0,38 mmol), hidrocloruro del éster etílico de ácido (1*R*,2*R*,4*S*)-4-amino-2-butil-ciclohexanocarboxílico (documento WO 9009374) (101 mg, 0,38 mmol), hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (95 mg, 0,50 mmol), hidrato de 1-hidroxibenzotriazol (60 mg, 0,40 mmol) y trietilamina (0,12 ml, 0,87 mmol) en diclorometano (3 ml), se agitó a temperatura ambiente durante 16 horas. La mezcla se evaporó a presión reducida, el residuo se trató con solución de bicarbonato de sodio y se extrajo con acetato de etilo. Los extractos orgánicos reunidos se secaron ($MgSO_4$) y se evaporaron a presión reducida para dar una goma. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando acetato de etilo:pentano (50:50) como eluyente, y se destiló azeotrópicamente con diclorometano para proporcionar el compuesto del título, 190 mg; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,88 (m, 6H), 1,20-1,40 (m, 13H), 1,40-2,10 (m, 25H), 2,16-2,30 (m, 2H), 4,18 (m, 3H). 5,83 (d, 1H).

224 227

Preparación 49

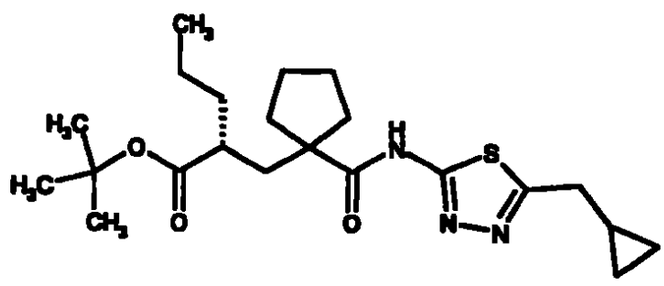
Ácido (1*R*,2*R*,4*S*)-4-[({1-[2-(terc-butoxicarbonil)pentil]ciclopentil}carbonil)amino]-2-butilciclohexanocarboxílico



Una mezcla del éster etílico de la Preparación 48 (190 mg, 0,39 mmol) y solución de hidróxido de sodio 1 N (0,85 ml, 0,85 mmol) en metanol (1,5 ml) se agitó a temperatura ambiente durante 22 horas. La mezcla de reacción se aciduló a pH 1 utilizando ácido clorhídrico (2 N), luego se repartió entre acetato de etilo y agua. Se separaron las capas y la fase orgánica se secó ($MgSO_4$) y se evaporó a presión reducida para proporcionar el compuesto del título, 130 mg, 72%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0,86 (m, 6H), 1,20-2,12 (m, 36H), 2,24 (m, 2H), 4,18 (m, 1H), 5,82 (d, 1H); LRMS: m/z 464 $(M-H)^-$.

Preparación 50

(2*R*)-1-{[1-({[5-(Ciclopropilmetil)-1,3,4-tiadiazol-2-il]amino}carbonil)ciclopentil]metil}-pentanoato de terc-butilo

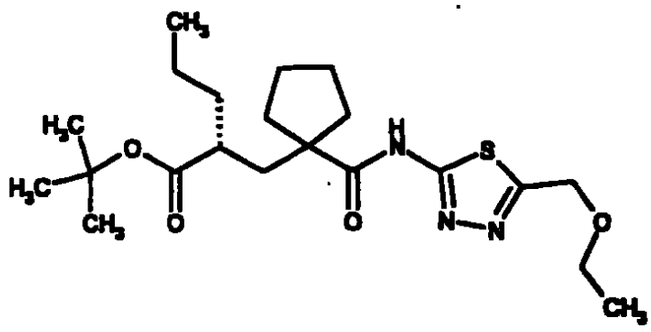


El compuesto del título se preparó a partir del ácido de la Preparación 2 y la amina de la Preparación 31, con 65% de rendimiento, siguiendo el procedimiento

descrito en la Preparación 33; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,35 (m, 2H), 0,63 (m, 2H), 0,80 (m, 3H), 1,10 (m, 1H), 1,20-1,94 (m, 20H), 2,19 (m, 4H), 2,93 (t, 2H), 3,50 (s, 1H); LRMS: m/z 422,4 (MH^+); $[\alpha]_D$ = -14,15° (c = 0,082, metanol).

Preparación 51

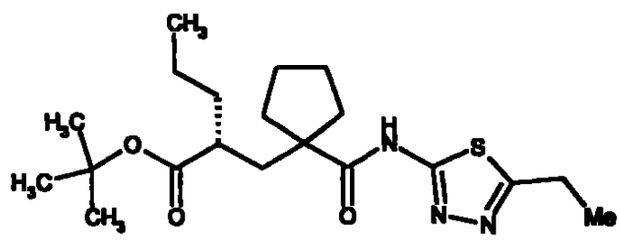
(2*R*)-2-{[1-({[5-(Etoximetil)-1,3,4-tiadiazol-2-il]amino}carbonil)ciclopentil]metil}-pentanoato de terc-butilo



El compuesto del título se preparó a partir del ácido de la Preparación 2 y 5-(etoximetil)-1,3,4-tiadiazol-2-amina, con 51% de rendimiento, siguiendo el procedimiento descrito en la Preparación 33; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,10-1,78 (m, 25H), 1,82 (m, 1H), 2,19 (m, 5H), 3,48 (s, 1H), 4,82 (s, 2H), 10,16 (s ancho, 1H); LRMS: m/z 426,4 (MH^+); $[\alpha]_D$ = -12,50° (c = 0,08, metanol).

Preparación 51a

(2*R*)-2-[(1-{[(5-Etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]pentanoato de terc-butilo

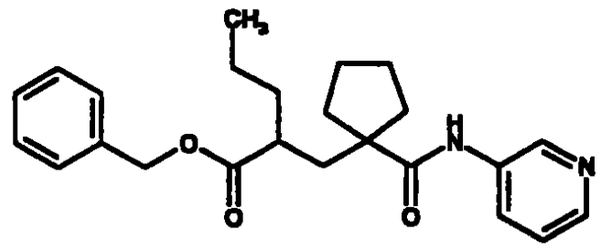


A una mezcla de diclorometano (2,39 l) y piridina (2,39 l) a -14°C se añadió

cloruro de tionilo (135 ml, 1,85 mol, 1,1 equiv.) con agitación a lo largo de un período de 1 hora bajo atmósfera de nitrógeno. Después de agitar la solución de color naranja resultante durante 5 minutos, se añadió una solución del producto de la Preparación 2 (477 g, 1,67 mol) en diclorometano (477 ml) a lo largo de un período de 45 minutos, produciendo enturbiamiento de la mezcla de reacción. Después de agitar la mezcla de reacción durante 3 horas, se añadió trietilamina (424 g, 4,19 mol) a lo largo de un período de 20 minutos, seguido por 4-dimetilaminopiridina (20,5 g, 168 mmol). Luego a la mezcla resultante se añadió 2-amino-5-etil-1,3,4-tiadiazol (vendido por Lancaster) (282 g, 2,18 mol) en 3 porciones a lo largo de un período de 10 minutos. Luego la reacción se dejó calentar a temperatura ambiente y se agitó durante 43 horas. Luego se añadieron n-heptano (2,4 l) y agua desionizada (1 l) a la mezcla de reacción. Luego se añadió ácido clorhídrico concentrado (3,56 l) con agitación a lo largo de un período de 0,5 horas mientras enfriaba en un baño de agua con hielo. Luego las capas se separaron y a la capa orgánica se añadieron agua desionizada (2 l) y ácido clorhídrico concentrado (250 ml) con agitación. Las capas se separaron de nuevo, y a la capa orgánica se añadieron agua desionizada (2 l) y ácido clorhídrico concentrado (250 ml) con agitación. Luego a la capa orgánica se añadió solución acuosa saturada de carbonato de potasio (1 ml) con agitación. La fase orgánica se recogió y luego se lavó con salmuera saturada (2 x 1 l). Luego la solución resultante se concentró a vacío para dar el compuesto del título (546 g, 1,38 mol, 85% de rendimiento) como un aceite viscoso de color pardo oscuro que se utilizó en la etapa siguiente sin más purificación; LRMS (APCI negativo); m/z $[M-H]^-$ 394; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0,76 (t, 3H), 1,08-1,23 (m, 3H), 1,23-1,27 (m, 2H), 1,32 (s, 9H), 1,34-1,42 (m, 2H), 1,42-1,53 (m, 4H), 1,53-1,68 (m, 2H), 1,87 (dd, 1H), 1,95-2,06 (m, 1H), 2,06-2,24 (m, 3H), 2,98 (q, 2H), 12,15 (s amcho, 1H).

Preparación 52

2-[{1-[(3-Piridinilamino)carbonil]ciclopentil}metil]pentanoato de bencilo

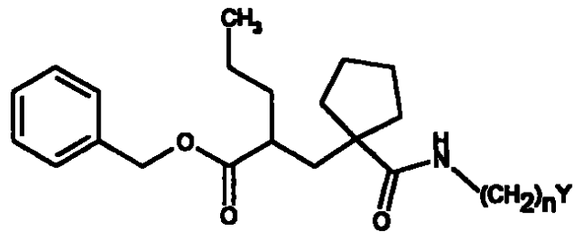


Se añadió trietilamina (0,11 ml, 0,78 mmol) a una mezcla del cloruro de ácido de la Preparación 3 (200 mg, 0,60 mmol) y 2-aminopiridina (61 mg, 0,65 mmol) en diclorometano (3 ml), y la reacción se agitó a temperatura ambiente durante 16 horas. La mezcla se evaporó a presión reducida, el residuo se repartió entre una solución de bicarbonato de sodio (5 ml) y acetato de etilo (20 ml), y se separaron las capas. La fase orgánica se secó ($MgSO_4$) y se evaporó a presión reducida para dar una goma. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando acetato de etilo como eluyente, para proporcionar el compuesto del título, 130 mg; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0,82 (t, 3H), 1,21 (m, 3H), 1,40 (m, 1H), 1,43-1,72 (m, 6H), 1,81 (d, 1H), 1,98 (m, 1H), 2,18 (m, 1H), 2,24 (m, 1H), 2,46 (m, 1H), 4,98 (m, 2H), 7,20-7,38 (m, 6H), 7,42 (s, 1H), 8,06 (d, 1H), 8,35 (d, 1H), 8,56 (s, 1H).

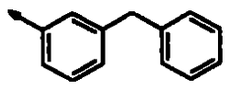
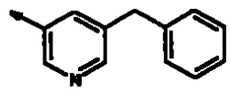
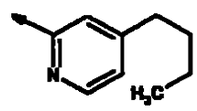
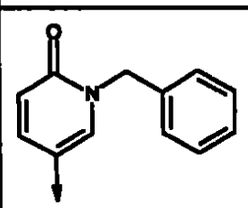
Preparaciones 53 a 56

Los compuestos de fórmula IV, es decir los compuestos de fórmula general IV donde Prot es bencilo y R^1 es propilo, se prepararon a partir del cloruro de ácido de la Preparación 3 y la amina indicada, siguiendo un procedimiento similar al descrito en la Preparación 52.

228



(IVd)

Prep.	-$(CH_2)_nY$	Amina precursora	Datos
53[1]		vendido por Trans World Chemicals	^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,84 (t, 3H), 1,24 (m, 2H), 1,40-1,76 (m, 7H), 1,84 (dd, 1H), 1,98 (m, 1H), 2,19 (dd, 1H), 2,28 (m, 1H), 2,56 (m, 1H), 3,98 (s, 2H), 4,99 (dd, 2H), 6,98 (d, 1H), 7,19-7,42 (m, 15H).
54		Preparación 27	^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,85 (t, 3H), 1,24 (m, 3H), 1,39-1,78 (m, 6H), 1,82 (dd, 1H), 1,98 (m, 2H), 2,20 (dd, 1H), 2,25 (m, 1H), 2,50 (m, 1H), 3,98 (s, 2H), 4,98 (dd, 2H), 7,18-7,40 (m, 10H),7,45 (s, 1H), 7,98 (s, 1H), 8,23 (s, 1H), 8,42 (s, 1H).
55		Preparación 30	^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,80 (t, 3H), 0,92 (t, 3H), 1,21 (m, 2H), 1,30-1,70 (m, 12H), 1,82 (dd, 1H), 2,04 (m, 1H), 2,20 (m, 2H), 2,50 (m, 1H), 2,58 (t, 2H), 4,98 (dd, 2H), 6,83 (d, 1H), 7,30 (m, 5H), 7,90 (s, 1H), 8,08 (s, 1H), 8,15 (d, 1H).
56[2]		Preparación 28	^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,84 (t, 3H), 1,25 (m, 2H), 1,27-1,99 (m, 10H), 2,07-2,30 (m, 2H), 2,47 (m, 1H), 4,99 (s, 2H), 5,10 (dd, 2H), 6,59 (d, 1H), 7,15 (d, 1H), 7,34 (m, 11H), 8,10 (s, 1H).

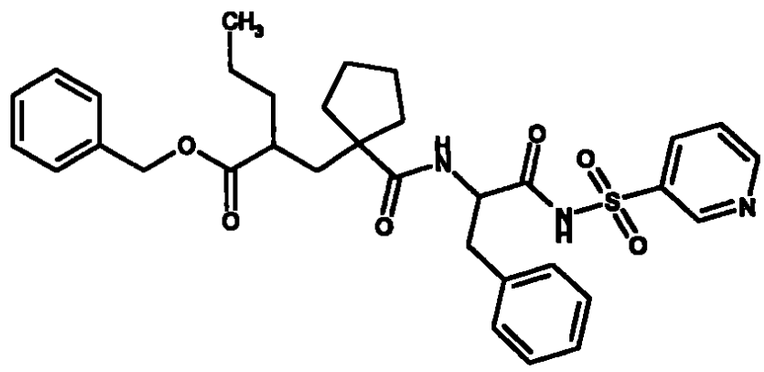
1 = diclorometano utilizado como eluyente de la columna

2 = se utiliza N-metilmorfolina como base

229 .

Preparación 57

2-({1-[({1-Bencil-2-oxo-2-[(3-piridilsulfonil)amino]etil}amino)carbonil]ciclopentil}metil)-pentanoato de bencilo

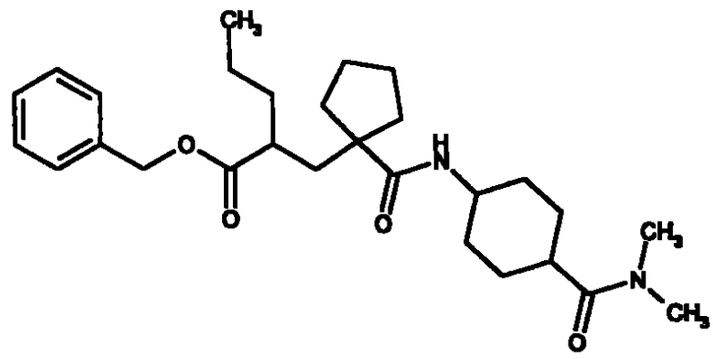


El hidrocloruro de amina de la Preparación 25 (828 mg, 2,19 mmol) y N-metilmorfolina (2,21 g, 21,9 mmol) se añadieron a una solución enfriada con hielo del cloruro de ácido de la Preparación 3 (737 mg, 2,19 mmol) en diclorometano (50 ml), y la reacción se agitó a temperatura ambiente durante 24 horas. La mezcla de reacción se evaporó a presión reducida, el residuo se repartió entre acetato de etilo (50 ml) y agua (50 ml), y se separaron las capas. La fase orgánica se lavó con salmuera (25 ml), se secó ($MgSO_4$) y se evaporó a presión reducida. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de acetato de etilo:metanol (100:0 a 95:5) para dar el compuesto del título como una espuma de color crema, 975 mg, 73%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0,72 (m, 3H), 0,94-2,20 (m, 17H), 2,84 (m, 1H), 3,00 (m, 1H), 4,18 (m, 1H), 5,00 (m, 2H), 6,95 (m, 2H), 7,02 (m, 3H), 7,38 (m, 6H), 8,06 (m, 1H), 8,60 (m, 1H), 8,87 (s, 1H).

230

Preparación 58

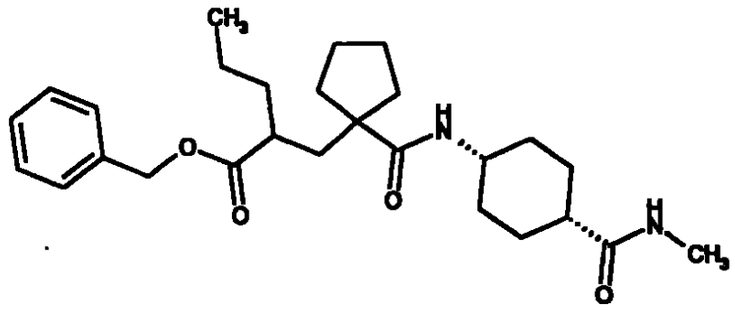
cis-2-({1-[({4-[(Dimetilamino)carbonil]ciclohexil}amino)carbonil]ciclopentil}metil)-pentanoato de bencilo



Una mezcla de ácido cis-4-{[(1-{2-[(benciloxi)carbonil]pentil}ciclopentil)-carbonil]amino}ciclohexanocarboxílico (documento EP 274234) (200 mg, 0,45 mmol), hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (112 mg, 0,58 mmol), hidrato de 1-hidroxibenzotriazol (70 mg, 0,46 mmol) y dimetilamina (0,56 ml, 2 M en tetrahidrofurano, 1,12 mmol) en diclorometano (5 ml) se agitó a temperatura ambiente durante 16 horas. La mezcla se concentró a presión reducida y el residuo se repartió entre una solución de bicarbonato de sodio y acetato de etilo, y se separaron las capas. La fase orgánica se secó ($MgSO_4$) y se evaporó a presión reducida para dar una goma. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando acetato de etilo como eluyente para proporcionar el compuesto del título, 150 mg; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0,82 (t, 3H), 1,22 (m, 3H), 1,32-1,88 (m, H), 2,00 (m, 4H), 2,40 (m, 1H), 2,60 (m, 1H), 2,97 (s, 3H), 3,04 (s, 3H), 4,04 (m, 1H), 5,12 (s, 2H), 5,80 (d ancho, 1H), 7,37 (m, 5H).

Preparación 59

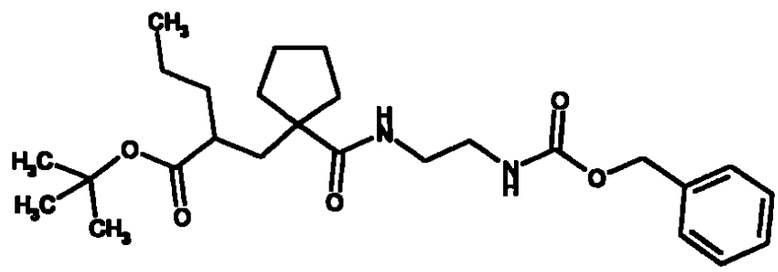
cis-2-({1-[({4-[(Metilamino)carbonil]ciclohexil}amino)carbonil]ciclopentil}metil)pentanoato de bencilo



El compuesto del título se preparó con 49% de rendimiento a partir del ácido cis-4-{[(1-{2-[(benciloxi)carbonil]pentil}ciclopentil)carbonil]amino}ciclohexanocarboxílico (documento EP 274234) y metilamina (2 M en tetrahidrofurano), siguiendo el procedimiento descrito en la Preparación 58; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0,82 (t, 3H), 1,17-2,12 (m, 22H), 2,21 (m, 1H), 2,41 (m, 1H), 2,80 (d, 3H), 4,00 (m, 1H), 5,12 (s, 2H), 5,61 (m, 1H), 5,79 (d, 1H), 7,38 (m, 5H).

Preparación 60

2-[(1-{[(2-{[(Benciloxi)carbonil]amino}etil)amino]carbonil}ciclopentil)metil]pentanoato de terc-butilo

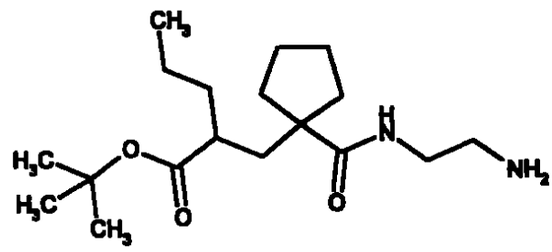


El compuesto del título se obtuvo como un aceite amarillo con 55% de rendimiento, a partir del ácido de la Preparación 1 y N-benciloxicarbonil-1,2-diaminoetano, siguiendo un procedimiento similar al descrito en la Preparación 44; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,84 (t, 3H), 1,20-1,38 (m, 3H), 1,40-1,74 (m, 17H), 1,90 (m,

2H), 2,04 (m, 1H), 2,20 (m, 1H), 3,32 (m, 3H), 3,44 (m, 1H), 5,10 (s, 2H), 5,61 (m, 1H), 6,20 (m, 1H), 7,36 (m, 5H).

Preparación 61

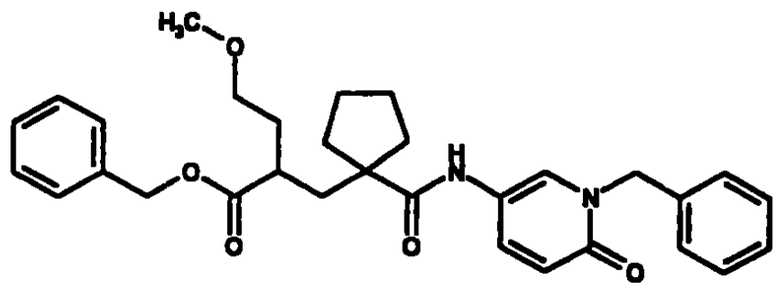
2-[(1-{[(2-Aminoetil)amino]carbonil}ciclopentil)metil]pentanoato de terc-butilo



Una mezcla del carbamato de la Preparación 60 (1,43 g, 3,10 mmol) y paladio al 10% sobre carbón vegetal (200 mg) en etanol (8 ml) se hidrogenó a temperatura ambiente y 1 atmósfera durante 18 horas. La mezcla de reacción se filtró a través de Arbocel®, y el filtrado se evaporó a presión reducida para proporcionar el compuesto del título, 920 mg, 92%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,84 (t, 3H), 1,20-1,38 (m, 3H), 1,40-1,54 (m, 12H), 1,61 (m, 5H), 1,92-2,12 (m, 3H), 2,20 (m, 1H), 2,98 (m, 2H), 3,38 (m, 1H), 3,42 (m, 1H), 3,97 (m, 2H), 6,65 (m, 1H); LRMS: m/z 326,8 (M^+).

Preparación 62

2-[(1-{[(1-Bencil-6-oxo-1,6-dihidro-3-piridinil)amino]carbonil}ciclopentil)metil]-4-metoxibutanoato de bencilo

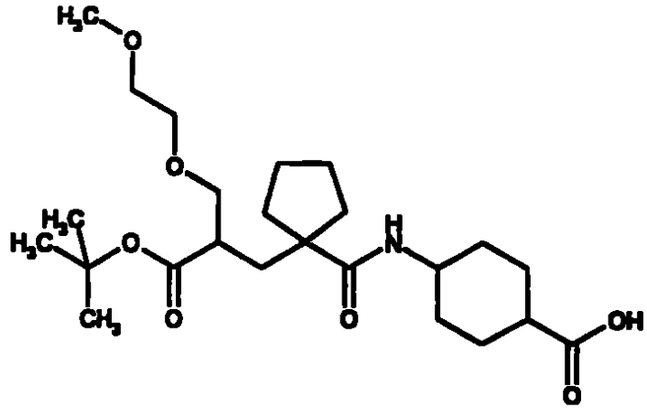


Se añadió cloruro de oxalilo (0,26 ml, 3,0 mmol) a una solución enfriada con hielo de ácido 1-{2-[(benciloxi)carbonil]-4-metoxibutil}ciclopentanocarboxílico

(documento EP 274234) (1,0 g, 3,0 mmol) y N,N-dimetilformamida (2 gotas) en diclorometano (20 ml), y la reacción se agitó a temperatura ambiente durante 2 horas. La solución se concentró a presión reducida y el residuo se destiló azeotrópicamente con diclorometano (3 x 10 ml). El producto se disolvió en diclorometano (20 ml) y luego se enfrió en un baño de hielo. Se añadieron la amina de la Preparación 28 (600 mg, 3 mmol) y N-metilmorfolina (0,6 ml, 5,45 mmol) y la reacción se agitó a temperatura ambiente durante 18 horas. La mezcla de reacción se concentró a presión reducida y se repartió entre agua y éter. La capa orgánica se lavó con ácido clorhídrico (2 N), con solución de bicarbonato de sodio, luego con agua, se secó ($MgSO_4$) y se evaporó a presión reducida. El sólido verde residual se purificó por cromatografía en columna a presión media sobre gel de sílice utilizando acetato de etilo:hexano (90:10) como eluyente para proporcionar el compuesto del título, 880 mg, 57%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1,37-2,28 (m, 12H), 2,46-2,64 (m, 1H), 3,20 (s, 3H), 3,31 (m, 2H), 4,97 (dd, 2H), 5,08 (dd, 2H), 6,57 (d, 1H), 7,12 (m, 1H), 7,18-7,48 (m, 10H), 8,08 (d, 1H).

Preparación 63

Ácido 4-{[(1-{3-terc-butoxi-2-[(2-metoxietoxi)metil]-3-oxopropil}ciclopentil)carbonil]-amino}ciclohexanocarboxílico

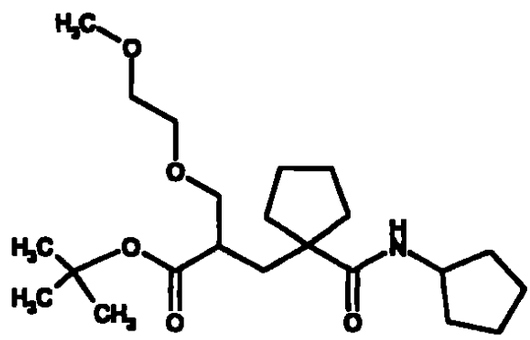


Una mezcla de 4-{[(1-{3-terc-butoxi-2-[(2-metoxietoxi)metil]-3-oxopropil}-ciclopentil)carbonil]amino}ciclohexanocarboxilato de bencilo (documento EP 274234) y paladio al 10% sobre carbón vegetal (250 mg) en agua (10 ml) y etanol (50 ml) se hidrogenó a 3,5 kg/cm^2 y temperatura ambiente durante 18 horas. La mezcla de

reacción se filtró a través de Solkafloc®, el filtrado se concentró a presión reducida y el residuo se destiló azeotrópicamente con tolueno (3 x) y luego con diclorometano (3 x) para dar el compuesto del título, 2,0 g, 96%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1,48 (s, 9H), 1,53-1,84 (m, 14H), 1,94-2,10 (m, 5H), 2,60 (m, 2H), 3,40 (s, 3H), 3,41-3,63 (m, 5H), 3,96 (m, 1H), 5,90 (d ancho, 1H).

Preparación 64

3-{1-[(Ciclopentilamino)carbonil]ciclopentil}-2-[(2-metoxietoxi)metil]propanoato de terc-butilo

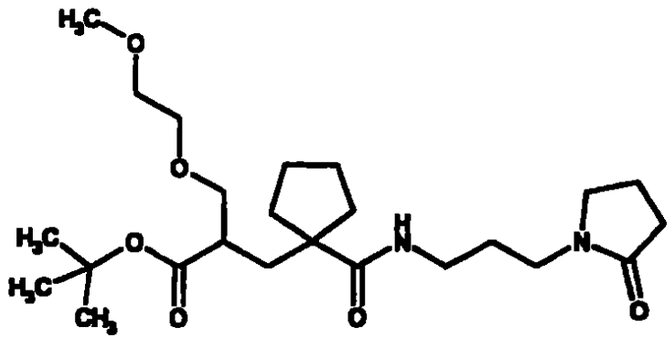


Se añadieron hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (197 mg, 1,07 mmol), hidrato de 1-hidroxibenzotriazol (139 mg, 1,07 mmol), N-metilmorfolina (0,18 ml, 1,64 mmol) y ciclopentilamina (101µl, 1,07 mmol) a una solución de ácido 1-{3-terc-butoxi-2-[(2-metoxietoxi)metil]-3-oxopropil}ciclopentanocarboxílico (documento EP 274234) (400 mg, 1,07 mmol) en diclorometano (5 ml) y la reacción se agitó a temperatura ambiente durante 22 horas. La reacción se inactivó por adición de agua, se extrajo con diclorometano (3 x) y los extractos orgánicos reunidos se secaron ($MgSO_4$) y se evaporaron a presión reducida. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando acetato de etilo:pentano (30:70) como eluyente para proporcionar el compuesto del título como un aceite claro, 320 mg, 78%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,22-2,02 (m, 27H), 2,58 (m, 1H), 3,36 (s, 3H), 3,40 (m, 1H), 3,46 (m, 2H), 3,57 (m, 3H), 4,10-4,20 (m, 1H), 5,80 (s ancho, 1H).

235

Preparación 65

3-(2-Metoxietoxi)-2-{[1-({[3-(2-oxo-1-pirrolidinil)propil]amino}carbonil)ciclopentil]-metil}propanoato de terc-butilo

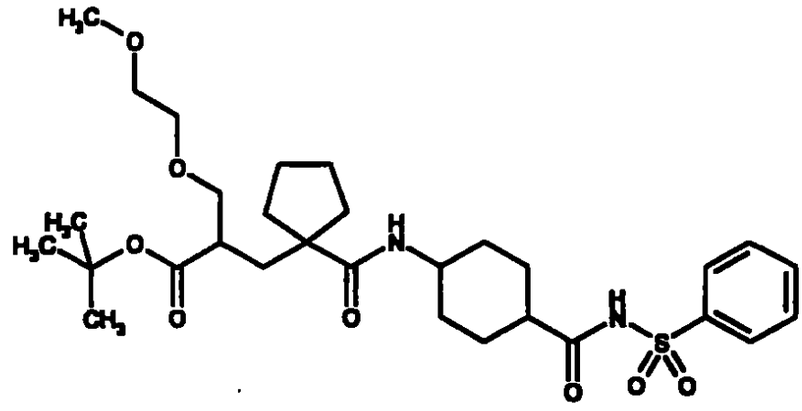


El compuesto del título se obtuvo como un aceite claro con 97% de rendimiento a partir del ácido 1-{3-terc-butoxi-2-[(2-metoxietoxi)metil]-3-oxopropil}-ciclopentanocarboxílico (documento EP 274234) y 1-(3-aminopropil)-2-pirrolidinona, siguiendo un procedimiento similar al descrito en la Preparación 64, excepto que se utilizó diclorometano:metanol (95:5) como eluyente de la columna, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,41 (s, 9H), 1,50 (m, 2H), 1,60-1,70 (m, 7H), 1,78 (m, 1H), 1,90 (m, 1H), 2,20 (m, 4H), 2,40 (m, 2H), 2,58 (m, 1H), 3,14 (m, 1H), 3,20 (m, 1H), 3,38 (m, 6H), 3,42-3,60 (m, 6H), 7,00 (m, 1H).

236

Preparación 66

cis-3-(2-Metoxietoxi)-2-[(1-{[(4-{[(fenilsulfonil)amino]carbonil}ciclohexil)amino]carbonil}ciclopentil)metil]propanoato de terc-butilo

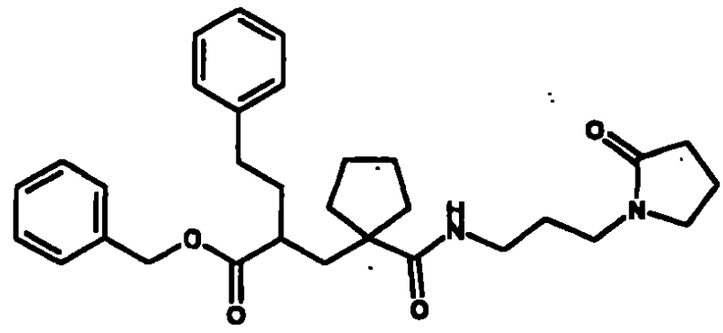


Se añadieron N,N-diciclohexilcarbodiimida (199 mg, 0,97 mmol), 4-dimetilaminopiridina (118 mg, 0,97 mmol) y bencenosulfonamida (152 mg, 0,97 mmol) a una suspensión enfriada con hielo del ácido de la Preparación 63 (400 mg, 0,878 mmol) en diclorometano (12 ml) y N,N-dimetilformamida (0,5 ml), y la reacción se agitó a temperatura ambiente durante 20 horas. La mezcla se concentró a presión reducida y el residuo se suspendió en acetato de etilo frío. El material insoluble resultante se separó por filtración, el filtrado se lavó con ácido clorhídrico (1 N) y agua, luego se secó ($MgSO_4$) y se evaporó a presión reducida. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando un gradiente de elución de diclorometano:metanol (95:5 a 90:10) para proporcionar el compuesto del título como una espuma blanca, 480 mg, 92%; ^1H NMR ($CDCl_3$, 400 MHz) δ: 1,44 (s, 9H), 1,63 (m, 13H), 1,80 (m, 2H), 1,88 (m, 1H), 1,98 (m, 2H), 2,36 (m, 1H), 2,57 (m, 1H), 3,38 (s, 3H), 3,40 (m, 1H), 3,51 (t, 2H), 3,58 (m, 3H), 3,95 (m, 1H), 5,92 (d, 1H), 7,56 (m, 2H), 7,62 (m, 1H), 8,05 (d, 2H), 8,75 (s ancho, 1H); LRMS: m/z 718 (MNa^+).

237 / 240

Preparación 67

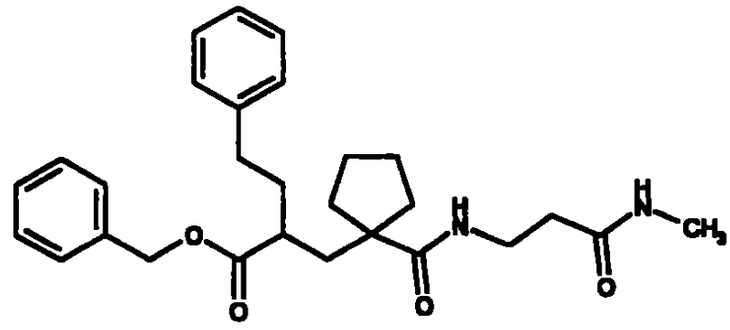
2-{[1-({[3-(2-Oxo-1-pirrolidinil)propil]amino}carbonil)ciclopentil]metil}-4-fenilbutanoato de bencilo



Se añadieron hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida (1,06 g, 5,53 mmol), hidrato de 1-hidroxibenzotriazol (0,60 g, 4,44 mmol) y 4-metilmorfolina (0,56 g, 5,54 mmol) secuencialmente a una solución enfriada de ácido 1-{2-[(benciloxi)carbonil]-4-fenilbutil}ciclopentanocarboxílico (documento EP 274234) (1,5 g, 3,94 mmol) en diclorometano seco (15 ml) a temperatura ambiente, seguido por N-(d-aminopropil)-2-pirrolidinona (0,56 g, 3,94 mmol), y la reacción se agitó a temperatura ambiente durante 18 horas. La mezcla se lavó con agua, ácido clorhídrico 2 N, solución acuosa saturada de bicarbonato de sodio, y luego se secó ($MgSO_4$) y se evaporó a presión reducida. El aceite amarillo residual se purificó por cromatografía en columna sobre gel de sílice utilizando acetato de etilo:pentano (50:50) como eluyente para proporcionar el compuesto del título como una goma clara, 800 mg, 40%; ^1H NMR ($CDCl_3$, 300 MHz) d: 1,37-2,20 (m, 16H), 2,34-2,58 (m, 5H), 2,92-3,46 (m, 6H), 5,07 (d, 1H), 5,18 (d, 1H), 6,98-7,47 (m, 10H).

Preparación 68

2-{[1-({[3-(Metilamino)-3-oxopropil]amino}carbonil)ciclopentil]metil}-4-fenilbutanoato de bencilo

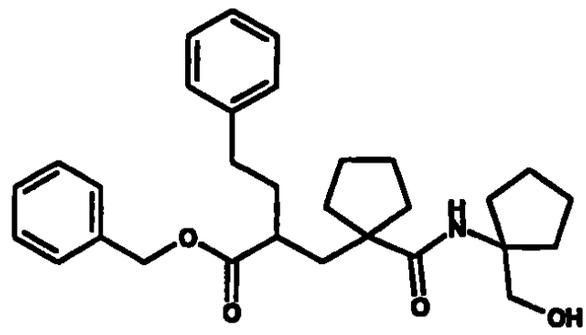


Se añadieron hidrocloruro de 1-(3-dimetilaminopropil)-3-carbodiimida (122 mg, 0,64 mmol), hidrato de 1-hidroxibenzotriazol (86 mg, 0,64 mmol) y 4-metilmorfolina (173 µl, 1,59 mmol) secuencialmente a una solución enfriada de ácido 1-{2-[(benciloxi)carbonil]-4-fenilbutil}ciclopentanocarboxílico (documento EP 274234) (202 mg, 0,53 mmol) en N,N-dimetilformamida (5 ml) a temperatura ambiente, seguido por el hidrocloruro de amina de la Preparación 23 (146 mg, 1,06 mmol), y la reacción se agitó a 90°C durante 18 horas. La solución enfriada se concentró a presión reducida y el residuo se repartió entre agua (20 ml) y acetato de etilo (100 ml). Las capas se separaron, la fase orgánica se lavó con agua (3 x 30 ml), salmuera (25 ml), se secó ($MgSO_4$) y se evaporó a presión reducida para dar un aceite claro. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando diclorometano:-metanol (98:2) como eluyente para proporcionar el compuesto del título como un aceite incoloro, 162 mg, 67%; ^1H NMR ($CDCl_3$, 400 MHz) δ: 1,38-1,53 (m, 2H), 1,53-1,96 (m, 8H), 2,02 (m, 2H), 2,27 (t, 2H), 2,46 (m, 3H), 2,76 (d, 3H), 3,44 (m, 2H), 5,13 (s, 2H), 5,79 (s ancho, 1H), 6,38 (m, 1H), 7,06 (d, 2H), 7,18 (m, 1H), 7,22 (m, 2H), 7,38 (m, 5H); LRMS: m/z 465,5 (MH^+).

239

Preparación 69

2-{[1-({[1-(Hidroximetil)ciclopentil]amino}carbonil)ciclopentil]metil}-4-fenilbutanoato de bencilo

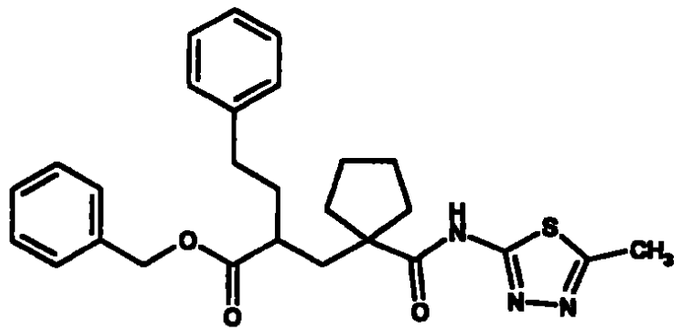


El compuesto del título se obtuvo como un sólido cristalino (48%) a partir del ácido 1-{2-[(benciloxi)carbonil]-4-fenilbutil}ciclopentanocarboxílico (documento EP 274234) y 1-amino-1-ciclopentanometanol, siguiendo un procedimiento similar al descrito en la Preparación 68, excepto que la mezcla de reacción se agitó a temperatura ambiente durante 18 horas y el producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando acetato de etilo:pentano como eluyente; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1,38 (m, 2H), 1,50-1,95 (m, 16H), 2,01 (m, 2H), 2,45 (m, 3H), 3,49 (dd, 1H), 3,60 (dd, 1H), 4,58 (m, 1H), 5,10 (s, 2H), 5,67 (s, 1H), 7,01 (d, 2H), 7,14 (m, 1H), 7,20 (m, 2H), 7,36 (m, 5H); LRMS: m/z 478,3 (MH^+).

240 22

Preparación 70

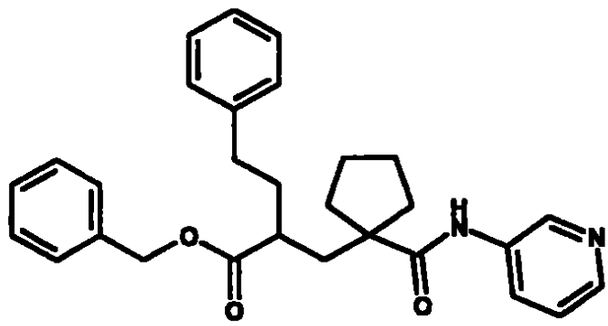
2-[(1-{[(5-Metil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]-4-fenilbutanoato de bencilo



El compuesto del título se obtuvo como un aceite claro con 74% de rendimiento a partir del ácido 1-{2-[(benciloxi)carbonil]-4-fenilbutil}ciclopentanocarboxílico (documento EP 274234) y 2-amino-5-metil-1,3,4-tiadiazol, siguiendo un procedimiento similar al descrito en la Preparación 68; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,58-1,76 (m, 7H), 1,83-1,98 (m, 3H), 2,03 (m, 1H), 2,20 (m, 1H), 2,35 (m, 1H), 2,44 (m, 3H), 2,65 (s, 3H), 5,02 (dd, 2H), 7,00 (d, 2H), 7,15 (m, 1H), 7,19 (m, 2H), 7,35 (m, 5H); LRMS: m/z 478,7 (MH^+).

Preparación 71

4-Fenil-2-({1-[(3-piridinilamino)carbonil]ciclopentil}metil)butanoato de bencilo

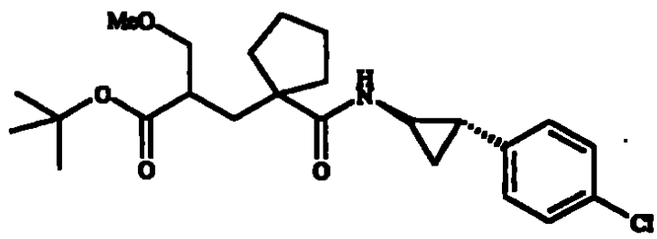


Se añadió cloruro de oxalilo (2,29 ml, 26,3 mmol) a una solución del ácido 1-{2-[(benciloxi)carbonil]-4-fenilbutil}ciclopentanocarboxílico (documento EP 274234) (5,0 g, 13,14 mmol) y N,N-dimetilformamida (2 gotas) en diclorometano (25 ml), y la

solución se agitó durante 2,5 horas. La mezcla se evaporó a presión reducida, el residuo se destiló azeotrópicamente con diclorometano para dar un aceite amarillo. Luego, éste se disolvió en diclorometano (50 ml) y se añadió una solución de este cloruro de ácido (10 ml, 2,45 mmol) a una solución enfriada con hielo de trietilamina (248 mg, 2,45 mmol) y 3-aminopiridina (253 mg, 2,70 mmol) en diclorometano seco (10 ml), y la reacción se agitó a temperatura ambiente durante 18 horas. La solución se lavó con agua (3 x), se secó ($MgSO_4$) y se evaporó a presión reducida. El producto bruto se purificó por cromatografía en columna sobre gel de sílice utilizando acetato de etilo:hexano (40:60) como eluyente, y se repitió utilizando un gradiente de elución de éter:hexano (90:10 a 100:0). El producto se cristalizó en acetato de etilo:hexano para proporcionar el compuesto del título, 740 mg, 66%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1,38-2,07 (m, 10H), 2,10-2,37 (m, 2H), 2,42-2,63 (m, 3H), 5,02 (s, 2H), 6,94-7,44 (m, 10H), 7,50 (s, 1H), 8,03 (d, 1H), 8,36 (d, 1H), 8,52 (s, 1H).

Preparación 72

***trans*-3-[1-({[2-(4-Clorofenil)ciclopropil]amino}carbonil)ciclopentil]-2-(metoximetil)propanoato de terc-butilo**

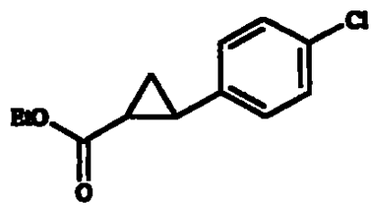


El producto de la Preparación 94 (286 mg, 1 mmol), *trans*-2-(4-clorofenil)-ciclopropilamina (véase la Preparación 76) (203 mg, 1 mmol), 1-(3-dimetilaminopropil)-3-etilcarbodiimida (211 mg, 1,1 mmol), trietilamina (1 ml) y HOBt (148 mg, 1,1 mmol) se combinaron en 5 ml de DCM a temperatura ambiente y se agitaron durante 16 h. La mezcla de reacción se lavó con agua (20 ml), se secó sobre $MgSO_4$ y se purificó por cromatografía en columna utilizando EtOAc:pentano 1:8 y luego 1:5 como eluyente para proporcionar el producto del título como una película incolora (122 mg, 40%); R_f 1:5 (EtOAc:pentano) 0,2; ^1H NMR (400 MHz, $CDCl_3$) δ: 1,05-1,2 (m, 2H), 1,4 (s, 9H), 1,55-1,75 (m, 4H), 1,9-2,0 (m, 4H), 2,4-2,5 (m, 1H), 2,75-2,85 (m, 1H), 3,3 (s, 3H), 3,4-3,5 (m, 1H), 6,3 (m, 1H), 7,05 (d, 2H), 7,2 (d, 2H); HRMS: m/z

M+H, Encontrado 436,2242. $C_{24}H_{35}NO_4Cl$ requiere 436,2249.

Preparación 73

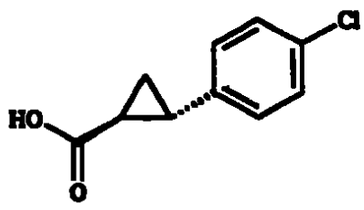
2-(4-Clorofenil)ciclopropanocarboxilato de etilo



Una mezcla de 4-cloroestireno (10,1 ml, 96 mmol) y acetato de rodio dímero (1 g, 4,5 mmol) en tolueno (50 ml) se calentó a 85°C antes de añadir diazoacetato de etilo (11,3 ml, 94 mmol) durante 30 minutos y luego el producto completo se calentó a 80°C durante 1 h más antes de la concentración a vacío. Luego el residuo se purificó por cromatografía en columna utilizando DCM:pentano 1:2 como eluyente para dar el producto del título como un aceite incoloro (7,8 g, 37%); R_f 1:2 (DCM:pentano) 0,35; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1,15-1,3 (m, 4H), 1,5-1,7 (m, 1H), 1,8-1,9 (m, 1H), 2,4-3,55 (m, 1H), 4,2 (q, 2H), 6,95 (d, 2H), 7,20-7,28 (m, 2H); LRMS: m/z M+NH_4^+ 242.

Preparación 74

Ácido *trans*-2-(4-clorofenil)ciclopropanocarboxílico

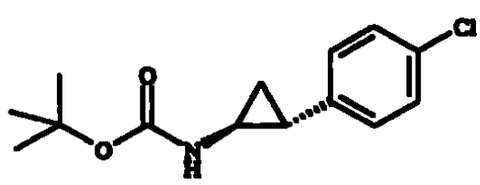


El producto de la Preparación 73 (7,8 g, 37 mmol) se disolvió en EtOH (75 ml) a temperatura ambiente en nitrógeno y se añadió poco a poco metóxido de sodio (8,1 g, 150 mmol) durante 15 min. Después de completar la adición, el producto completo se calentó a reflujo durante 18 h. La mezcla de reacción se concentró a

vacío, y el residuo resultante se diluyó con DCM y agua (150 ml, mezcla 2:1). Se separó la capa orgánica, y la capa acuosa se extrajo nuevamente con DCM (2 x 50 ml). Los extractos orgánicos combinados se secaron sobre $MgSO_4$ y se evaporaron para proporcionar el éster *trans* (4,96 g, 62%). La acidulación de la capa acuosa con HCl concentrado a pH 1 dio como resultado un precipitado blanco, que se filtró y se secó a vacío para proporcionar el producto de hidrólisis (el ácido correspondiente) como un polvo blanco (1,95 g, 27%). La disolución del éster en MeOH (50 ml), agua (50 ml) y LiOH (1,34 g, 32 mmol) dio una solución clara que se calentó a aproximadamente 70°C durante una noche. La mezcla de reacción se enfrió, se concentró a vacío y se aciduló con HCl concentrado a pH 1. El precipitado blanco resultante se extrajo con EtOAc (3 x 50 ml) y los extractos orgánicos combinados se secaron sobre $MgSO_4$ y se evaporaron a sequedad, para proporcionar el ácido (4 g, 96%). Este ácido se combinó con el producto hidrolizado de la etapa anterior para dar un total de 5,95 g; ^1H NMR (400 MHz, $CDCl_3$) δ: 1,3-1,4 (m, 1H), 1,6-1,7 (m, 1H), 1,8-1,9 (m, 1H), 2,5-2,6 (m, 1H), 7,00 (d, 2H), 7,26 (d, 2H); LRMS: m/z M-H 195.

Preparación 75

***trans*-2-(4-Clorofenil)ciclopropilcarbamato de terc-butilo**



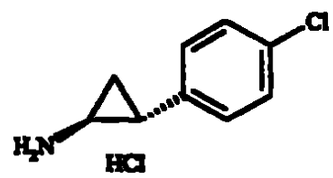
El producto de la Preparación 74 (1,5 g, 7,65 mmol), DPPA (1,8 ml, 8,4 mmol) y trietilamina (1,25 ml, 12 mmol) se combinaron en 20 ml de terc-butanol en nitrógeno y se calentaron a aproximadamente 90°C durante 48 h antes de retirar la fuente de calor y la mezcla se dejó enfriar a temperatura ambiente. La mezcla se diluyó con EtOAc (20 ml) y solución saturada de Na_2CO_3 (20 ml) y luego se separó la capa orgánica. La capa acuosa se extrajo de nuevo con EtOAc (20 ml) y las capas orgánicas combinadas se secaron sobre $MgSO_4$, se filtraron y se evaporaron. El residuo resultante se purificó por cromatografía en columna utilizando 1:10, luego 1:2 de EtOAc:pentano como eluyente para proporcionar el producto del título como un sólido blanco (1,7 g, 83%); R_f (EtOAc:pentano 1:2) 0,9; ^1H NMR (400 MHz, $CDCl_3$)

244

δ 1,2-1,3 (m, 1H), 1,4 (s, 10H), 1,9-2,0 (m, 1H), 2,6 (s ancho, 1H), 4,8 (s ancho, 1H), 7,0 (d, 2H), 7,2 (d, 2H); HRMS: m/z M+Na, Encontrado 290,0923. $C_{14}H_{18}NO_2ClNa$ requiere 290,0918.

Preparación 76

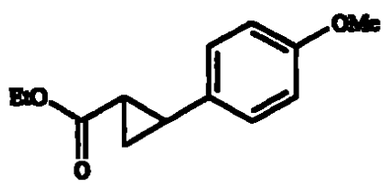
2-(4-Clorofenil)ciclopropilamina



El producto de la Preparación 75 se recogió en EtOAc, se enfrió a 0°C y se burbujeó gas cloruro de hidrógeno a la solución durante 30 minutos. Luego la solución se concentró a vacío para dar el producto del título (1,29 g, 6,3 mmol); R_f 1:3 (EtOAc:pentano); ^1H NMR (400 MHz, CD_3OD) δ: 1,3-1,4 (m, 1H), 1,4-1,5 (m, 1H), 2,3-2,4 (m, 1H), 2,8-2,9 (m, 1H), 7,15 (d, 2H), 7,3 (d, 2H).

Preparación 77

2-(4-Metoxifenil)ciclopropanocarboxilato de etilo

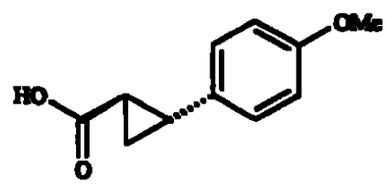


Una mezcla de 4-metoxiestireno (25,5 ml, 192 mmol), acetato de rodio dímero (2 g, 4,5 mmol) y tolueno (100 ml) se agitó a temperatura ambiente en nitrógeno durante 20 minutos y luego se calentó a 85°C. Después de que la reacción alcanzara esta temperatura, se añadió gota a gota diazoacetato de etilo (19,8 ml, 188 mmol) durante 50 minutos a razón de una gota cada 2 ó 3 segundos para mantener la temperatura interna de reacción alrededor de 95°C. Después de que se completara la adición, la mezcla se calentó durante 1 h a 85°C y se enfrió a temperatura ambiente. La mezcla se filtró a través de arbocel y se evaporó parda dar un aceite que

se purificó por cromatografía en columna utilizando DCM:pentano (1:2) como eluyente para proporcionar el producto del título (13 g, 31%) que tenía una mezcla 3:1 de isómeros *trans*:*cis*; R_f 0,22 (DCM:pentano) 1:2; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1,20-1,38 (m, 5H), 1,83 (ddd, 1H), 2,50 (ddd, H), 3,80 (s, 3H), 4,16 (q, 2H), 6,82 (d, 2H), 7,03 (d, 2H).

Preparación 78

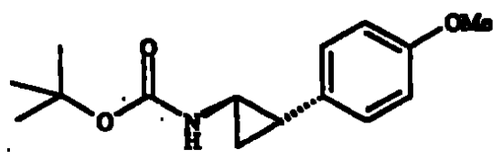
Ácido *trans*-2-(4-metoxifenil)ciclopropanocarboxílico



Se añadió metóxido de sodio (14,8 g, 273,8 mmol) a una solución del producto de la Preparación 77 (13 g, 59 mmol) en EtOH (135 ml) mientras se agitaba a temperatura ambiente en una atmósfera de nitrógeno. Una vez completada la adición, la mezcla se calentó a reflujo suave durante 1 h, tiempo mediante el cual el análisis por TLC indicó que no había ningún isómero *cis* remanente. La reacción se enfrió a temperatura ambiente y se añadió agua (100 ml) en una porción. Luego el producto completo se agitó a temperatura ambiente durante 63 h y luego se evaporó a bajo volumen para separar el MeOH antes de acidular con HCl concentrado a pH 1. La suspensión se extrajo con EtOAc (2 x 100 ml), y los extractos se secaron ($MgSO_4$) y se evaporaron para dar un sólido amarillo, que se purificó por cromatografía en columna utilizando EtOAc:pentano 1:1 para proporcionar el producto del título (8,9 g, 78%); R_f 1:1 (EtOAc:pentano) 0,4; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1,30-1,41 (m, 1H), 1,58-1,69 (m, 1H), 1,79-1,90 (m, 1H), 2,53-2,63 (m, 1H), 6,83 (d, 2H), 7,04 (d, 2H).

Preparación 79

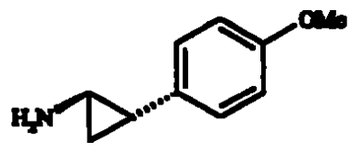
***trans*-2-(4-Metoxifenil)ciclopropilcarbamato de terc-butilo**



Se añadió DPPA (11 ml, 50,9 mmol) a una mezcla agitada del producto de la Preparación 78 (8,9 g, 46,3 mmol), trietilamina (10,1 ml, 72,7 mmol) y terc-BuOH (75 ml). Luego el producto completo se calentó a 90°C durante 43 h y el terc-BuOH se separó por evaporación y el residuo oleoso resultante se trató con 120 ml de K_2CO_3 saturado y luego se extrajo con EtOAc (2 x 100 ml). Los extractos orgánicos combinados se evaporaron a presión reducida para dar un sólido de color pardo que se purificó por cromatografía en columna utilizando DCM:MeOH (98:2) como eluyente para proporcionar el producto del título (5,8 g, 48%) como un aceite claro; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1,07-1,14 (m, 2H), 1,44 (s, 9H), 1,93-2,06 (m, 1H), 2,62-2,71 (m, 1H), 3,80 (s, 3H), 4,72-4,88 (m, 1H), 6,80 (d, 2H), 7,08 (d, 2H).

Preparación 80

***Trans*-2-(4-metoxifenil)ciclopropilamina**

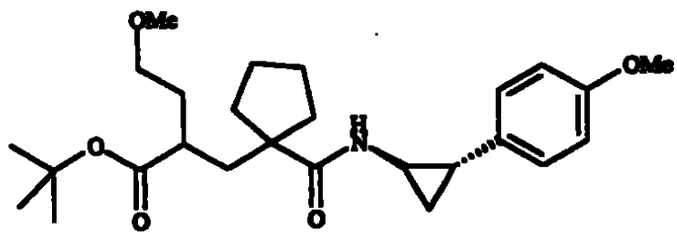


Se añadió TFA (20 ml) a una mezcla agitada del producto de la Preparación 79 (5,8 g, 22,0 mmol) y DCM (15 ml) a temperatura ambiente en nitrógeno. La reacción se agitó durante 16 h, y después de ese tiempo el disolvente se separó a presión reducida, y el aceite resultante se trató con K_2CO_3 acuoso saturado hasta llegar a alcanzar un pH de 10 (aproximadamente 150 ml requeridos). Esta solución opaca se extrajo con EtOAc (2 x 150 ml) y luego los extractos se secaron sobre $MgSO_4$ y se evaporaron para dar un sólido de color beige. Este sólido se purificó por

cromatografía en columna utilizando DCM:MeOH 99:1, 95:5 como eluyente para dar el producto del título como un sólido blanco (3,2 g, 89%); R_f DCM:MeOH (19:1) 0,18; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0,87-2,05 (m, 3H), 1,79-1,90 (m, 1H), 2,43-2,54 (m, 1H), 3,80 (s, 3H), 6,80 (d, 2H), 6,98 (d, 2H).

Preparación 81

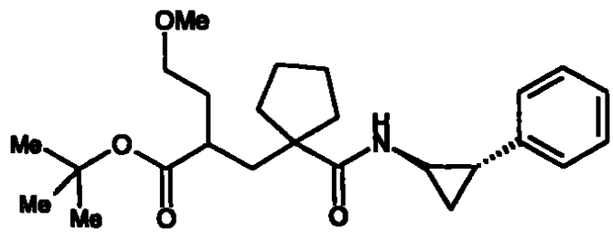
4-Metoxi-2-{[1-({[2-(4-metoxifenil)ciclopropil]amino}carbonil)ciclopentil]metil}-butanoato de terc-butilo



El ácido 1-{2-[(terc-butoxi)carbonil]-4-metoxibutil}ciclopentanocarboxílico (véase el documento EP 274234 para la preparación) (210 mg, 0,66 mmol), trietilamina (1 ml), HOBt (140 mg, 0,73 mmol) y el producto de la Preparación 80 (107 mg, 0,66 mmol) se combinaron secuencialmente en DCM a temperatura ambiente en nitrógeno. Luego se añadió WSCDI (98 mg, 0,73 mmol) a la mezcla y el producto completo se agitó durante una noche durante unas 16 horas. La reacción se diluyó con agua, la capa orgánica se separó y luego se lavó con salmuera, se secó sobre $MgSO_4$, se filtró y se evaporó para dar un aceite amarillo. Éste se purificó por cromatografía en columna utilizando EtOAc:pentano 1:3 como eluyente para proporcionar el producto del título (113 mg, 38%); R_f 1:10 (EtOAc:pentano) 0,2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1,0-1,01 (m, 2H), 1,40 (s, 9H), 1,35-1,45 (m, 2H), 1,50-1,80 (m, 7H), 1,85-2,10 (m, 4H), 2,25-2,35 (m, 1H), 2,70-2,80 (m, 1H), 3,2 (s, 3H), 3,25-3,35 (m, 2H), 3,7 (s, 3H), 6,05 (s ancho, 1H), 6,70 (d, 2H), 7,05 (d, 2H); LRMS: m/z 446 (M+H).

Preparación 81a

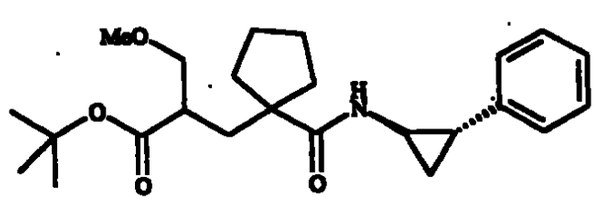
4-Metoxi-2-{[1-({[2-fenilciclopropil]amino}carbonil)ciclopentil]metil}butanoato de terc-butilo



El producto del título se preparó de manera análoga a la Preparación 81, reemplazando el producto de la Preparación 80 por 1-*S*-amino-2-*R*-fenilciclopropano (*J. Med. Chem.*, 1986, 29, 2044); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1,1 (m, 1H), 1,2 (m, 1H), 1,4 (s 9H), 1,6 (m, 8H), 1,8 (m, 1H), 1,9 (m, 2H), 2,1 (m, 2H), 2,4 (q, 1H), 2,8 (q, 1H), 3,2 (s, 3H), 3,3 (q, 2H), 6,1 (s ancho, 1H), 7,1 (d, 3H), 7,2 (d, 2H); LRMS: m/z 416 (M+H); LRMS m/z Encontrado 416.2794. $C_{25}H_{32}NO_4$ requiere 416.2796.

Preparación 82

3-Metoxi-2-[(1-{[(2-fenilciclopropil)amino]carbonil}ciclopentil)metil]propanoato de terc-butilo

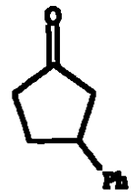


El producto de la Preparación 94 (200 mg, 0,66 mmol), trietilamina (1 ml), HOBt (98 mg, 0,73 mmol) y 1-*S*-amino-2-*R*-fenilciclopropano (*J. Med. Chem.*, 1986, 29, 2044) (123 mg, 0,73 mmol) se combinaron secuencialmente en DCM (6 ml) a temperatura ambiente en nitrógeno. Luego se añadió WSCDI (98 mg, 0,73 mmol) a la mezcla y el producto completo se agitó durante una noche durante unas 16 h. la reacción se diluyó con agua, la capa orgánica se separó y luego se lavó con salmuera,

se secó sobre $MgSO_4$, se filtró y se evaporó para dar un aceite amarillo. Éste se purificó por cromatografía en columna utilizando EtOAc:pentano 1:3 como eluyente para proporcionar el compuesto del título (164 mg, 62%); R_f 1:10 (EtOAc:pentano) 0,2; ^1H NMR (400 MHz, $CDCl_3$) δ: 1,0-1,1 (m, 2H), 1,4 (s, 9H), 1,5-1,8 (m, 6H), 1,8-2,05 (m, 4H), 2,3-2,4 (m, 1H), 2,7-2,8 (m, 1H), 3,2 (s, 3H), 3,25-3,5 (m, 1H), 3,7 (s, 3H), 6,05 (s, 1H), 6,7 (d, 2H), 7,05 (d, 2H); LRMS: m/z M+H, 446.

Preparación 83

3-Fenilciclopentanona

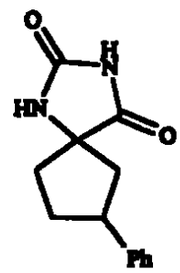


Se recogió bromuro de fenilmagnesio (0,27 mol) en 200 ml de éter seco, y se enfrió a 0°C en una atmósfera de nitrógeno. Se añadió yoduro de cobre (I) (25,5 g, 0,13 mol) en una porción, y la suspensión se agitó a 0°C durante 20 minutos. Luego se añadió gota a gota ciclopenten-2-ona a lo largo de 10-15 minutos y la solución resultante se agitó a 0°C durante 10 min, y luego se dejó calentar a temperatura ambiente en el curso de 1 h. La mezcla de reacción se añadió a 100 ml de una mezcla de una solución saturada de cloruro de amonio y amoníaco concentrado, cuyo pH estaba inicialmente limitado a 9. El producto completo se agitó a temperatura ambiente durante 30 minutos y luego se filtró, y luego las capas del filtrado se separaron. La capa acuosa se extrajo con éter (2 x 70 ml) y los extractos se combinaron con la capa orgánica original. Luego las capas de éter amontonadas se lavaron con salmuera, se secaron sobre $MgSO_4$, se filtraron y se evaporaron para dar un aceite amarillo pálido. Luego este aceite se cromatografió utilizando éter/pentano 1:3 para proporcionar el producto del título (2,9 g, 14%); R_f EtOAc:pentano (1:2) 0,65; ^1H NMR (300 MHz, $CDCl_3$) δ: 1,92-2,10 (m, 1H), 2,18-2,59 (m, 4H), 2,67 (dd, 1H), 3,38-3,52 (m, 1H), 7,19-7,47 (m, 5H).

250

Preparación 84

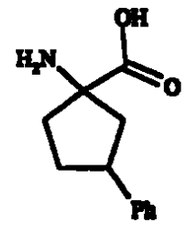
7-Fenil-1-3,diazaespiro[4.4]nonano-2,4-diona



El producto de la Preparación 83 (5,8 g), cianuro de potasio (2,75 g) y carbonato de amonio (9,1 g) se calentaron en 80 ml de EtOH acuoso al 50% durante 7 h, y luego a temperatura ambiente durante 48 h. La mezcla se filtró y el sólido se lavó a fondo con agua (3 x 50 ml). El filtrado se concentró, el pH se ajustó a 2 utilizando HCl concentrado y la suspensión resultante se separó por filtración y se lavó con agua (3 x 50 ml). Los sólidos amontonados se recristalizaron en EtOH y agua (300 ml:100 ml) para dar el producto del título como una mezcla 1:1 de pares diastereoisómeros (6,32 g); R_f 0,6 en EtOAc; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1,60-2,58 (m, 6H), 3,08-3,37 (m, 1H), 6,83-7,44 (m, 5H), 8,33 (d, 1H), 10,60 (s, 1H); Anál. Encontrado: C, 68,09; H, 6,21; N, 12,25%. $C_{13}H_{14}N_2O_2$ requiere C, 67,81; H, 613; N, 12,17%.

Preparación 85

Ácido 1-amino-3-fenilciclopentanocarboxílico

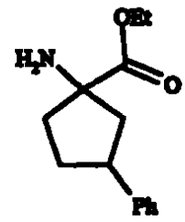


El producto del título de la Preparación 84 (6,2 g), hidróxido de bario octahidratado (17,2 g) y agua (100 ml) se calentaron juntos en una bomba a 160°C durante 7 h, y luego se dejaron estar durante una noche a temperatura ambiente. La

mezcla de reacción se aciduló a pH 1 utilizando H_2SO_4 concentrado. Luego la suspensión resultante se filtró y el sólido se lavó con agua (100 ml). Luego el filtrado se alcalinizó a aproximadamente pH 6 utilizando una solución concentrada de amoníaco, la suspensión se enfrió en un baño de hielo y luego se filtró. El sólido se lavó con agua y se secó a vacío para dar el producto del título (2,9 g); p.f. 265°C (con descomposición); R_f 0,5 en metilisobutilcetona:ácido acético:agua (2:1:1); ^1H NMR (300 MHz, $CDCl_3$) δ: 2,06-3,14 (m, 6H), 3,42-3,73 (m, 1H), 7,12-7,44 (m, 5H); Anál. Encontrado C, 69,54; H, 7,26; N, 6,73%. $C_{12}H_{15}NO_2$ requiere C, 70,22; H, 7,37; N, 6,82%.

Preparación 86

1-Amino-3-fenilciclopentanocarboxilato de etilo

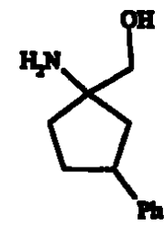


El producto de la Preparación 85 (500 mg, 2,4 mmol) se recogió en 70 ml de EtOH saturado con cloruro de hidrógeno a 0°C y luego se agitó a temperatura ambiente durante 16 h. Luego se burbujeó gas nitrógeno a la solución durante 10 minutos, y el disolvente se evaporó para dar un sólido de color beige. Éste se trató con solución acuosa saturada de $NaHCO_3$ (10 ml) y se extrajo con EtOAc (2 x 10 ml). Los extractos orgánicos reunidos se secaron y se evaporaron para dar el producto del título (360 mg, 63%); R_f DMC:MeOH (97:3) 0,23; ^1H NMR (400 MHz, $CDCl_3$) δ: 1,31 (t, 3H), 1,50-2,37 (m, 4H), 2,38-244 (m, 1H), 2,63 (dd, 1H), 3,22-3,66 y 3,43-3,57 (m, 1H), 4,20 (q, 2H), 7,20-7,35 (m, 5H).

252

Preparación 87

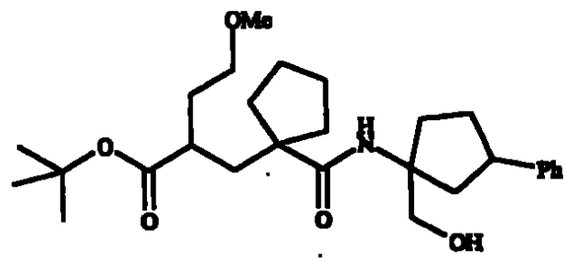
(1-Amino-3-fenilciclopentil)metanol



Se añadió poco a poco borohidruro de sodio (190 mg) a una solución agitada del producto de la Preparación 86 (390 mg, 1,67 mmol) en 8 ml de una solución al 50% de EtOH acuoso y luego se calentó a 50°C durante 3 h. Luego la mezcla de reacción se evaporó para dar una suspensión de un aceite en agua. Este aceite se extrajo con EtOAc (20 ml) y se evaporó a presión reducida para proporcionar el producto del título (295 mg); R_f (éter:pentano 1:2) 0,65; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1,30-2,36 (m, 6H), 3,02-3,17 (m, 1H), 3,33-3,50 (m, 2H), 7,14-7,37 (m, 5H).

Preparación 88

2-{[1-({[1-(Hidroximetil)-3-fenilciclopentil]amino}carbonil)ciclopentil]metil}-4-metoxibutanoato de terc-butilo

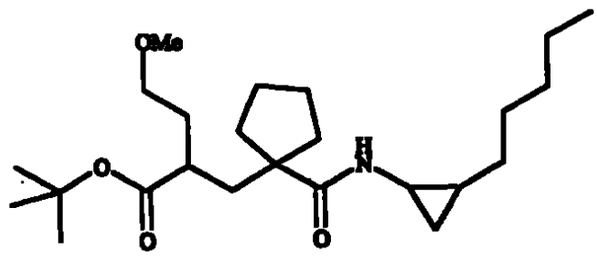


El producto del título se preparó por un método similar al descrito en la Preparación 33 a partir del producto de la Preparación 99 y el producto del título de la Preparación 87; R_f EtOAc:pentano (1:4) 0,25; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0,80-0,88 (m, 3H), 1,16-2,60 (m, 21H), 1,42 (s, 9H), 3,04-3,32 (m, 1H), 3,57-3,84 (m, 2H), 4,56-4,77 (m, 1H), 5,94 (t ancho, 1H), 7,16-7,28 (m, 5H).

253 252

Preparación 89

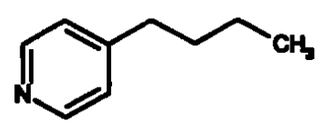
4-Metoxi-2-[(1-{[(2-pentilciclopropil)amino]carbonil}ciclopentil)metil]butanoato de terc-butilo



El producto del título de la Preparación 99 (105 mg, 0,33 mmol), trietilamina (0,5 ml), HOBt (49 mg, 0,36 mmol) y 1-amino-2-pentilciclopropano (100 mg, 0,33 mmol) se combinaron secuencialmente en DCM a temperatura ambiente en nitrógeno. Luego se añadió WSCDI (70 mg, 0,36 mmol) a la mezcla y la mezcla se agitó durante una noche durante 16 h. La reacción se diluyó con agua, la capa orgánica se separó y luego se lavó con salmuera, se secó sobre $MgSO_4$, se filtró y se evaporó para dar un aceite amarillo. Éste se purificó por cromatografía en columna utilizando EtOAc:pentano 1:3 como eluyente para dar el producto del título (96 mg, 71%); R_f 1:6 (EtOAc:pentano) 0,2; ^1H NMR (400 MHz, $CDCl_3$) δ: 0,4-0,6 (m, 2H), 0,7-0,9 (m, 4H), 1,05-1,15 (m, 1H), 1,2-1,3 (m, 4H), 1,3-1,5 (m, 14H), 1,5-2,0 (m, 10H), 2,2-2,4 (m, 2H), 3,2-3,35 (m, 5H), 5,7-5,9 (s ancho, 1H); LRMS: m/z M+H, 410.

Preparación 90

4-Butilpiridina

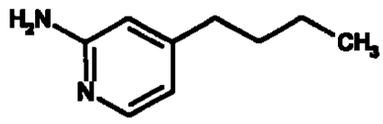


Se formó diisopropilamiduro de litio por la adición de n-butil-litio (43 ml de una solución 2,5 M en hexanos) a una solución agitada de diisopropilamina (10,9 g) en THF seco (100 ml) a -30°C en nitrógeno. Después de 1 h de agitación a esta temperatura, la solución se enfrió a -78°C y se añadió una solución de 4-metilpiridina

(10 g) en 20 ml de THF seco, seguida por agitación continuada a -78°C durante 1 h. Se añadió gota a gota yodopropano (20 g) durante 45 minutos como una solución en 20 ml de THF seco, seguida por agitación continuada de la mezcla completa durante 1 h. Se añadieron 20 ml de una solución acuosa saturada de cloruro de amonio, y luego la reacción se extrajo con éter (2 x 100 ml). Los extractos de éter combinados se lavaron con agua (75 ml), se secaron sobre $MgSO_4$ y se evaporaron para dar un aceite claro. Este aceite se purificó por destilación a vacío con aspiración de agua (aproximadamente 30 mm de Hg) y el producto del título se recogió como la fracción que se destiló a 84-90°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 0,93 (t, 3H, Me), 1,30-1,42 (m, 2H), 1,57-1,66 (m, 2H), 2,60 (t, 2H), 7,06 (d, 2H), 8,46 (d, 2H).

Preparación 90a

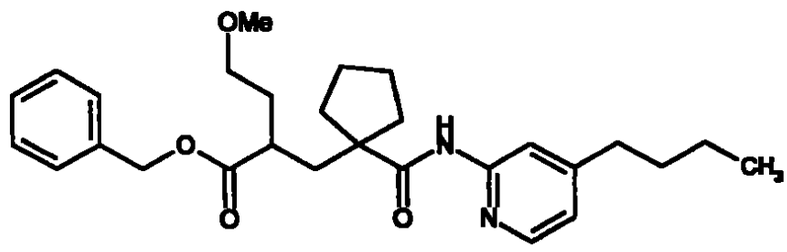
2-Amino-4-butilpiridina



El producto del título se preparó a partir del producto de la Preparación 90 de acuerdo con el método descrito en el Journal of the Chemical Society, 1946, pág. 936.

Preparación 91

2-[(1-{[(4-Butil-2-piridinil)amino]carbonil}ciclopentil)metil]-4-metoxibutanoato de bencilo

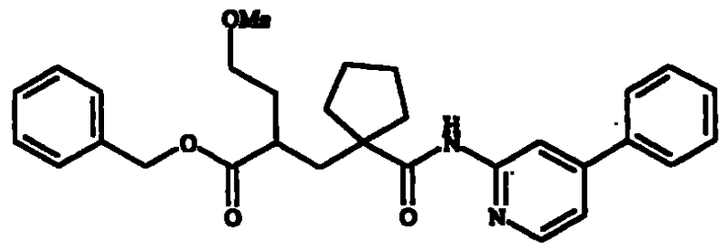


El producto del título se preparó por un procedimiento similar al descrito en

la Preparación 62 a partir de ácido 1-{2-[(benciloxi)carbonil]-4-metoxibutil}-ciclopentanocarboxílico (EP 274234, Ejemplo 15) y el producto de la preparación 90a; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0,89 (t, 3H, Me), 1,36 (q, 2H, CH_2), 1,47-2,26 (m, 14H), 2,55-2,68 (m, 3H), 3,17 (s, 3H, OMe), 3,24 (t, 2H, CH_2OMe), 4,91 (d, 1H, CHPh), 5,00 (d, 1H, CHPh), 6,83 (d, 1H, Ar), 7,27-7,35 (m, 5H), 7,94 (s ancho, 1H, NH), 8,07 (s, 1H, Ar), 8,13 (d, 1H, Ar).

Preparación 92

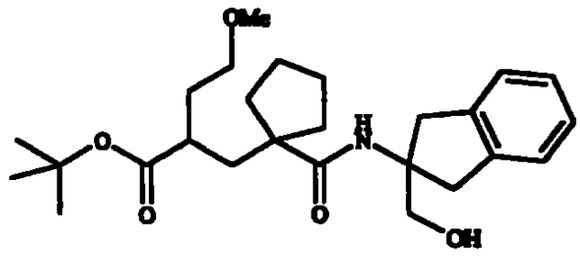
2-[(1-{[(4-Fenil-2-piridinil)amino]carbonil}ciclopentil)metil]-4-metoxibutanoato de bencilo



El producto del título se preparó por un procedimiento similar al descrito en la Preparación 62 a partir de ácido 1-{2-[(benciloxi)carbonil]-4-metoxibutil}-ciclopentanocarboxílico (EP 274234, Ejemplo 15) y 2-amino-4-fenilpiridina (véase Journal of Medicinal Chemistry, 1978, pág. 874); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,43-2,34 (m, 10H), 2,60-2,68 (m, 1H), 3,17 (s, 3H, OMe), 3,26 (t, 2H, CH_2), 4,93 (d, 1H, CHPh), 5,02 (d, 1H, CHPh), 7,18-7,32 (m, 5H, Ph), 7,38-7,46 (m, 3H), 7,61-7,69 (m, 2H), 8,02 (s ancho, 1H, NH), 8,29 (d, 1H, Ar), 8,57 (s, 1H, Ar).

Preparación 93

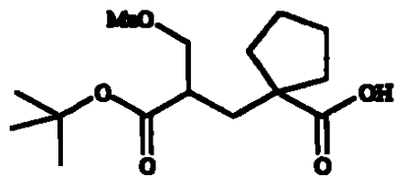
2-{[1-({[2-(Hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)ciclopentil]metil}-4-metoxibutanoato de terc-butilo



El producto del título se preparado por un procedimiento similar al descrito en la Preparación 33 a partir del producto de la preparación 99 y 2-amino-2-hidroximetil-2,3-dihidroindeno (WO9110644; Ejemplo 8a); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,40 (s, 9H), 1,44-2,00 (m, 12H), 2,37-2,43 (m, 1H), 2,99 (d, 1H), 3,08 (d, 1H), 3,20-3,38 (m, 7H), 3,65 (dd, 1H), 3,84 (dd, 1H), 4,40 (t, 1H), 6,00 (s, 1H), 7,10-7,18 (m, 4H).

Preparación 94

Éster terc-butílico de ácido 3-(1-carboxiciclopentil)-2-(metoximetil)propenoico

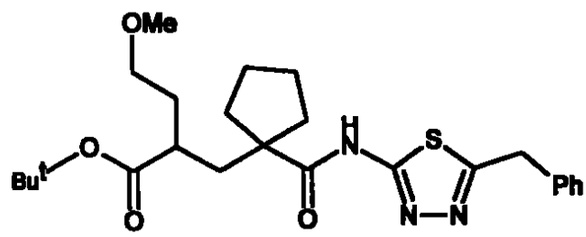


El producto del título de la Preparación 2, etapa b) (10 g, 41,3 mmol) se recogió en THF a -78°C y se añadió gota a gota diisopropilamiduro de litio (43 ml, 86,7 mmol, solución en THF 2 M). La mezcla se agitó a -78°C durante 40 minutos, y después de ese tiempo se añadió gota a gota éter metil-clorometílico. Luego la solución se dejó calentar lentamente a temperatura ambiente durante una noche y se inactivó por adición de HCl 2 N (100 ml). Los extractos orgánicos se extrajeron con EtOAc (2 x 100 ml), se secaron ($MgSO_4$) y se purificaron por cromatografía en columna utilizando 2%, luego 3% y después 5% de MeOH en DCM para proporcionar

el producto del título como un aceite amarillo (6,2 g, 53%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1,40 (9H, s), 1,40-1,50 (4H, m), 1,20-1,80 (1H, m), 1,80-1,90 (1H, m), 2,00 (1H, dd), 2,00-2,05 (3H, m), 2,20 (1H, dd), 2,50-2,60 (1H, m), 3,30 (1H, s), 3,30-3,40 (1H, m), 3,40 (1H, t); LRMS: m/z MNH_4^+ 304.

Preparación 95

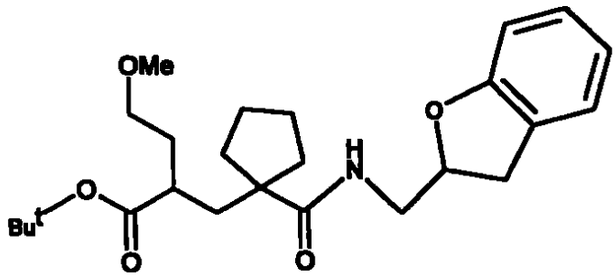
2-[(1-{[(5-Bencil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]-4-metoxibutanoato de terc-butilo



El producto de la Preparación 98 se hizo reaccionar con el producto del título de la Preparación 99 utilizando un procedimiento similar al descrito en la Preparación 33 para proporcionar la amida como una espuma blanca; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0,80 (t, 3H), 1,20-1,35 (m, 2H), 1,37 (s, 9H), 1,40-2,20 (m, 13H), 4,30 (s, 2H), 7,30 (m, 5H); LRMS m/z 450 (M+H).

Preparación 96

2-[(1-{[(2,3-Dihidro-1-benzofuran-2-ilmetil)amino]carbonil}ciclopentil)metil]-4-metoxibutanoato de terc-butilo



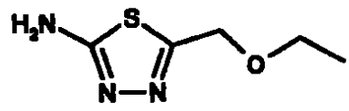
El compuesto del título se preparó a partir de 2-aminometil-2,3-dihidrobenzofurano (J. Med. Chem., 1968, 11(4), página 844) y el producto del título de la

258

Preparación 99 utilizando un procedimiento similar al descrito en la Preparación 33; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1,4 (s, 10H), 1,45-2,00 (m, 12H), 2,05 (m, 1H), 2,30 (m, 1H), 2,90 (m, 1H), 3,05 (m, 5H), 3,30 (m, 2H), 3,55-3,65 (m, 1H), 4,80 (m, 1H), 6,15 (m, 1H), 6,70 (d, 1H), 6,80 (t, 1H), 7,00 (d, 1H), 7,05 (m, 1H); LRMS m/z 432 (M+H).

Preparación 97

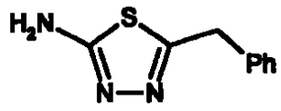
5-(Etoximetil)-1,3,4-tiadiazol-2-amina



Se preparó siguiendo un procedimiento similar a la Preparación 31 a partir de ácido etoxiacético.

Preparación 98

5-Bencil-1,3,4-tiadiazol-2-amina

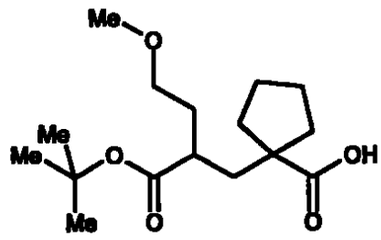


El compuesto del título se preparó siguiendo un procedimiento similar a la Preparación 31 a partir de ácido fenilacético; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4,10 (m, 2H), 7,30 (m, 5H); Anál. Encontrado C, 56,73; H, 4,72; N, 21,67%. $C_9H_9N_3S$ requiere C 56,62; H, 4,74; N, 21,97%.

259

Preparación 99

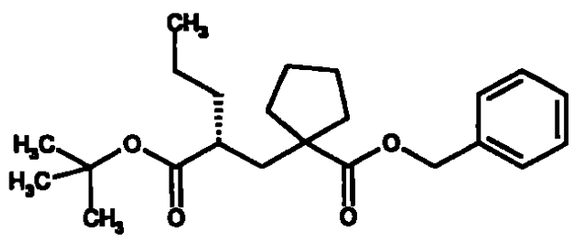
Ácido 1-[2-(terc-butoxicarbonil)-4-metoxibutil]ciclopentanocarboxílico



Una solución del producto del título de la Preparación 2, etapa b), en tetrahidrofurano seco (100 ml) se añadió a una solución agitada de diisopropilamiduro de litio (130 ml) en una mezcla de hexano (52 ml) y tetrahidrofurano (200 ml) a -78°C en nitrógeno. Después de 1 hora, se añadió una solución de éter 2-bromoetilmetílico en tetrahidrofurano (100 ml) manteniendo la temperatura a -78°C. La mezcla de reacción se dejó calentar a temperatura ambiente durante una noche. La mezcla de inactivó con agua (100 ml) y se aciduló a pH 1 con ácido clorhídrico 2 M y se extrajo con acetato de etilo (2 x 150 ml). Los extractos orgánicos reunidos se secaron sobre sulfato de magnesio y se concentraron a vacío para dar el ácido bruto que se cromatografió sobre sílice. La elución con proporciones crecientes de metanol en diclorometano (diclorometano puro a 1:50) dio un aceite (7,7 g, 25,6 mmol, 52%). Rf 0,3 metanol, diclorometano 1:20. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1,4 (s, 9H), 1,4-1,7 (m, 7H), 1,75-1,95 (m, 2H), 2,02-2,15 (, 3H), 2,3-2,4 (m, 1H), 3,3 (s, 3H), 3,3-3,4 (m, 2H); LRMS m/z 299 ($M-H^+$).

Preparación 100

1[2-(terc-Butoxicarbonil)pentil]ciclopentanocarboxilato de bencilo

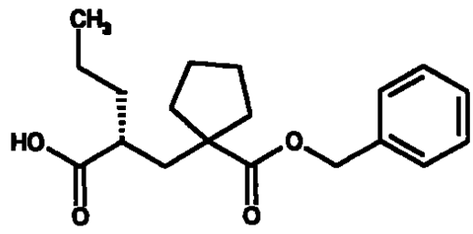


El producto de la Preparación 2 (513 mg, 1,80 mmol) se disolvió en metanol

(5 ml de metanol acuoso al 75%) y se añadió carbonato de cesio (300 mg, 0,95 mmol) en una porción a temperatura ambiente. Después de 5 minutos, los disolventes se separaron a presión reducida y el residuo se destiló azeotrópicamente con tolueno (2 x 5 ml) y luego se disolvió de nuevo en 7 ml de DMF seca en una atmósfera de nitrógeno. Se recogió bromuro de bencilo en 3 ml de DMF seca y se añadió lentamente con agitación, antes de agitar la mezcla de reacción a temperatura ambiente durante 3 horas. La mezcla se vertió en acetato de etilo (40 ml) y se lavó con agua (40 ml), HCl 1 N (20 ml) y agua (2 x 20 ml). La capa orgánica se secó ($MgSO_4$) y se evaporó para formar un aceite denso que se purificó por cromatografía en columna utilizando DCM:pentano 1:2, después EtOAc:pentano 1:2 como eluyente para proporcionar el producto del título (430 mg, 64%); ^1H NMR (400 MHz, $CDCl_3$) 0,83 (t, 3H), 1,17-1,32 (m, 3H), 1,42 (s, 9H), 1,36-1,68 (m, 7H), 1,80 (dd, 1H), 1,97-2,12 (m, 4H), 5,10 (app. q, 2H), 7,36 (5H).

Preparación 101

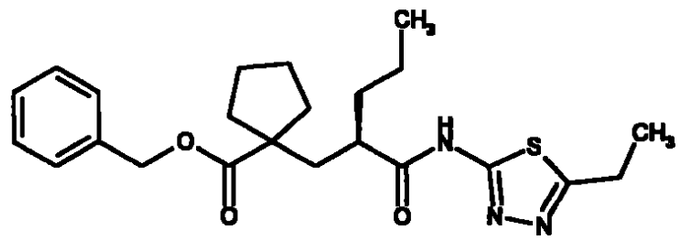
Ácido 2-({1-[(benciloxi)carbonil]ciclopentil}metil)pentanoico



El producto de la Preparación 100 (430 mg, 1,15 mmol) se recogió en TFA (2 ml) bajo atmósfera de nitrógeno y se agitó durante 16 h. La mezcla se evaporó a sequedad y luego el residuo se purificó por cromatografía en columna, utilizando DCM:MeOH 95:5 para proporcionar el producto del título (353 mg, 97%); ^1H NMR (400 MHz, $CDCl_3$) 0,82 (t, 3H), 1,20-1,74 (m, 10H), 1,80 (dd, 1H), 2,04-2,13 (m, 3H), 2,24-2,41 (m, 1H), 5,10 (app. q, 2H), 7,37 (m, 5H).

Preparación 102

1-(2-{[(5-Etil-1,3,4-tiadiazol-2-il)amino]carbonil}pentil)ciclopentanocarboxilato de bencilo



El producto de la Preparación 101 (353 mg, 1,11mmol), 2-amino-5-etil-1,3,4-tiadiazol (vendido por Lancaster) (150 mg, 1,15 mmol), WSCDI (255 mg, 1,20 mmol), HOBt (173 mg, 1,20 mmol) y 4-metilmorfolina (0,24 ml, 1,20 mmol) se mezclaron en 5 ml de acetonitrilo y se agitaron bajo nitrógeno durante 16 h a temperatura ambiente. Después de este tiempo, la mezcla se calentó a 50°C durante 3 h y luego a 80°C durante 3 h. La mezcla se enfrió a temperatura ambiente, se evaporó, se disolvió en EtOAc (10 ml) y se lavó con $NaHCO_3$ (10 ml). La capa orgánica se secó ($MgSO_4$) y se evaporó para proporcionar una goma que se purificó por cromatografía en columna para dar el producto del título (430 mg, 90%); ^1H NMR (400 MHz, $CDCl_3$) 0,79 (t, 3H), 1,15-1,24 (m, 3H), 1,37 (t, 3H), 1,42-1,63 (m, 7H), 1,83 (dd, 1H), 2,00-2,20 (m, 3H), 2,42-2,51 (m, 1H), 2,97 (q, 2H), 5,01 (app. q, 2H), 7,30 (m, 5H).

262

Análisis de NEP:

Preparación y análisis de endopeptidasa neutra soluble (NEP) de corteza renal canina, de rata, de conejo y humana

Se obtuvo NEP soluble a partir de la corteza renal y se analizó su actividad midiendo la velocidad de escisión del substrato Abz-D-Arg-Arg-Leu-EDDnp para NEP, para generar su producto fluorescente, Abz-D-Arg-Arg.

Procedimiento experimental:

1 Materiales

Todo el agua se desioniza dos veces.

1.1 Tejidos:

Riñón humano	IIAM (Pennsylvania, EE.UU.)
Riñón de rata	Fuente de tejido propia
Riñón de conejo	Fuente de tejido propia
Riñón canino	Fuente de tejido propia

1.2. Medio de homogeneización:

Manitol 100 mM y Tris 20 mM a pH 7,1.

Se diluyen 2,42 g de Tris (Fischer TP630/60) en 1 litro de agua y el pH se ajusta a 7,1 utilizando HCl 6 M a temperatura ambiente. A esto se añaden 18,22 g de Manitol (Sigma M-9546).

1.3 Tampón Tris (tampón NEP):

Se diluyen 50 ml de Tris 50 mM pH 7,4 (Sigma T2663) en 950 ml de agua.

1.4 Substrato (Abz-D-Arg-Arg-Leu-EDDnp):

Hecho a medida a partir de SNPE, y se guarda como un polvo a -20°C. Se prepara una solución madre 2 mM resuspendiendo suavemente el substrato en tampón Tris, esta suspensión no se someterá a vórtice ni a sonicación. Alícuotas de 600 µl de la suspensión de partida 2 mM se guardan a -20°C durante un período de hasta un mes. (Medeiros, M.A.S., Franca, M.S.F. y col., (1997), Brazilian Journal of Medical and Biological Research, 30, 1157-1162).

1.5 Producto total:

Muestras que corresponden a una conversión de substrato en producto del 100% se incluyen en la placa para permitir la determinación del porcentaje de recambio de substrato. El producto total se genera incubando 1 ml de substrato 2 mM con 20 µl de la solución madre de enzima durante 24 horas a 37°C.

1.6 Solución madre:

Se prepara una solución madre 300 µM de Fosforamidón (Sigma R7385) en tampón NEP y se guarda en alícuotas de 50 µl a -20°C.

1.7 Dimetilsulfóxido (DMSO).

1.8 Cloruro de magnesio: $MgCl_2.6H_2O$ (Fisher M0600/53).

1.9 Placas de ensayo de fondo plano de 96 pocillos, negras (Costar 3915).

1.10 Hojas autoadhesivas Topseal A (Packard 6005185).

1.11 Tubos para centrífuga.

2 Equipo específico

2.1 Centrífuga Sorvall RC-5B (rotor SS34 GSA, pre-enfriada a 4°C).

2.2 Minipimera Braun Minipimer.

2.3 Centrífuga Bekcman CS-6R.

2.4 Fluorímetro automático Fluostar Galaxy.

2.5 Incubadora de agitación orbital Wesbart 1589.

3 Métodos

3.1 Preparación del tejido

3.2 Se obtiene NEP de perro, rata, conejo y ser humano a partir de corteza renal utilizando una adaptación del método de Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Los riñones congelados se dejan descongelar a temperatura ambiente y se diseccíona la corteza separándola de la médula.

3.4 La corteza se pica finamente y se homogeneiza en aproximadamente 10 volúmenes de tampón de homogeneización (1.2) utilizando un Braun Minipimer (2.2).

3.5 Se añade cloruro de magnesio (1.8) (20,3 mg/g de tejido) al material homogeneizado y se agita en un baño de agua con hielo durante 15 minutos.

3.6 El material homogeneizado se centrifuga a 1.500 g (3.820 rpm) durante 12

minutos en una centrífuga Beckman (2.3) antes de separar el sobrenadante en un tubo de centrífuga sin utilizar previamente y de descartar el sedimento.

3.7 El sobrenadante se centrifuga a 15.000 g (12.100 rpm) durante 12 minutos en una centrífuga Sovall (2.1) y el sobrenadante se desecha.

3.8 La capa de color rosa pálido en la parte superior del sedimento restante se separa y se resuspende en tampón de homogeneización que contiene cloruro de magnesio (9 mg de MgCl en 5 ml de tampón por 1 g de tejido).

3.9 La suspensión se centrifuga a 2.200 g (4.630 rpm) durante 12 minutos en una centrífuga Beckman (2.3) antes de desechar el sedimento.

3.10 El sobrenadante se centrifuga a 15.000 g (12.100 rpm) durante 12 minutos utilizando la centrífuga Sorvall (2.1) y el sobrenadante se desecha.

3.11 El sedimiento final se resuspende en tampón de homogeneización que contiene cloruro de magnesio (0,9 mg de MgCl en 0,5 ml de tampón por 1 g de tejido). Se obtiene una suspensión homogénea utilizando un picadora Braun (2.2). Después ésta se congela en alícuotas de 100 µl para ser analizadas en busca de su actividad NEP.

4 <u>Determinación de actividad NEP</u>

La actividad de la NEP previamente dispuesta en alícuotas se mide por su capacidad de escindir el substrato peptídico específico NEP.

4.1 Se prepara una solución al 4% de DMSO/tampón NEP (4 ml de DMSO en 96 ml de tampón NEP).

4.2 El substrato, el producto total, la enzima y las soluciones de partida de Fosforamidón se dejan descongelar sobre hielo.

4.3 A cada pocillo se añaden 50 µl de solución al 4% de DMSO/tampón NEP.

4.4 La solución madre de substrato 2 mM se diluye 1:40 para preparar una solución 50 µM. Se añaden 100 µl de substrato 50 µM a cada pocillo (concentración final 25 µM).

4.5 Se añaden 50 µl de una gama de diluciones de enzimas para iniciar la reacción (habitualmente se utilizan 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200). A pocillos utilizados como blancos, se añaden 50 µl de tampón NEP.

4.6 El producto total 2 mM se diluye 1:80 para preparar una solución 25 µM. Se añaden 200 µl de producto 25 µM a los cuatro primeros pocillos de una

nueva placa.

4.7 Se incuban las placas a 37°C en una incubadora de agitación orbital durante 60 minutos.

4.8 La solución madre de Fosforamidón 300 µM se diluye 1:100 hasta 300 nM. La reacción se detiene por adición de 100 µl de Fosforamidón 300 nM y se incuba a 37°C en una incubadora de agitación orbital durante 20 minutos antes de leerse en el Fluostar (ex320/em420).

5 Análisis de inhibición de NEP

5.1 Las soluciones de partida de substrato, producto total, enzima y Fosforamidón se dejan descongelar sobre hielo.

5.2 Las soluciones de partida de compuesto se preparan en DMSO al 100% y se diluyen 1:25 en tampón NEP para dar una solución de DMSO al 4%. Todas las diluciones adicionales se llevan a cabo en una solución de DMSO al 4% (4 ml de DMSO en 96 ml de tampón NEP).

5.3 Se añaden 50 µl de compuesto por duplicado a la placa de 96 pocillos y 50 µl de DMSO al 4%/tampón NEP a los pocillos utilizados como testigos y como blancos (véase el apéndice para la extensión en placas). Alternativamente véase el apéndice para las diluciones robóticas.

5.4 La solución madre de substrato 2 mM se diluye 1:40 en tampón NEP para preparar una solución 50 µM (para una placa, son suficientes 275 µl de substrato 2 mM para 10,73 ml de tampón).

5.5 La solución madre de enzima se diluye en tampón NEP (determinado a partir de comprobaciones de la actividad).

5.6 La solución madre de producto total 2 mM se diluye 1:80 en tampón NEP para preparar una solución 25 µM. Se añaden 200 µl a los cuatro primeros pocillos de una placa separada.

5.7 La solución madre de Fosforamidón 300 µM se diluye 1:1.000 para hacer una solución madre 300 nM (11 µl de Fosforamidón a 10,99 ml de tampón NEP).

5.8 A cada pocillo en la placa de 96 pocillos se añade lo siguiente:

Tabla: reactivos que se añaden a la placa de 96 pocillos:

266 279

	Compuesto/ DMSO	Tampón Tris	Substrato	Enzima NEP	Producto total
Muestras	2 µl de compuesto	50 µl	100 µl	50 µl	Ninguno
Testigos	2 µl de DMSO	50 µl	100 µl	50 µl	Ninguno
Blancos	2 µl de DMSO	100 µl	100 µl	Ninguno	Ninguno
Totales	2 µl de DMSO	Ninguno	Ninguno	Ninguno	200 µl

5.9 La reacción se inicia por adición de enzima NEP antes de incubar a 37°C durante 1 hora en una incubadora de agitación orbital.

5.10 La reacción se detiene con 100 µl de Fosforamidón 300 nM y se incuba a 37°C durante 20 minutos en una incubadora de agitación orbital antes de leerse en el Fluostar (ex320/em420).

6. <u>Cálculos</u>

La actividad de la enzima NEP se determina en presencia y ausencia de compuesto y se expresa como porcentaje.

% de actividad testigo (recambio de enzima) =

$$\frac{\text{FU media de testigos - FU media de blancos}}{\text{FU media de totales - FU media de blancos}} \times 100$$

% de actividad con inhibidor =

$$\frac{\text{FU media de compuesto - FU media de blancos}}{\text{FU media de totales - FU media de blancos}} \times 100$$

Actividad expresada como % de testigo =

$$\frac{\text{\% de actividad con inhibidor}}{\text{\% de actividad testigo}} \times 100$$

Una curva dosis-respuesta sigmoidal se ajusta a los porcentajes de

actividades (% de testigo) frente a concentración de compuesto y se calculan los valores de CI50 utilizando ajuste de curva LabStats en Excel.

Análisis de ACE:

Preparación y análisis de enzima que convierte angiotensina (ACE) soluble, a partir de corteza renal porcina y humana

Se obtiene actividad de ACE soluble a partir de la corteza renal y se determina midiendo la velocidad de escisión del substrato para Abz-Gly-p-nitro-Phe-Pro-OH para ACE para generar su producto fluorescente, Abz-Gly.

1 **Materiales**

Todo el agua se desioniza dos veces.

1.1	Riñón humano	IIAM (Pennsylvania, EE.UU.) o Banco de tejido humano UK (UK HTB).
1.2	ACE de riñón porcino	Sigma (A2580)

1.3 Tampón 1 de homogeneización:

Manitol 10 mM y Tris 20 mM a pH 7,1.

Se diluyen 2,42 g de Tris (Fischer TP630/60) en 1 litro de agua y el pH se ajusta a 7,1 utilizando HCl 6 M a temperatura ambiente. A esto se añaden 18,22 g de Manitol (Sigma M-9546).

1.4 Tampón 2 de homogeneización

Manitol 100 mM, Tris 20 mM a pH 7,1 y $MgCl_2.6H_2O$ 10 mM (Fisher M0600/53).

A 500 ml de tampón de homogeneización 1 (1.4) se añaden 1,017 g de $MgCl_2$.

1.5 Tampón Tris (tampón ACE)

Tris 50 mM y NaCl 300 mM a pH 7,4.

Se ajusta el volumen de 50 ml de Tris 50 mM pH 7,4 (Sigma T2663) y 17,52 g de NaCl (Fisher S/3160/60) a 1.000 con agua.

1.6 Substrato (Abz-D-Gly-p-nitro-Phe-Pro-OH) (Bachem M-1100)

El substrato de ACE se guarda como un polvo a -20°C. Se prepara una solución madre 2 mM resuspendiendo cuidadosamente el substrato en tampón ACE, y esta suspensión no se someterá a vórtice ni a sonicación. Alícuotas de 400 µl de la suspensión de partida 2 mM se guardan a -20°C

durante un período de hasta un mes.

1.7 Producto total:

En la placa, se incluyen muestras que corresponden a una conversión de substrato en producto del 100% para permitir la determinación del porcentaje de recambio de substrato (véanse los cálculos). El producto total se genera incubando 1 ml de substrato 2 mM con 20 µl de la solución madre de enzima durante 24 horas a 37°C.

1.8 Solución de parada.

Se diluye EDTA 0,5 M (Promega CAS[6081/92/6]) 1:250 en tampón ACE para preparar una solución 2 mM.

1.9 Dimetilsulfóxido (DMSO).

1.10 Cloruro de magnesio: $MgCl_2.6H_2O$ (Fisher M0600/53).

1.11 Placas de ensayo de fondo plano de 96 pocillos, negras (Costar 3915 o Packard).

1.12 Hojas autoadhesivas Topseal A (Packard 6005185).

1.13 Tubos para centrífuga.

2 Equipo específico

2.1 Centrífuga Sorvall RC-5B (rotor SS34 GSA, pre-enfriada a 4°C).

2.2 Mezcladora Braun Minipimer.

2.3 Centrífuga Bekcman CS-6R.

2.4 Fluorímetro automático Fluostar Galaxy

2.5 Incubadora de agitación orbital Wesbart 1589.

3 Métodos

3.1 Preparación del tejido

3.2 Se obtiene ACE humana a partir de corteza renal utilizando una adaptación del método de Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Los riñones congelados se dejan descongelar a temperatura ambiente y se diseccíona la corteza separándola de la médula.

3.4 La corteza se pica finamente y se homogeneiza en aproximadamente 10 volúmenes de tampón de homogeneización 1 (1.4) utilizando una Braun Minipimer (2.2).

3.5 Se añade cloruro de magnesio (1.11) (20,3 mg/g de tejido) al material

269 27L

homogeneizado y se agita en un baño de agua con hielo durante 15 minutos.

3.6 El material homogeneizado se centrifuga a 1.500 g (3.820 rpm) durante 12 minutos en una centrífuga Beckman (2.3) antes de separar el sobrenadante en un tubo de centrífuga sin utilizar previamente y de descartar el sedimento.

3.7 El sobrenadante se centrifuga a 15.000 g (12.100 rpm) durante 12 minutos en una centrífuga Sorvall (2.1) y el sobrenadante se desecha.

3.8 La capa de color rosa pálido en la parte superior del sedimento remanente se separa y se resuspende en tampón 2 de homogeneización (1.5) (5 ml de tampón por 1 g de tejido).

3.9 La suspensión se centrifuga a 2.200 g (4.630 rpm) durante 12 minutos en una centrífuga Beckman antes de desechar el sedimento.

3.10 El sobrenadante se centrifuga a 15.000 g (12.100 rpm) durante 12 minutos utilizando la centrífuga Sorvall y el sobrenadante se desecha.

3.11 El sedimiento final se resuspende en tampón 2 de homogeneización (0,5 ml de tampón por 1 g de tejido). Se obtiene una suspensión homogénea utilizando un picadora Braun (2.2). Después ésta se descongela en alícuotas de 100 µl que se someten a determinación de la actividad en NEP.

4 <u>Determinación de actividad de ACE</u>

La actividad de la ACE previamente distribuida en alícuotas se mide por su capacidad de escindir el substrato peptídico específico para ACE.

ACE porcina (1.2) se congela y resuspende en tampón ACE (1.6) a 0,004 U/µl, y esto se descongela en alícuotas de 50 µl.

4.1 Se prepara una solución al 4% de DMSO/tampón ACE (4 ml de DMSO en 96 ml de tampón ACE).

4.2 El substrato (1.7), el producto total (1.8) y la enzima (1.1, 1.2 y 1.3) se dejan sobre hielo para descongelarse.

4.3 Se añaden 50 µl de solución al 4% de DMSO/tampón ACE a cada pocillo.

4.4 La solución madre de substrato 2 mM se diluye 1:100 para preparar una solución 20 µM. Se añaden 100 µl de substrato 20 µM a cada pocillo (concentración final en el ensayo 10 µM).

4.5 Se añaden 50 µl de una gama de diluciones de enzimas para iniciar la reacción (habitualmente se utilizan 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200). Se añaden 50 µl de tampón ACE a los pocillos utilizados como

270

blancos.

4.6 El producto total 2 mM se diluye 1:200 para preparar una solución 10 µM. Se añaden 200 µl de producto 10 µM a los cuatro primeros pocillos de una nueva placa.

4.7 Se incuban las placas a 37°C en una incubadora de agitación orbital durante 60 minutos.

4.8 La reacción enzimática se detiene por adición de 100 µl de EDTA 2 mM en tampón ACE y se incuba a 37°C en una incubadora de agitación orbital durante 20 minutos antes de leerse en el fluorímetro automático BMG Fluostar Galaxy (ex320/em420).

5 <u>Análisis de inhibición de ACE</u>

5.1 Las soluciones de partida de substrato, producto total y enzima se dejan descongelar sobre hielo.

5.2 Las soluciones de partida de compuesto se preparan en DMSO al 100% y se diluyen 1:25 en tampón ACE para dar una solución de DMSO al 4%. Todas las diluciones adicionales se llevan a cabo en una solución de DMSO al 4%/tampón ACE (4 ml de DMSO en 96 ml de tampón ACE).

5.3 Se añaden 50 µl de compuesto por duplicado a la placa de 96 pocillos y se añaden 50 µl de DMSO al 4%/tampón ACE a los pocillos utilizados como testigos y como blancos (véase el apéndice para la extensión en placas).

5.4 Las Etapas 5.2 y 5.3 se pueden llevar a cabo o bien de forma manual o utilizando robots multisonda Packard (véase el apéndice 2 para detalles).

5.5 La solución madre de substrato 2 mM se diluye 1:100 en tampón ACE para preparar una solución 20 µM (concentración final 10 µM en el análisis) (para una placa, es suficiente añadir 110 µl de substrato 2 mM a 10,89 ml de tampón).

5.6 La solución madre de enzima se diluye en tampón ACE, como se determina a partir de comprobaciones de la actividad (4.0).

5.7 La solución madre de producto total 2 mM se diluye 1:200 en tampón ACE para preparar una solución 10 µM. Se añaden 200 µl a los cuatro primeros pocillos de una placa separada.

5.8 La solución madre de EDTA 0,5 mM se diluye 1:250 para preparar una solución madre 2 mM (44 µl de EDTA para 10,96 ml de tampón ACE).

271

5.9 A cada pocillo de la placa de 96 pocillos se añaden los reactivos siguientes:

Tabla 1: reactivos que se añaden a la placa de 96 pocillos.

	Compuesto/ DMSO	Tampón Tris	Substrato	Enzima NEP	Producto total
Muestras	2 µl de compuesto	50 µl	100 µl	50 µl	Ninguno
Testigos	2 µl de DMSO	50 µl	100 µl	50 µl	Ninguno
Blancos	2 µl de DMSO	100 µl	100 µl	Ninguno	Ninguno
Totales	2 µl de DMSO	Ninguno	Ninguno	Ninguno	200 µl

5.10 Se añaden por duplicado 50 µl de la concentración más alta de cada compuesto utilizado en el análisis a la misma placa de 96 pocillos que los totales (5.7). Se añaden 150 µl de tampón ACE para determinar la fluorescencia del compuesto.

5.11 La reacción se inicia por adición de enzima ACE antes de incubar a 37°C durante 1 hora en una incubadora de agitación orbital.

5.12 La reacción se detiene por adición de 100 µl de EDTA 2 mM y se incuba a 37°C durante 20 minutos en una incubadora de agitación orbital antes de leerse en el fluorímetro automático BMG Fluostar Galaxy (ex320/em420).

6. <u>Cálculos</u>

La actividad de la enzima ACE se determina en presencia y ausencia de compuesto y se expresa como porcentaje (FU = unidades de florescencia)..

(i) % de actividad testigo (recambio de enzima) =

$$\frac{\text{FU media de testigos - FU media de blancos}}{\text{FU media de totales - FU media de blancos}} \times 100$$

(ii) % de actividad con inhibidor =

$$\frac{\text{FU media de compuesto - FU media de blancos}}{\text{FU media de totales - FU media de blancos}} \times 100$$

(iii) (Actividad expresada como % de testigo =

$$\frac{\text{\% de actividad con inhibidor}}{\text{\% de actividad testigo}} \times 100$$

o

$$\frac{\text{FU media de compuesto - FU media de blancos}}{\text{FU media de testigos - FU media de blancos}} \times 100$$

(iv) % de inhibición = 100 - % de testigo

(v) Para compuestos fluorescentes, la FU media de los pocillos utilizados como blancos que contienen compuesto (5.10) se deduce de la FU media de los valores de compuesto utilizados para calcular el porcentaje de actividad.

Una curva dosis-respuesta sigmoidal se ajusta a los porcentajes de actividades (% de testigo) frente a concentración de compuesto y se calculan los valores de CI50 utilizando ajuste de curva LabStats en Excel.

Los ejemplos específicos dados aquí tenían una CI50 frente a NEP menor que 5.000 nM.

Además, muchos de los ejemplos ensayados tenían una selectividad para NEP al menos 300 veces mayor que para ACE.

<u>Modelo animal de respuesta de excitación</u>

Los autores de la invención han desarrollado un modelo animal que simula la respuesta de excitación fisiológica observada durante la excitación sexual femenina y refleja directamente los datos clínicos obtenidos con mujeres voluntarias. El modelo utiliza tecnologías láser Doppler para registrar pequeños cambios en el flujo sanguíneo vaginal y clitoral inducidos por la estimulación del nervio pélvico o por neurotransmisores vasoactivos. Durante la excitación sexual, hay un incremento en el flujo sanguíneo genital resultante de la inervación incrementada del nervio pélvico. El incremento estimulado por el nervio pélvico en el flujo sanguíneo vaginal y clitoral, observado en

el modelo animal, representa los efectos vasculares endógenos observados durante la excitación sexual femenina. Por lo tanto, se puede utilizar este modelo para, en primer lugar, identificar los mecanismos implicados en la regulación del flujo sanguíneo vaginal y clitoral y, en segundo lugar, utilizar el modelo para validar nuevas estrategias para la intensificación del flujo sanguíneo genital.

El incremento de flujo sanguíneo vaginal y clitoral logrado por estimulación del nervio pélvico, observado en el modelo animal, representa los efectos vasculares enfógenos observados durante la excitación sexual femenina. Por lo tanto, este modelo puede ser utilizado en primer lugar para identificar los mecanismos implicados en la regulación del flujo sanguíneo vaginal y clitoral y, en segundo lugar, para usar el modelo con objeto de validar nuevas estrategias para la intensificación del flujo sanguíneo genital.

El producto del título del ejemplo 29 se administró utilizando este modelo animal de acuerdo con el siguiente protocolo para demostrar una intensificación en los incrementos de flujo sanguíneo genital logrados por estimulación del nervio pélvico en conejas.

Se estudiaron dos rutas de administración: a) administración intravenosa y b) administración tópica. Ambos estudios utilizaron el modelo de coneja anestesiada de acuerdo con el siguiente protocolo.

Métodos

Protocolo anestésico

Conejas de Nueva Zelanda (~2,5 kg) se premedicaron con una combinación de Medetomidina (Domitor®) 0,5 ml/kg *i.m.* y Ketamina (Vetalar®) 0,25 ml/kg *i.m.* mientras se mantenía suministro de oxígeno mediante una mascarilla facial. Las conejas se traqueotomizaron utilizando un tubo endotraqueal sin doblar Portex™, 3 ID, conectado a un respirador y manteniéndose un ritmo de respiración de 30 ó 40 exalaciones por minuto, con un volumen corriente de aire movilizado de aproximadamente 18-20 ml, y una presión máxima de las vías respiratorias de 10 cm de H_2O. Después se comenzó la anestesia con Isoflurane y se continuó la respiración con O_2 a 2 l/min. La vena marginal derecha de la oreja se canuló utilizando un catéter de calibre 23G ó 24G y se perfundió solución Ringer con lactato a 0,5 ml/min. La coneja se mantuvo con un 3% de Isofurane durante la cirugía invasiva, rebajándola a 2%

como anestesia de mantenimiento.

Estimulación del nervio pélvico

Se hizo una incisión en la línea media ventral en la cavidad abdominal. La incisión tenía aproximadamente 5 cm de longitud justo encima del pubis. La grasa y la masa muscular se diseccionaron bruscamente para dejar a la vista el nervio hipogástrico que desciende por la cavidad corporal. Fue esencial mantener cerrada la curva lateral de la pared del pubis con el fin de evitar dañar la vena y la arteria femoral que caen por encima del pubis. Los nervios ciático y pélvico están a nivel más profundo y se localizaron después de hacer una disección adicional en el lado dorsal de la coneja. Una vez que se identifica el nervio ciático, el nervio pélvico se localiza fácilmente. La expresión "*nervio pélvico*" es poco utilizada. Los libros de anatomía sobre la materia no llegan a identificar los nervios con suficiente detalle. Sin embargo, la estimulación del nervio produce un incremento en el flujo sanguíneo vaginal y clitoral e inervación de la región pélvica. El nervio pélvico se liberó de tejido circundante y se colocó un electrodo estimulador bipolar *Harvard* alrededor del nervio. El nervio se levantó ligeramente para producir alguna tensión, y después el electrodo se aseguró en su sitio. Se puso aproximadamente 1 ml de aceite de parafina ligero en torno al nervio y el electrodo. Esto sirve de lubricante protector para el nervio y evita la contaminación del electrodo con sangre. El electrodo se conectó a un estimulador *Grass* S88. El nervio pélvico fue estimulado utilizando los siguientes parámetros: 5V, amplitud de pulso 0,5 ms, duración de estímulo 10 segundos a intervalo de frecuencias de 2 a 16 Hz. Se obtuvieron respuestas reproducibles cuando el nervio se estimulaba cada 15-20 minutos.

Se determinó una curva de frecuencia-respuesta al comienzo de cada experimento con el fin de determinar la frecuencia óptima que convenía utilizar como respuesta submáxima, normalmente 4 Hz. Los compuestos de ensayo se infundieron, a través de la vena femoral, utilizando una bomba de infusión *Harvard* 22 permitiendo un ciclo continuo de estimulación de 15 minutos.

Inserción de sondas Doppler láser

Se hizo una incisión en la línea central ventral, en el extremo caudal del pubis, para exponer el área púbica. Se separó el tejido conjuntivo, y se expuso la túnica del clítoris, asegurando que la pared quedara libre de pequeños vasos

sanguíneos. También se expuso la pared vaginal externa separando tejido conjuntivo. Se insertó una sonda de flujo Doppler láser 3 cm en la vagina, de modo que fuera visible la mitad del eje de la sonda. Se colocó una segunda sonda de modo que cayera justo por encima de la pared clitoral externa. La posición de esta sonda se ajustó después hasta que se obtuvo una señal. Se colocó una segunda sonda justo por encima de la superficie de un vaso sanguíneo sobre la pared vaginal externa. Ambas sondas se sujetaron en su sitio.

El flujo sanguíneo vaginal y clitoral se registró o bien en forma de números directamente a partir del Flujímetro utilizando el programa de adquisición de datos *Po-ne-mah* (Ponemah Physiology Platform, Gould Instrument Systems Inc.), o bien indirectamente a partir del trazado de un registrador gráfico Gould. Al comienzo del experimento se hace una calibración (0-125 ml/min/100 g de tejido).

Administración de inhibidores

a) Administración intravenosa

Canulación de los vasos sanguíneos

Se afeitó la superficie de la ingle izquierda de la coneja y se hizo una incisión vertical de aproximadamente 5 cm de longitud a lo largo del muslo. La vena y la arteria femoral se expusieron, aislaron y después se canularon con un catéter de PVC (17G) para la infusión de fármacos y compuestos. Se repitió la canulación para la arteria femoral, insertando el catéter a una profundidad de 10 cm para garantizar que el catéter llegaba hasta la aorta abdominal. Este catéter arterial se conectó a un sistema Gould para registrar la presión sanguínea. A través del catéter arterial también se extrajeron las muestras para el análisis de gases en sangre. Se midieron las presiones sistólica y diastólica y se calculó la presión arterial media utilizando la fórmula (diastólica x 2 + sistólica) ÷ 3. El ritmo cardíaco se midió mediante el oxímetro de pulso y el sistema informático de adquisición de datos *Po-ne-mah* (Ponemah Physiology Platform, Gould Instrument Systems Inc.).

Se preparó en solución salina o solución al 5% de glucosa (200 µl, 50% de glucosa en 1,8 ml de agua para inyección) el compuesto del título del ejemplo 29. El inhibidor y los testigos con vehículo se infundieron utilizando una bomba Harvard 22, infundiendo a razón de 500 µl/min a través

de un grifo de 3 vías en la vena femoral. Después de la infusión, el catéter se lavó con solución salina heparinizada (Hepsaline) de modo que no quedara nada de inhibidor de NEP en el catéter.

*b) **Administración tópica de inhibidores***

Se preparó una formulación tópica mezclando el producto del Ejemplo 29 a 90% de saturación en 50% de propilenglicol/50% de agua. La mezcla se hizo viscosa con carboximetilcelulosa (CMC) dando una concentración final de aproximadamente 2,5 mg/ml. La administración de 0,2 ml proporciona una dosis máxima de 0,5 mg.

Esta formulación se aplicó tópicamente a la pared vaginal interna vía un aplicador diseñado por la solicitante. Básicamente, se cortaron trozos de tubo (DI 3 mm, DE 4 mm) de 10 cm de longitud y se unieron a jeringas de 1 ml. Cada jeringa se llenó con un gel utilizado como testigo (que no contenía ingrediente activo) o con la formulación descrita anteriormente. El tubo se insertó 2 cm en la vagina se inyectaron 0,2 ml del gel con el debido cuidado para no alterar la sonda láser Doppler. La adición de gel no produjo distensión considerable de la vagina durante los períodos de estimulación o no estimulación.

Resultados y discusión

Modelo animal de excitación sexual

Se ha desarrollado ahora un robusto modelo reproducible de la fisiología de la excitación sexual. Utilizando este modelo de conejas anestesiadas, los autores de la invención han sido capaces de medir pequeños cambios en el flujo sanguíneo genital utilizando la tecnología láser Doppler. Se utiliza la estimulación del nervio pélvico para estimular los efectos neuronales de la excitación sexual.

La FSAD está asociada y puede ser el resultado de un flujo sanguíneo genital disminuido.

Nuestros resultados demuestran que el compuesto del título del Ejemplo 29 mejoró significativamente los incrementos en el flujo sanguíneo genital, producidos por estimulación del nervio pélvico, a dosis clínicamente representativas. Esta mejora se observa tanto con administración intravenosa como tópica.

La Figura 1 muestra el efecto sobre el flujo sanguíneo vaginal y clitoral de la

277 282

administración intravenosa del producto del título del Ejemplo 29. La administración intravenosa intensificó los incrementos estimulados por el nervio pélvico (PNS) en el flujo sanguíneo genital en el modelo de excitación sexual en conejas anestesiadas. PNS repetitivo a intervalos de 15 minutos indujo incrementos reproducibles en el flujo sanguíneo genital (barras rayadas). La administración de un inhibidor de NEP (barras en gris) intensificó el incremento máximo en el flujo sanguíneo clitoral y vaginal inducido por frecuencias de estimulación submáximas (p.ej. 4 Hz) en comparación con los incrementos observados durante estimulaciones testigo o testigos de vehículo equiparables en el tiempo (barra rayada).

Se observaron las siguientes intensificaciones simultáneas después de un bolo iv de 1,0 mg/kg: 131% de incremento en flujo sanguíneo clitoral y 92% de incremento en flujo sanguíneo vaginal (n = 3). Los datos se expresan como valor medio ± desviación típica; todos los cambios se vigilaron utilizando tecnología láser Doppler.

La Figura 2 muestra el efecto sobre el flujo sanguíneo vaginal a lo largo del tiempo de la administración del producto del título del Ejemplo 29 tópicamente. No hubo ningún cambio apreciable para flujo sanguíneo vaginal no estimulado/basal, ni tampoco se observó cambio alguno sobre el flujo sanguíneo vaginal estimulado después de la inserción inicial del tubo o de la aplicación de los 0,2 ml del gel vehículo.

La estimulación repetitiva del nervio pélvico a frecuencias de estimulación submáximas a intervalos de 15 minutos induce incrementos reproducibles en el flujo sanguíneo vaginal (círculo negro). La aplicación intravaginal de una concentración establecida del producto del título del Ejemplo 29 (0,2 mg/ml) intensificó el incremento máximo en el flujo sanguíneo vaginal (círculos blancos) en comparación con los incrementos de flujo medio con el testigo. El producto del título del Ejemplo 29 no tuvo ningún efecto sobre el flujo sanguíneo vaginal (no estimulado) (cuadrados blancos) comparado con el flujo testigo (cuadrado negro). Todos los cambios se vigilaron utilizando tecnologías láser Doppler y los datos se expresan como valor medio + d.t. de la media (n = 4), *** $P<0,001$, ensayo de la t de Studen.

Este estudio demuestra que el producto del título del Ejemplo 29, cuando se aplicó tópicamente a la vagina intensificó significativamente los incrementos de flujo sanguíneo vaginal bajo estimulación del nervio pélvico. Es interesante que la intensificación ocurría a concentración plasmática libre del producto del título del Ejemplo 29, que no sería de esperar que causara una potenciación del flujo sanguíneo

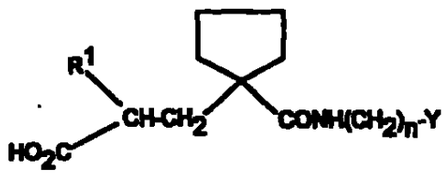
vaginal.

En conclusión, este estudio demuestra que la aplicación tópica, intravaginal, de los compuestos de la invención intensifican los incrementos en el flujo sanguíneo vaginal bajo estimulación del nervio pélvico.

279

REIVINDICACIONES

1. Un compuesto de fórmula I, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, útiles en la preparación de medicamentos para el tratamiento de la disfunción sexual femenina:



(I)

en la que:

R^1 es alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: halo, hidroxi, alcoxi C_{1-6}, hidroxialcoxi C_{2-6}, alcoxi C_{1-6}-(alcoxi C_{1-6}), cicloalquilo C_{3-7}, cicloalquenilo C_{3-7}, arilo, ariloxi, (alcoxi C_{1-4})-ariloxi, heterociclilo, heterociclíloxi, $-NR^2R^3$, NR^4COR^5, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ y $-CO_2$-(alquilo C_{1-4}); o R^1 es cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes de dicha lista, sustituyentes que pueden ser iguales o diferentes, cuya lista incluye además alquilo C_{1-6}; o R^1 es alcoxi C_{1-6}, $-NR^2R^3$ o $-NR^4SO_2R^5$;

donde

R^2 y R^3 son cada uno independientemente H, alquilo C_{1-4}, cicloalquilo C_{3-7} (opcionalmente sustituido con hidroxi o alcoxi C_{1-4}), arilo, (alquil C_{1-4})-arilo, alcoxi C_{1-6}-arilo o heterociclilo; o R^2 y R^3 junto con el nitrógeno al que están unidos forman un grupo pirrolidinilo, piperidino, morfolino, piperazinilo o N-(alquil C_{1-4})piperazinilo;

R^4 es H o alquilo C_{1-4};

R^5 es alquilo C_{1-4}, CF_3, arilo, (alquil C_{1-4})-arilo, (alcoxi C_{1-4})-arilo, heterociclilo, alcoxi C_{1-4} o $-NR^2R^3$ donde R^2 y R^3 son como se han definido previamente;

R^6 es alquilo C_{1-4}, arilo, heterociclilo o NR^2R^3 donde R^2 y R^3 son como se

han definido previamente, y

R^7 es alquilo C_{1-4}, cicloalquilo C_{3-7}, arilo o heterociclilo; p es 0, 1, 2 ó 3; n es 0, 1 ó 2;

el enlace $-(CH_2)_n-$ está opcionalmente sustituido con alquilo C_{1-4}, alquilo C_{1-4} sustituido con uno o más grupos fluoro o fenilo, alcoxi C_{1-4}, hidroxi, hidroxi-(alquilo C_{1-3}), cicloalquilo C_{3-7}, arilo o heterociclilo;

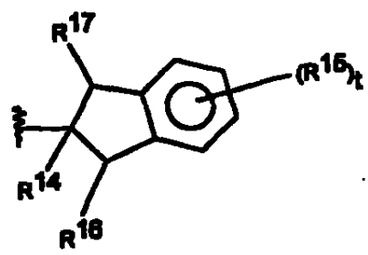
Y es el grupo



donde A es $-(CH_2)_q-$, donde q es 1, 2, 3 ó 4 para completar un anillo carbocíclico de 3 a 7 miembros que puede ser saturado o insaturado; R^8 es H, alquilo C_{1-6}, $-CH_2OH$, fenilo, fenil-(alquilo C_{1-4}) o $CONR^{11}R^{12}$; R^9 y R^{10} son cada uno independientemente H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, alquilo C_{1-6}, fenilo (opcionalmente sustituido con alquilo C_{1-4}, halo o alcoxi C_{1-4}) o fenil-(alquilo C_{1-4}), donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}, o R^9 y R^{10} juntos forman un dioxolano; R^{11} y R^{12}, que pueden ser iguales o diferentes, son H, alquilo C_{1-4}, R^{13} o $S(O)_rR^{13}$, donde r es 0, 1 ó 2 y R^{13} es fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-alquilo C_{1-4} donde el fenilo está opcionalmente sustituido con alquilo C_{1-4}; o

Y es el grupo $-C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se han definido previamente, excepto que R^{11} y R^{12} no son ambos H; o

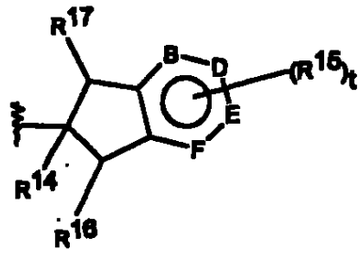
Y es el grupo



281

donde R^{14} es H, CH_2OH o $C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se han definido previamente; cuando está presente R^{15}, que puede ser igual o diferente a cualquier otro R^{15}, es OH, alquilo C_{1-4}, alcoxi C_{1-4}, halo o CF_3; t es 0, 1, 2, 3 ó 4; y R^{16} y R^{17} son independientemente H o alquilo C_{1-4}; o

Y es el grupo



donde uno o dos de B, D, E o F es un nitrógeno, siendo los demás carbono; y R^{14} a R^{17} y t son como se han definido previamente; o

Y es un anillo heterocíclico de 5 a 7 miembros opcionalmente sustituido, que puede ser saturado, insaturado o aromático y que contiene un nitrógeno, oxígeno o azufre y opcionalmente uno, dos o tres átomos de nitrógeno adicionales en el anillo y que puede estar opcionalmente condensando con benceno y opcionalmente sustituido con:

alcoxi C_{1-6}; hidroxi, oxo; amino; mono- o di-(alquil C_{1-4})-amino; alcanoil C_{1-4}-amino; o

alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: alcoxi C_{1-6}, haloalcoxi C_{1-6}, alquil C_{1-6}-tio, halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo; o

cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: alquilo C_{1-6}, alcoxi C_{1-6}, haloalcoxi C_{1-6}, alquil C_{1-6}-tio, halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo;

donde, cuando hay una sustitución oxo en el anillo heterocíclico, el anillo contiene solamente uno o dos átomos de nitrógeno y la sustitución

oxo es adyacente a un átomo de nitrógeno del anillo; o

Y es $-NR^{18}S(O)_uR^{19}$, donde R^{18} es H o alquilo C_{1-4}; R^{19} es arilo, aril-alquilo C_{1-4} o heterociclilo; y u es 0, 1, 2 ó 3.

2. Un compuesto de fórmula (I), sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que R^1, n e Y son como se han definido en la reivindicación 1, con la condición de que Y no sea el grupo $-C(O)NR^{11}R^{12}$ y, cuando R^1 es propilo o feniletilo, R^{14} no sea $-CH_2OH$.

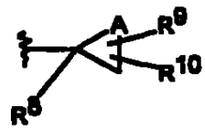
3. Un compuesto de fórmula (I), sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que R^1, n e Y son como se han definido en la reivindicación 1, con la excepción de que Y no sea el grupo $-C(O)NR^{11}R^{12}$ y R^{14} no sea H o CH_2OH.

4. Un compuesto según una cualquiera de las reivindicaciones 2 ó 3, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que R^1 es alquilo C_{1-6}, alcoxi C_{1-6}, alcoxi C_{1-6}-alquilo C_{1-3}, alcoxi C_{1-6}-alcoxi C_{1-6}-alquilo C_{1-3} o alquilo C_{1-6} sustituido con arilo.

5. Un compuesto según la reivindicación 4, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que R^1 es alquilo C_{1-6}, alcoxi C_{1-6}, alcoxi C_{1-6}-alquilo (C_{1-3}) o alcoxi C_{1-6}-alcoxi C_{1-6}-alquilo C_{1-3}.

6. Un compuesto según la reivindicación 5, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que R^1 es alquilo C_{1-6} o alcoxi C_{1-6}-alquilo C_{1-3}.

7. Un compuesto según una cualquiera de las reivindicaciones 2 a 6, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que, cuando Y es el grupo



288

y el anillo carbocíclico está totalmente saturado, entonces uno de R^9 o R^{10} es $-CH_2OH$, $-C(O)NR^{11}R^{12}$, alquilo C_{1-6}, fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-(alquilo C_{1-4}) donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}.

8. Un compuesto según la reivindicación 7, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que el anillo carbocíclico es de 5, 6 ó 7 miembros, donde uno de R^9 o R^{10} es $-C(O)NR^{11}R^{12}$, siendo el otro alquilo C_{1-6}, fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-(alquilo C_{1-4}) donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}.

9. Un compuesto según la reivindicación 7 u 8, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que R^9 y R^{10} están unidos a los átomos de carbono adyacentes en el anillo.

10. Un compuesto según una cualquiera de las reivindicaciones 7 a 9, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que R^8 es CH_2OH.

11. Un compuesto según una cualquiera de las reivindicaciones 2 a 6, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que cuando Y es el grupo $-NR^{18}S(O)_uR^{19}$, preferiblemente R^{18} es H.

12. Un compuesto según una cualquiera de las reivindicaciones 2 a 6 u 11, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que R^{19} es bencilo o fenilo.

13. Un compuesto según una cualquiera de las reivindicaciones 2 a 6, u 11 o 12, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que u es 2.

14. Un compuesto según una cualquiera de las reivindicaciones 2 a 6, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que Y es un anillo heterocíclico de 5 a 7 miembros, opcionalmente sustituido.

15. Un compuesto según la reivindicación 14, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que el anillo heterocíclico de 5 a 7 miembros es un anillo aromático opcionalmente sustituido.

16. Un compuesto según la reivindicación 15, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que dicho anillo aromático es piridilo, pirazinilo, pirimidinilo, piridazinilo, pirazolilo, triazolilo, tetrazolilo, oxadiazol, tiazol, tiadiazol, oxazolilo, indolilo, isoindolinilo, quinolinilo, isoquinolinilo, piridona, quinoxalinilo y quinazolinilo, estando cada uno de ellos opcionalmente sustituido como se ha definido en la reivindicación 1.

17. Un compuesto según la reivindicación 16, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que el anillo aromático es oxadiazol, piridona o tiadiazol, cada uno de los cuales puede estar sustituido como se ha definido en la reivindicación 1.

18. Un compuesto según la reivindicación 17, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que el anillo aromático se selecciona entre 1,2,5-oxadiazol, 1,3,4-oxadiazol, 2-piridona y 1,3,4-tiadiazol, cada uno de los cuales puede estar sustituido como se ha definido en la reivindicación 1.

19. Un compuesto según una cualquiera de las reivindicaciones 14 a 18, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que el anillo heterocíclico de 5 a 7 miembros está sustituido con uno o más de alquilo C_{1-6}, fenilo o fenil-alquilo C_{1-4}.

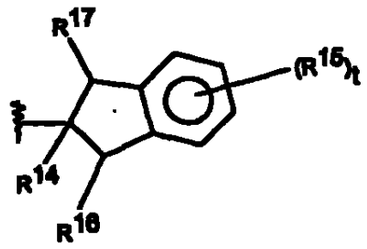
20. Un compuesto según la reivindicación 19, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que el anillo heterocíclico de 5 a 7 miembros está sustituido con alquilo C_{1-4} o bencilo.

21. Un compuesto según las reivindicaciones 17 a 20, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que cuando Y es una piridona, dicha piridona está N-sustituida.

285

22. Un compuesto según la reivindicación 14, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que Y es una lactama unida en el nitrógeno.

23. Un compuesto según una cualquiera de las reivindicaciones 2 a 6, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que Y es

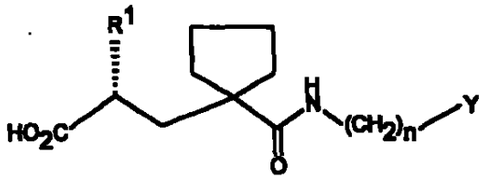


donde R^{14} es CH_2OH o $C(O)NR^{11}R^{12}$.

24. Un compuesto según una cualquiera de las reivindicaciones 2 a 6 ó 23, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que R^{16} y R^{17} son hidrógeno.

25. Un compuesto según una cualquiera de las reivindicaciones 2 a 6, 23 ó 24, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en el que t es 0.

26. Un compuesto de fórmula Ie, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables,



(Ie)

en la que R^1, Y y n son como se han definido en una cualquiera de las reivindicaciones 2 a 25.

27. Un compuesto, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, seleccionado del grupo formado por:

ácido 2-[(1-{[(1-bencil-6-oxo-1,6-dihidro-3-piridinil)amino]carbonil}ciclo-pentil)-metil]-4-metoxibutanoico (Ejemplo 35),

ácido 2-{[1-({[3-(2-oxo-1-pirrolidinil)propil]amino}carbonilciclopentil]metil}-4-fenilbutanoico (Ejemplo 40),

ácido (+)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}-4-fenilbutanoico (Ejemplo 44),

ácido 2-[(1-{[(5-metil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]-4-fenilbutanoico (Ejemplo 43),

ácido cis-3-(2-metoxietoxi)-2-[(1-{[(4-{[(fenilsulfonil)amino]carbonil}ciclohexil)-amino]carbonil}ciclopentil)metil]propanoico (Ejemplo 38),

ácido (+)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}pentanoico (Ejemplo 31),

ácido (2*R*)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]-pentanoico o ácido (-)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)-metil]pentanoico (Ejemplo 29);

ácido (2S)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)metil]-pentanoico o ácido (+)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2-il)amino]carbonil}ciclopentil)-metil]pentanoico(Ejemplo 30) ,

ácido 2-({1-[(3-bencilanilino)carbonil]ciclopentil}metil)pentanoico (Ejemplo 21),

ácido 2-[(1-{[(1-bencil-6-oxo-1,6-dihidro-3-piridinil)amino]carbonil}ciclopentil)-metil]pentanoico (Ejemplo 22),

ácido 2-{[1-({[(1R,3S,4R)-4-(aminocarbonil)-3-butilciclohexil]amino}carbonil)-ciclopentil]metil}pentanoico (Ejemplo 9),

ácido *trans*-3-[1-({[2-(4-clorofenil)ciclopropil]amino}carbonil)ciclopentil]-2-(metoximetil)propanoico (Ejemplo 46);

ácido *trans*-3-[1-({[2-(4-metoxifenil)ciclopropil]amino}carbonil)ciclopentil]-2-(metoxietil)propanoico (Ejemplo 47);

ácido *trans*-3-[1-({[2-pentilciclopropil]amino}carbonil)ciclopentil]-2-(metoxi-

etil)propanoico (Ejemplo 48);

ácido 3-[1-({[5-bencil-[1,3,4]-tiadiazol-2-il]amino}carbonil)ciclopentil]-2-(metoxietil)propanoico (Ejemplo 49);

ácido 3-[1-({[4-butilpiridin-2-il]amino}carbonil)ciclopentil]-2-(metoxietil)-propanoico (Ejemplo 50);

ácido 3-[1-({[4-fenilpiridin-2-il]amino}carbonil)ciclopentil]-2-(metoxietil)-propanoico (Ejemplo 51);

ácido 3-[1-({[1-hidroximetil-3-fenilciclopentil]amino}carbonil)ciclopentil]-2-(metoxietil)propanoico (Ejemplo 52);

ácido 2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}-4-metoxibutanoico (Ejemplo 53);

ácido *trans*-3-[1-({[2-fenilciclopropil]amino}carbonil)ciclopentil]-2-(metoxietil)-propanoico (Ejemplo 54);

ácido (*R*)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}-4-metoxibutanoico (Ejemplo 55); y

ácido (*S*)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}-4-metoxibutanoico (Ejemplo 56).

28. Uso según la reivindicación 1, en el que la disfunción sexual femenina tratada incluye al menos disfunción de excitación sexual femenina (FSAD).

29. Uso según las reivindicación 1 ó 28, en el que el medicamento se administra sistémicamente.

30. Uso según la reivindicación 29, en el que el medicamento se administra oralmente.

31. Uso de un compuesto según una cualquiera de las reivindicaciones 2 a 27, en la preparación de un medicamento para el tratamiento o la profilaxis de un proceso para el que se puede obtener una respuesta terapéutica beneficiosa por la inhibición de endopeptidasa neutra.

32 Un compuesto según una cualquiera de las reivindicaciones 2 a 27, para uso en medicina.

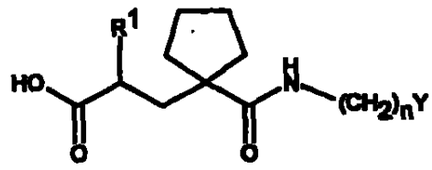
288

33. Una formulación farmacéutica que incluye un compuesto según una cualquiera de las reivindicaciones 2 a 27, junto con un excipiente farmacéuticamente aceptable.

34. Un método para el tratamiento o la profilaxis de disfunción sexual femenina, que incluye administrar al paciente una cantidad terapéuticamente eficaz de un compuesto según una cualquiera de las reivindicaciones 1 a 27.

35. Una formulación farmacéutica para disfunción sexual femenina, que incluye una cantidad terapéuticamente eficaz de un compuesto según una cualquiera de las reivindicaciones 2 a 27 junto con un excipiente farmacéuticamente aceptable.

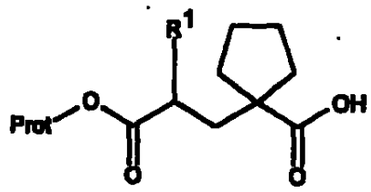
36. Un procedimiento para preparar un compuesto de fórmula I o sus sales



(I)

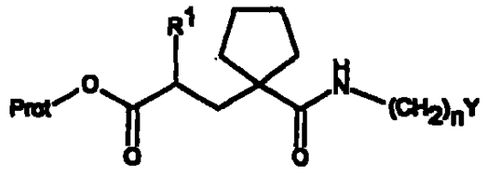
en la que R^1, n e Y son como se definen en las reivindicaciones 2 a 27, que comprende las etapas de:

a) hacer reaccionar un compuesto de fórmula II



(II)

en la que Prot es un grupo protector adecuado, con un compuesto de fórmula $Y(CH_2)_nNH_2$ (III), para dar un compuesto de fórmula IV,



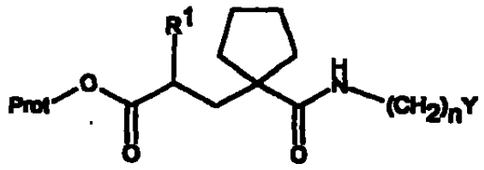
(IV)

y después

b) hacer reaccionar el compuesto de fórmula IV en condiciones adecuadas de desprotección para dar el compuesto de fórmula I; y después

c) opcionalmente formar una sal.

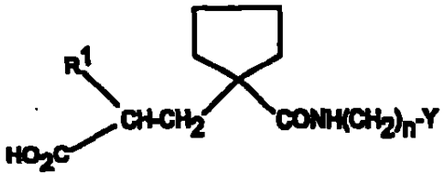
37. Un compuesto de fórmula IV



(IV)

en la que R^1, n e Y son como se han definido en una cualquiera de las reivindicaciones 2 a 27 y en la que Prot es un grupo protector.

38. El uso de un compuesto de fórmula I, sus sales, solvatos, polimorfos o profármacos farmacéuticamente aceptables, en la preparación de un medicamentos para el tratamiento de disfución sexual femenina:



(I)

290

en la que:

R^1 es alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: halo, hidroxi, alcoxi C_{1-6}, hidroxialcoxi C_{2-6}, alcoxi C_{1-6}-(alcoxi C_{1-6}), cicloalquilo C_{3-7}, cicloalquenilo C_{3-7}, arilo, ariloxi, (alcoxi C_{1-4})-ariloxi, heterociclilo, heterociclíloxi, $-NR^2R^3$, NR^4COR^5, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ y $-CO_2$-(alquilo C_{1-4}); o R^1 es cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes de dicha lista, sustituyentes que pueden ser iguales o diferentes, cuya lista incluye además alquilo C_{1-6}; o R^1 es alcoxi C_{1-6}, $-NR^2R^3$ o $-NR^4SO_2R^5$;

donde

R^2 y R^3 son cada uno independientemente H, alquilo C_{1-4}, cicloalquilo C_{3-7} (opcionalmente sustituido con hidroxi o alcoxi C_{1-4}), arilo, (alquil C_{1-4})-arilo, alcoxi C_{1-6}-arilo o heterociclilo; o R^2 y R^3 junto con el nitrógeno al que están unidos forman un grupo pirrolidinilo, piperidino, morfolino, piperazinilo o N-(alquil C_{1-4})piperazinilo;

R^4 es H o alquilo C_{1-4};

R^5 es alquilo C_{1-4}, CF_3, arilo, (alquil C_{1-4})-arilo, (alcoxi C_{1-4})-arilo, heterociclilo, alcoxi C_{1-4} o $-NR^2R^3$ donde R^2 y R^3 son como se han definido previamente;

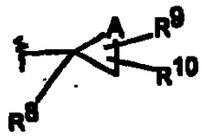
R^6 es alquilo C_{1-4}, arilo, heterociclilo o NR^2R^3 donde R^2 y R^3 son como se han definido previamente, y

R^7 es alquilo C_{1-4}, cicloalquilo C_{3-7}, arilo o heterociclilo; p es 0, 1, 2 ó 3;

n es 0, 1 ó 2;

el enlace $-(CH_2)_n-$ está opcionalmente sustituido con alquilo C_{1-4}, alquilo C_{1-4} sustituido con uno o más grupos fluoro o fenilo, alcoxi C_{1-4}, hidroxi, hidroxi-(alquilo C_{1-3}), cicloalquilo C_{3-7}, arilo o heterociclilo;

Y es el grupo

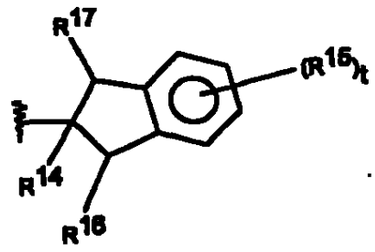


291

donde A es $-(CH_2)_q-$, donde q es 1, 2, 3 ó 4 para completar un anillo carbocíclico de 3 a 7 miembros que puede ser saturado o insaturado; R^8 es H, alquilo C_{1-6}, $-CH_2OH$, fenilo, fenil-(alquilo C_{1-4}) o $CONR^{11}R^{12}$; R^9 y R^{10} son cada uno independientemente H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, alquilo C_{1-6}, fenilo (opcionalmente sustituido con alquilo C_{1-4}, halo o alcoxi C_{1-4}) o fenil-(alquilo C_{1-4}), donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}, o R^9 y R^{10} juntos forman un dioxolano; R^{11} y R^{12}, que pueden ser iguales o diferentes, son H, alquilo C_{1-4}, R^{13} o $S(O)_rR^{13}$, donde r es 0, 1 ó 2 y R^{13} es fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-alquilo C_{1-4} donde el fenilo está opcionalmente sustituido con alquilo C_{1-4}; o

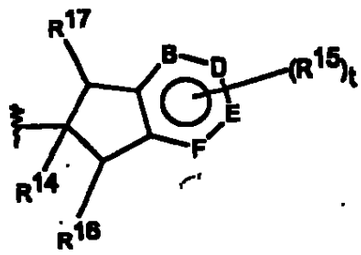
Y es el grupo $-C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se han definido previamente, excepto que R^{11} y R^{12} no son ambos H; o

Y es el grupo



donde R^{14} es H, CH_2OH o $C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se han definido previamente; cuando está presente R^{15}, que puede ser igual o diferente a cualquier otro R^{15}, es OH, alquilo C_{1-4}, alcoxi C_{1-4}, halo o CF_3; t es 0, 1, 2, 3 ó 4; y R^{16} y R^{17} son independientemente H o alquilo C_{1-4}; o

Y es el grupo



donde uno o dos de B, D, E o F es un nitrógeno, siendo los demás carbono; y R^{14} a R^{17} y t son como se han definido previamente; o

Y es un anillo heterocíclico de 5 a 7 miembros opcionalmente sustituido, que puede ser saturado, insaturado o aromático y que contiene un nitrógeno, oxígeno o azufre y opcionalmente uno, dos o tres átomos de nitrógeno adicionales en el anillo y que puede estar opcionalmente condensando con benceno y opcionalmente sustituido con:

alcoxi C_{1-6}; hidroxi, oxo; amino; mono- o di-(alquil C_{1-4})-amino; alcanoil C_{1-4}-amino; o

alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: alcoxi C_{1-6}, haloalcoxi C_{1-6}, alquil C_{1-6}-tio, halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo; o

cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: alquilo C_{1-6}, alcoxi C_{1-6}, haloalcoxi C_{1-6}, alquil C_{1-6}-tio, halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo;

donde, cuando hay una sustitución oxo en el anillo heterocíclico, el anillo contiene solamente uno o dos átomos de nitrógeno y la sustitución oxo es adyacente a un átomo de nitrógeno del anillo; o

Y es $-NR^{18}S(O)_uR^{19}$, donde R^{18} es H o alquilo C_{1-4}; R^{19} es arilo, arilalquilo C_{1-4} o heterociclilo; y u es 0, 1, 2 ó 3.

Figura 1

Los inhibidores de NEP incrementan el flujo sanguíneo genital

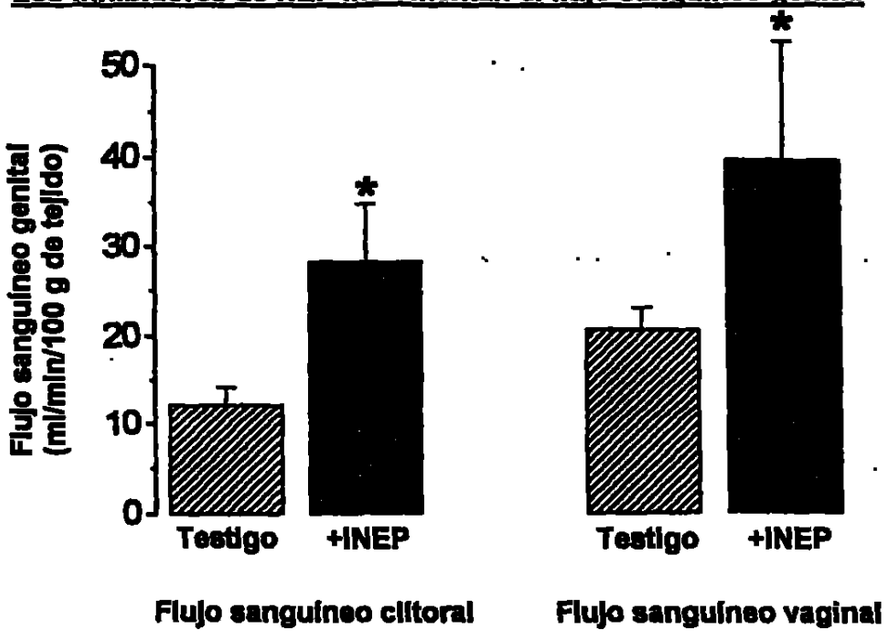
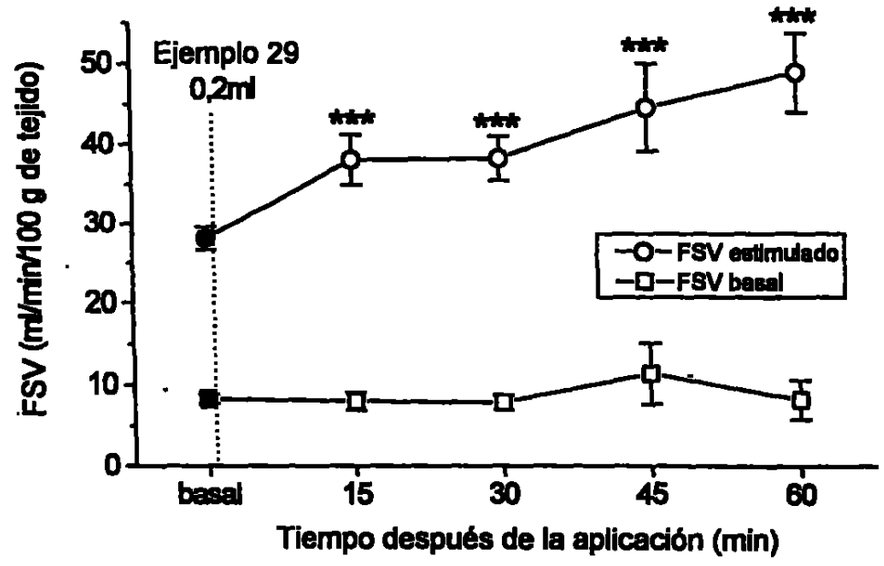


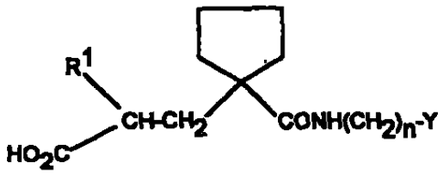
Figura 2



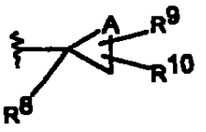
294

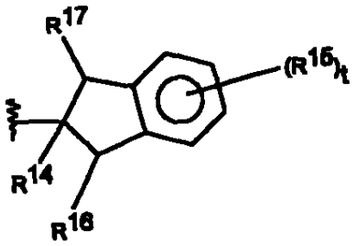
RESUMEN

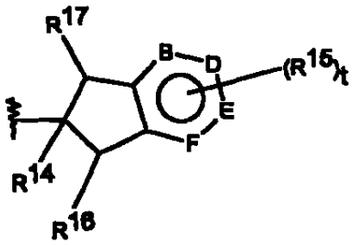
La invención proporciona compuestos de fórmula I en la que R^1 es alquilo C_{1-6} opcionalmente sustituido, cicloalquilo C_{3-7} opcionalmente sustituido, arilo opcionalmente sustituido o heterociclilo opcionalmente sustituido; n es 0, 1 ó 2; e Y es $-NR^{18}S(O)_uR^{19}$ o un grupo indicado a continuación.



(I)







PATENT COOPERATION TREATY

PCT

295

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PCS10910ARCS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB 01/ 01205	02/07/2001	06/07/2000
International Patent Classification (IPC) or national classification and IPC		
C07C237/22		
Applicant		
PFIZER LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 2 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of _____ sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/10/2001	19/06/2002
Name and mailing address of the IPEA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Netherlands Tel.: (+31-70) 340-2040 Fax: (+31-70) 340-3016	VERHULST W Tel. (+49-89) 2399 2828

Form PCT/IPEA/409 (cover sheet) (July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No PCT/ IB 01/ 01205

I. Basis of the report

The basis of this international preliminary examination is the application as originally filed.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question of whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable has not been the subject of the international preliminary examination in respect of the claims which have not been searched (Article 17(2)(a) or (3) and Rule 66.1(e) PCT); see also international search report).

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability

To the extent that the international preliminary examination has been carried out (see item III above), the following is pointed out:

In light of the documents cited in the international search report, it is considered that the invention as defined in the independent claims, which have been the subject of an international search report, meets the criteria mentioned in Article 33(1) PCT, i.e. appears to be novel, to involve an inventive step and to be industrially applicable.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 01/01205

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C237/22 A61K31/19 A61P15/00 C07C233/58 C07C233/60
C07C235/40 C07C275/52 C07C237/24 C07D317/58 C07D285/12
C07D207/27 C07D209/16 C07D207/14 C07D211/76 C07D213/75

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 33825 A (NEXMED HOLDINGS INC) 15 June 2000 (2000-06-15) claims	1-37
A	WO 91 07386 A (SCHERING CORP) 30 May 1991 (1991-05-30) cited in the application claims	1
A	WO 91 10644 A (PFIZER LTD ;PFIZER (US)) 25 July 1991 (1991-07-25) cited in the application claims	1
P,X	EP 1 097 706 A (PFIZER LTD ;PFIZER (US)) 9 May 2001 (2001-05-09) page 40 -page 41 page 68 -page 82	1-37

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 2001	04/12/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Pauwels, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 01/01205

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D213/71 C07C311/18 . C07C311/13 C07C311/51 C07D307/81

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 2001	

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Pauwels, G

Form PCT/ISA/210 (second sheet) (July 1998)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 34 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

---

Continuation of Box I.2

Claims Nos.: 1-27 (all partially)

The scope of claims 1-27 as far as the expression "prodrug" is concerned is so unclear that a meaningful International Search is impossible with regard to this expression.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 01/01205

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0033825	A	15-06-2000	AU	2358600 A	26-06-2000
			BR	9916076 A	04-09-2001
			EP	1137409 A2	04-10-2001
			WO	0033825 A2	15-06-2000
WO 9107386	A	30-05-1991	AU	6880291 A	13-06-1991
			CA	2069112 A1	22-05-1991
			EP	0502075 A1	09-09-1992
			JP	7005530 B	25-01-1995
			JP	5501111 T	04-03-1993
			WO	9107386 A1	30-05-1991
			US	5389610 A	14-02-1995
WO 9110644	A	25-07-1991	CA	2072126 A1	13-07-1991
			WO	9110644 A1	25-07-1991
			EP	0513016 A1	19-11-1992
			FI	922410 A	26-05-1992
			IE	910083 A1	17-07-1991
			JP	6045581 B	15-06-1994
			JP	5502231 T	22-04-1993
			PT	96446 A	15-10-1991
EP 1097706	A	09-05-2001	AU	7140700 A	17-05-2001
			AU	7140800 A	17-05-2001
			AU	7140900 A	10-05-2001
			AU	7141100 A	10-05-2001
			CN	1320426 A	07-11-2001
			EP	1097706 A1	09-05-2001
			EP	1097707 A1	09-05-2001
			EP	1097718 A1	09-05-2001
			EP	1097719 A1	09-05-2001
			HU	0004347 A2	28-06-2001
			HU	0004348 A2	28-06-2001
			HU	0004349 A2	28-06-2001
			HU	0004350 A2	28-06-2001
			JP	2001213802 A	07-08-2001
			JP	2001206855 A	31-07-2001
			JP	2001247478 A	11-09-2001
			JP	2001247479 A	11-09-2001
			NO	20005618 A	09-05-2001
			NO	20005661 A	09-05-2001
			NO	20005662 A	09-05-2001
			PL	343753 A1	21-05-2001
			PL	343754 A1	21-05-2001
			PL	343755 A1	21-05-2001

La Oficina de Patentes

Concept House

Cardiff Road

Newport

South Wales

NP10 8QQ

Yo, el suscrito, siendo un oficial debidamente autorizado de acuerdo con la Sección 74(1) y (4) de la Ley de Deregulación y Contratación de 1994, para firmar y emitir certificados en nombre del Contralor-General, por la presente certifico que lo anexado es una copia fiel de los documentos como fueron originalmente radicados en conexión con la solicitud de Patente identificada allí dentro.

De acuerdo con las Reglas de Patentes (Re-inscripción de Compañías) de 1982, sí una compañía nombrada en este certificado y cualquier documento acompañante se ha re-inscrito bajo la Ley de Compañías de 1980 con el mismo nombre con el cual fue inscrito inmediatamente antes de la re-inscripción, salvo la substitución como, o la inclusión como, la última parte del nombre de las palabras "compañía

pública limitada" o sus equivalentes en Galés, referencias al nombre de la compañía en este certificado y cualquier documento acompañante serán tratados como referencias al nombre con el cual está re-inscrito.

De acuerdo con las reglas, las palabras "compañía pública limitada" pueden ser reemplazadas por p.l.c., plc, P.L.C. ó PLC.

Una re-inscripción bajo la Ley de Compañías no constituye una nueva entidad legal sino apenas sujeta a la compañía a ciertas reglas de ley corporativa adicionales.

Sello ilegible.

Firmado: ilegible

Fecha: 10 de mayo de 2001

Una Agencia Ejecutiva del Departamento de Comercio e Industria

301

La Oficina de Patentes

Sello de la oficina de Patentes con fecha 6 de Julio de 2000

Su Referencia PCS10910WPM-PROV **0016684.3**

Oficina de Patentes

Petición para el otorgamiento de una Patente

Formulario 1/77 **Patentes 1977**

---

1. Titulo de la Invención COMPOSICIÓN FARMACEUTICA

1 Por favor suministre el título de la invención

---

2. Detalles de la Solicitud

Primer o único solicitante

2a Si usted está aplicando como sociedad por favor dé:

Nombre corporativo

PFIZER LIMITED

País (y estado de la incorporación, si es apropiado)

REINO UNIDO

302

2b. Si usted está aplicando como individuo o uno de una sociedad por favor de por completo:

Apellidos

Nombres

2c. En todos los casos, por favor suministre los siguientes detalles:

Dirección

RAMSGATE ROAD

SÁNDWICH

KENT

Reino Unido Código PostalCT13 9NJ

(si aplica)

País Reino Unido

Número de ADP

(si lo conoce) 68926 73001

Segundo solicitante (si lo hay)

2d. Sí usted está aplicando como sociedad por favor dé:

Nombre corporativo

País (y estado de la incorporación, si es apropiado)

2e. Si usted está aplicando como individuo o uno de una sociedad por favor de por completo:

Apellidos

Nombres

2f. En todos los casos, por favor suministre los siguientes detalles:

Dirección

Reino Unido Código Postal

(si aplica)

País

Número ADP de patente

(si lo conoce)

---

3. Dirección para los detalles del servicio

3a. Tiene usted designado un agente para encargarse de su solicitud?

Si [X] No → ir a 3b

↓

Por favor suministre los detalles a continuación:

Nombre del Agente

DR. RICHARD C. SEWELL

Dirección del Agente

PFIZER LIMITED

RAMSGATE ROAD

SANDWICH

KENT

Código postal CT13 9NJ

Número ADP del Agente 6892673001

3b. Si usted no ha designado un agente por favor suministre nombre y dirección en el Reino Unido en donde debe ser enviada toda la correspondencia:

Nombre

Dirección

Código Postal Teléfono diurno

Número ADP número (si esta disponible)

(si lo conoce)

4. Número de Referencia

4 Agente o solicitante
número de referencia PCS10910WPM-PROV
(Si aplica)

---

5. Prioridad de una solicitud anterior

5 Está usted reclamando prioridad de una solicitud que haya sido presentada en una fecha anterior a esta solicitud?

Si [X] No [X] → ir a 6

↓

Por favor suministre detalles abajo
Número de solicitud anterior o número de patente
Fecha de presentación (día mes año)
Y la Sección del Acto de patentes 1977 bajo el cual usted esta reclamando:
15(4) Divisional [ ] 8(3)[ ] 12(6)[ ] 37(4) [ ]

---

6. Declaración de prioridad

6 Si está declarando prioridad de solicitud(es) previas, por favor suministre:

País de presentación	Número de solicitud de prioridad (si lo conoce)	Fecha de radicación (día, mes, año)

---

7. Inventoría

7 Es usted (el solicitante o solicitantes) el único inventor ó los inventores comunes?

Por favor marque caja correcta

Si ☐ No ☒ → Una Declaración de Inventoría formulario de Patentes 7/77 debe ser presentado (véase regla 15)

---

8. Lista de comprobación

8a Por favor indicar el número de hojas para cada uno de los siguientes tipos de documentos contenidos en esta solicitud.

Hojas de continuación de este Formulario de Patentes 1/77

307

Reivindicaciones	11	Descripción	102
Resumen		Dibujo(s)	

8b. Cual de los siguientes documentos también acompañan la solicitud?

Documentos de prioridad (por favor indicar cuantos)

Traducción(es) de Documento(s) de Prioridad (por favor indicar cuantos)

Formulario de Patentes 7/77 Declaración de inventoría y Derecho de Otorgamiento (por favor indicar cuantos)

Formulario de Patentes 9/77 - Examen Preliminar/Búsqueda

Formulario de Patentes 10/77 - Petición para Examinación Substantiva

---

9. Petición

Yo/Nosotros pedimos el otorgamiento de una patente con base en esta solicitud.

Firma	Fecha
Richard Sewell	06/07/2000
	(dia, mes, año)

308

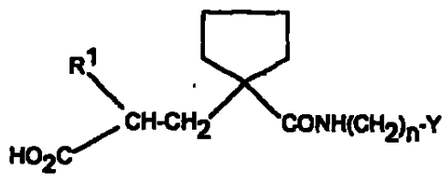
Por favor devolver el formulario completo, anexos y duplicados en donde han sido solicitados, junto con los honorarios descritos a :

The Comptroller
La Oficina de Patentes
Cardiff Road
NEWPORT
Gwent
NP9 1RH

The Comptroller
La Oficina de Patentes
25 Southampton Buildings
London
WC2A 1AY

REIVINDICACIONES:

1. El uso de un compuesto de fórmula I, o una sal, solvato, polimorfo o profármacos farmacéuticamente aceptable, en la preparación de un medicamento para el tratamiento de la disfunción sexual:



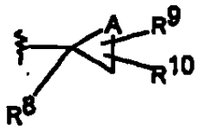
(I)

en donde:
R^1 es alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: halo, hidroxi, alcoxi C_{1-6}, hidroxialcoxi C_{2-6}, alcoxi C_{1-6}(alcoxi C_{1-6}), cicloalquilo C_{3-7}, cicloalquenilo C_{3-7}, arilo, ariloxi (alcoxi C_{1-4})ariloxi, heterociclilo, heterocicliloxi, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ y $-CO_2$(alquilo C_{1-4}); o R^1 es cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes de dicha lista, sustituyentes que pueden ser iguales o diferentes, cuya lista incluye además alquilo C_{1-6}; o R^1 es alcoxi C_{1-6}; $-NR^2R^3$ o $-NR^4SO_2R^5$;

donde
R^2 y R^3 son cada uno independientemente H, alquilo C_{1-4}, cicloalquilo C_{3-7}(opcionalmente sustituido con hidroxi o alcoxi C_{1-4}), arilo, (alquil C_{1-4})arilo, alcoxiarilo C_{1-6} o

heterociclilo; R^2 y R^3 junto con el nitrógeno al que están unidos forman un grupo pirrolidinilo, piperidino, morfolino, piperazinilo o N-(alquil C_{1-4}) grupo piperazinilo;
R^4 es H o alquilo C_{1-4};
R^5 es alquilo C_{1-4}, CF_3, arilo, (alquil C_{1-4})arilo, (alcoxi C_{1-4})arilo; heterociclilo, alcoxi C_{1-4} o $-NR^2R^3$ donde R^2 y R^3 son como se han definido previamente;
R^6 es alquilo C_{1-4}, arilo, heterociclilo o NR^2R^3 donde R^2 y R^3 son como se han definido previamente; y
R^7 es alquilo C_{1-4}, cicloalquilo C_{3-7}, arilo o heterociclilo;
n es 0, 1 ó 2; p es 0, 1, 2 ó 3;
el enlace $-(CH_2)_n-$ está opcionalmente sustituido con alquilo C_{1-4}, alquilo C_{1-4}, sustituido con uno o más grupos fluoro o fenilo, alcoxi C_{1-4}, hidroxi, hidroxi(alquilo C_{1-3}), cicloalquilo C_{3-7}, arilo ó heterociclilo;

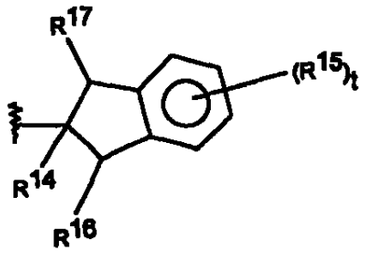
Y es el grupo



donde A es $-(CH_2)_q-$, donde q es 1, 2, 3 ó 4 para completar un anillo carbocíclico de 3 a 7 miembros que pueden ser saturado o insaturado; R^8 es H, alquilo C_{1-6}, $-CH_2OH$, fenilo, fenil(alquilo C_{1-4}) o $CONR^{11}R^{12}$; R^9 y R^{10} son cada uno independientemente H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, alquilo C_{1-6}, fenilo opcionalmente sustituido con alquilo C_{1-4}, o fenil(alquilo C_{1-4}), donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}, halo o alcoxi C_{1-4} o R^9 y R^{10} juntos forman un dioxolano; R^{11} y R^{12}, que pueden

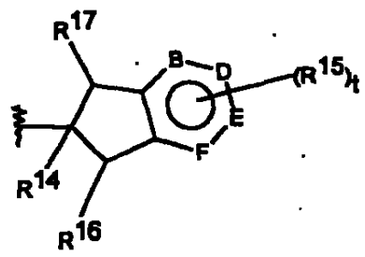
ser iguales o diferentes, son H, alquilo C_{1-4}, R^{13} o $S(O)_rR^{13}$, donde r es 0, 1 ó 2 y R^{13} es fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-alquilo C_{1-4} donde el fenilo esta opcionalmente sustituido con alquilo C_{1-4}; o
Y es el grupo $-C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se han definido previamente, excepto que R^{11} y R^{12} no son ambos H; o

Y es el grupo



donde R^{14} es H, CH_2OH o $C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se ha definido previamente; cuando está presente R^{15}, que puede ser igual o diferente a cualquier otro R^{15}, es OH, alquilo C_{1-4} , alcoxi C_{1-4}, halo o CF_3; t es 0, 1, 2, 3 ó 4; R^{16} Y R^{17} son independientemente H o alquilo C_{1-4}; o

Y es el grupo



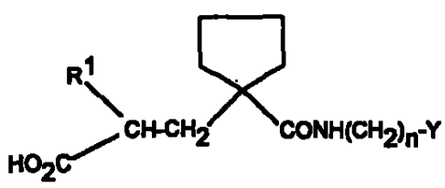
donde uno o dos de B, D o F es un nitrógeno, siendo los demás carbonos; y R^{14} a R^{17} y t son como se han definido previamente; o
Y es un anillo heterociclico de 5 a 7 miembros opcionalmente sustituido, que puede ser saturado, insaturado o aromático y que contiene un nitrógeno, oxígeno

o azufre y opcionalmente uno, dos o tres átomos de nitrógeno adicionales en el anillo y que puede estar opcionalmente condensado y opcionalmente sustituido con; alcoxi C_{1-6}; hidroxi, oxo, amino; mono o di-(alquilo C_{1-4})amino; alcanoilamino C_{1-4}; o
alquilo C_{1-6} que puede estar sustituido con uno o más grupos, que pueden ser iguales o diferentes, seleccionado de la lista: alcoxi C_{1-6}, haloalcoxi C_{1-6}; alquiltio C_{1-6}; halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo, o cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista, alquilo C_{1-6}, alcoxi C_{1-6}, haloalcoxi C_{1-6}, alquiltio C_{1-6}, halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo; donde, cuando hay una sustitución oxo en el anillo heterociclico, el anillo contiene solamente uno o dos átomos de nitrógeno y la sustitución oxo es adyacente a un átomo de nitrógeno del anillo; o

Y es $-NR^{18}S(O)_uR^{19}$, donde R^{18} es H o alquilo C_{1-4}; R^{19} es arilo, arilalquilo C_{1-4} o heterociclilo; y

u es 0, 1, 2 ó 3.

2. Un compuesto de fórmula (I), o una sal, solvato farmacéuticamente aceptable, o polimorfos de los mismos



(I)

en donde

R^1 es alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: halo, hidroxi, alcoxi C_{1-6}, hidroxialcoxi C_{2-6}, alcoxi C_{1-6}(alcoxi C_{1-6}), cicloalquilo C_{3-7}, cicloalquenilo C_{3-7}, arilo, ariloxi (alcoxi C_{1-4})ariloxi, heterociclilo, heterocicliloxi, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ y $-CO_2$(alquilo C_{1-4}); o R^1 es cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes de dicha lista, sustituyentes que pueden ser iguales o diferentes, cuya lista incluye además alquilo C_{1-6}; o R^1 es alcoxi C_{1-6}; $-NR^2R^3$ o $-NR^4SO_2R^5$;

donde

R^2 y R^3 son cada uno independientemente H, alquilo C_{1-4}, cicloalquilo C_{3-7}(opcionalmente sustituido con hidroxi o alcoxi C_{1-4}), arilo, (alquil C_{1-4})arilo, alcoxiarilo C_{1-6} o heterociclilo; R^2 y R^3 junto con el nitrógeno al que están unidos forman un grupo pirrolidinilo, piperidino, morfolino, piperazinilo o N-(alquil C_{1-4}) grupo piperazinilo;

R^4 es H o alquilo C_{1-4};

R^5 es alquilo C_{1-4}, CF_3, arilo, (alquilo C_{1-4})arilo, (alcoxi C_{1-4})arilo; heterociclilo, alcoxi C_{1-4} o $-NR^2R^3$ donde R^2 y R^3 son como se han definido previamente;

R^6 es alquilo C_{1-4}, arilo, heterociclilo o NR^2R^3 donde R^2 y R^3 son como se han definido previamente; y

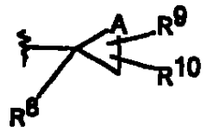
R^7 es alquilo C_{1-4}, cicloalquilo C_{3-7}, arilo o heterociclilo;

n es 0, 1 ó 2; p es 0, 1, 2 ó 3;

el enlace $-(CH_2)_n-$ está opcionalmente sustituido con alquilo C_{1-4}, alquilo C_{1-4}, sustituido con uno o más grupos fluoro o

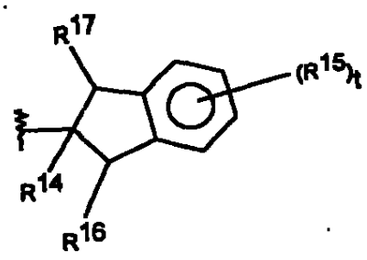
fenilo, alcoxi C_{1-4}, hidroxi, hidroxi(alquilo C_{1-3}), cicloalquilo C_{3-7}, arilo ó heterociclilo;

Y es el grupo



donde A es $-(CH_2)_q-$, donde q es 1, 2, 3 ó 4 para completar un anillo carbocíclico de 3 a 7 miembros que pueden ser saturado o insaturado; R^8 es H, alquilo C_{1-6}, $-CH_2OH$, fenilo, fenil(alquilo C_{1-4}) o $CONR^{11}R^{12}$; R^9 y R^{10} son cada uno independientemente H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, alquilo C_{1-6}, fenilo opcionalmente sustituido con alquilo C_{1-4}, o fenil(alquilo C_{1-4}), donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}, o R^9 y R^{10} juntos forman un dioxolano; R^{11} y R^{12}, que pueden ser iguales o diferentes, son H, alquilo C_{1-4}, R^{13} o $S(O)_rR^{13}$, donde r es 0, 1 ó 2 y R^{13} es fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-alquilo C_{1-4} donde el fenilo esta opcionalmente sustituido con alquilo C_{1-4}; o

Y es el grupo

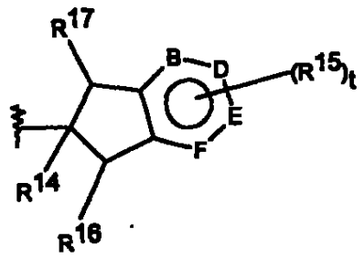


donde R^{14} es $C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se ha definido previamente; cuando está presente R^{15}, que puede ser igual o diferente a cualquier otro R^{15}, es OH, alquilo

C_{1-4} , alcoxi C_{1-4}, halo o CF_3; t es 0, 1, 2, 3 ó 4; R^{16} Y R^{17} son independientemente H o alquilo C_{1-4}; o

315

Y es el grupo



donde uno o dos de B, D o F es un nitrógeno, siendo los demás carbonos; y R^{14} a R^{17} y t son como se han definido previamente; o

Y es un anillo heterociclico de 5 a 7 miembros opcionalmente sustituido, que puede ser saturado, insaturado o aromatico y que contiene un nitrógeno, oxígeno o azufre y opcionalmente uno, dos o tres átomos de nitrógeno adicionales en el anillo y que puede estar opcionalmente condensado y opcionalmente sustituido con; alcoxi C_{1-6}; hidroxi, oxo, amino; mono o di-(alquilo C_{1-4})amino; alcanoilamino C_{1-4}; o

alquilo C_{1-6} que puede estar sustituido con uno o más grupos, que pueden ser iguales o diferentes, seleccionado de la lista: alcoxi C_{1-6}, haloalcoxi C_{1-6}; alquiltio C_{1-6}; halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo, o cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista, alquilo C_{1-6}, alcoxi C_{1-6}, haloalcoxi C_{1-6}, alquiltio C_{1-6}, halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo; donde, cuando hay una sustitución oxo en el anillo heterociclico, el anillo contiene solamente uno o dos

átomos de nitrógeno y la sustitución oxo es adyacente a un átomo de nitrógeno del anillo; o

Y es $-NR^{18}S(O)_uR^{19}$, donde R^{18} es H o alquilo C_{1-4}; R^{19} es arilo, arilalquilo C_{1-4} o heterociclilo; y

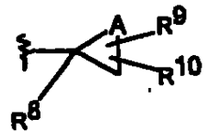
u es 0, 1, 2 ó 3.

3. Un compuesto con se define en la reivindicación 1 ó 2 en donde R^1 es alquilo C_{1-6}, alcoxi C_{1-6} ó alquilo C_{1-6} sustituido con arilo.

4. Un compuesto con se define en la reivindicación 3 en donde R^1 es alquilo C_{1-6}, alcoxi C_{1-6} ó alcoxi $C_{1-6}(C_{1-3})$alquilo.

5. Un compuesto como se define en la reivindicación 4 en donde R^1 es alquilo C_{1-4}.

6. Un compuesto según cualquiera de las reivindicaciones anteriores en el que, cuando Y es el grupo



y el anillo carbociclico está totalmente saturado, entonces uno de R^9 ó R^{10} es $-CH_2OH$, $-C(O)NR^{11}R^{12}$, alquilo C_{1-6}, fenilo opcionalmente sustituido con alquilo C_{1-4} o fenilo(alquilo C_{1-4})en donde el grupo fenilo es opcionalmente sustituido con alquilo C_{1-4}.

317

7. Un compuesto según la reivindicación 6, en donde el anillo carbociclico es de 5, 6 ó 7 miembros, donde uno de R^9 ó R^{10} es $-C(O)NR^{11}R^{12}$, siendo el otro alquilo C_{1-6}, fenilo opcionalmente sustituido con alquilo C_{1-4} o fenilo(alquilo C_{1-4})en donde el grupo fenilo es opcionalmente sustituido con alquilo C_{1-4}.

8. Un compuesto según la reivindicación 7, en donde R^9 y R^{10} están unidos a los átomos de carbono adyacentes en el anillo.

9. Un compuesto según una cualquiera de las reivindicaciones anteriores en donde R^8 es CH_2OH.

10. Un compuesto según una cualquiera de las reivindicaciones 1 a 5 en donde Y es el grupo $NR^{18}S(O)_uR^{19}$, entonces R^{18} es H.

11. Un compuesto como se define en cualquiera de las reivindicaciones 1 a 5 o 10 en donde R^{19} es benzilo o fenilo.

12. Un compuesto como se define en cualquiera de las reivindicaciones 1 a 5, 10 ó 11 en donde u es 2.

13. Un compuesto cono se define en la reivindicación 1 en donde Y es un anillo heterociclico de 5 a 7 miembros opcionalmente sustituido, que puede ser saturado, insaturado o aromatico y que contiene un nitrógeno, oxígeno o azufre y opcionalmente uno, dos o tres átomos de nitrógeno adicionales en el anillo y que puede estar opcionalmente condensado y opcionalmente sustituido con uno

o más alquilo C_{1-6}; fenilo, fenilalquil C_{1-4}, alcoxi C_{1-6} hidroxi, oxo, amino; mono o di-(alquilo C_{1-4})amino ó alcanoilamino C_{1-4}; con la condición de que cuando hay una sustitución oxo, el anillo contiene solamente uno o dos átomos de nitrógeno y la sustitución oxo es adyacente a un átomo de nitrógeno del anillo.

14. Un compuesto como se define en la reivindicación 13, en donde el anillo heterociclico es un anillo heteroaromático.

15. Un compuesto como se define en la reivindicación 14, en donde el anillo heteroaromático esta seleccionado de piridilo, pirazinilo, pirimidinilo, piridazinilo, pirazolilo, triazolilo, tetrazolilo, oxadiazol, tiazol, tiadiazol, oxazolilo, isoxazolilo, indolilo, isoindolinilo, quinolilo, piridona, quinoxalinilo y quinazolinilo, estando cada uno de ellos opcionalmente sustituidos como se han definido en la reivindicación 1.

16. Un compuesto como se define en la reivindicación 15, en donde el anillo heteroaromático está seleccionado de oxadiazole, piridona y tiadiazol.

17. Un compuesto como se define en la reivindicación 16, en donde el anillo heteroaromático está seleccionado de 1, 2, 5 oxadiazol, 1, 3, 4 oxadiazol, 2-piridona y 1, 3, 4 tiadiazol.

18. Un compuesto como se define en cualquiera de las reivindicaciones 13 a 17 en donde el anillo heterocicliclo

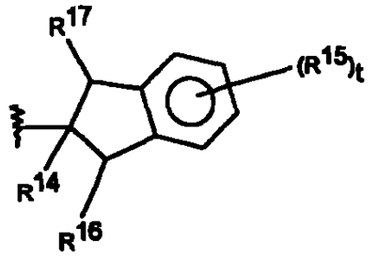
es sustituido con uno o mas alquilo C_{1-6}, fenilo o fenilalquil C_{1-4}.

19. Un compuesto como se define en la reivindicación 18, en donde la sustitución es alquilo C_{1-4} o benzilo.

20. Un compuesto como se define en la reivindicación 16 a 19, en el que cuando Y es una piridona, dicha piridina está N-sustituida (preferiblemente con benzilo o alquilo C_{1-4}).

21. Un compuesto como se define en la reivindicación 13, en donde Y es una lactama unida a el nitrógeno.

22. Un compuesto como se define en la reivindicación 1 y reivindicaciones dependientes de las mismas en donde Y es



donde R^{14} es H, CH_2OH ó $C(O)NR^{11}R^{12}$, R^{15} es uno o más de H, OH, alquilo C_{1-4}, alcoxi C_{1-4}, halo ó CF_3, y R^{16} y R^{17} son independientemente H ó alquilo C_{1-4}.

23. Un compuesto como se define en la reivindicación 22 en donde R^{14} es CH_2OH ó $C(O)NR^{11}R^{12}$.

24. Un compuesto como se define en la reivindicación 23 en donde R^{14} es $C(O)NR^{11}R^{12}$.

25. Un compuesto como se define en la reivindicación 2 y reivindicaciones 22 a 24 en donde R^{16} y R^{17} son H.

26. Un compuesto como se define en la reivindicación 2 y reivindicaciones 21 a 24 en donde t es 0.

27. Un compuesto de acuerdo con cualquiera de las reivindicaciones anteriores seleccionadas del grupo que consiste de:

2-[(1-{[(1-bencil-6-oxo-1,6-dihidro-3-piridinil) amino]carbonil}-ciclopentil)metil]-4-acido metoxibutanoico (Ejemplo 35),

2-{[1-({[3-(2-oxo-1-pirrolidinil)propil]amino} carbonilciclopentil]metil}-4-ácido fenilbutanoico (Ejemplo 40),

(+)-2-{[1-({[2-(hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)ciclopentil]-metil}-4-acido fenilbutanoico (Ejemplo 44),

2-[(1-{[(5-metil-1,3,4-tiadiazol-2-il)amino]carbonil} ciclopentil)-metil]-4-acido fenilbutanoico (Ejemplo 43),

cis-3-(2-metoxietoxi)-2-[(1-{[(4-{[(fenilsulfonil) amino]carbonil}ciclohexil)amino]carbonil}ciclopentil)metil] acido propanoico (Ejemplo 38),

(+)-2-{[1-({[2-hidroximetil)-2,3-dihidro-1*H*-inden-2-il]amino}carbonil)-ciclopentil]metil}acido pentanoico (Ejemplo 31),

(+)-2-[(1-{[(5-etil-1,3,4-tiadiazol-2il)amino] carbonil}ciclopentil)-metil]acido pentanoico (Ejemplo 30),

2-({1-[(3-bencilanilino)carbonil]ciclopentil}metil) acido pentanoico (Ejemplo 21),

2-[(1-{[(1-bencil-6-oxo-1,6-dihidro-3-piridinil)amino]carbonil}ciclopentil)-metil]acido pentanoico (Ejemplo 22), y

2-{[1-({[1R,3S,4R)-4-(aminocarbonil)-3-butilciclohexil]amino}carbonil)-ciclopentil]metil}acido pentanoico (Ejemplo 9).

28. Un compuesto como se define en cualquiera de las reivindicaciones anteriores en donde el carbono quiral unido a R^1 es preferiblemente el R-anantiomero.

29. Uso de un compuesto como se define en la reivindicación 2 y reivindicaciones dependientes de las mismas en la preparación de un medicamento para el tratamiento o profilaxis de una condición para la cual una respuesta terapéutica beneficiosa se puede obtener por la inhibición de endopeptidasa neutra.

30. Uso según la reivindicación 29 para el tratamiento o profilaxis de la disfunción sexual.

31. Uso de un compuesto de acuerdo con las reivindicaciones 1 a 30 en donde la disfunción sexual tratada es la disfunción sexual femenina.

32. Uso de acuerdo con la reivindicación 31 en donde la disfunción sexual femenina tratada incluye al menos despertar la disfunción sexual femenina.

33. Uso de acuerdo con cualquiera de las reivindicaciones 30 a 32 en donde el medicamento es administrado sistemáticamente.

34. Uso de acuerdo con cualquiera de las reivindicación 33 en donde el medicamento es administrado oralmente.

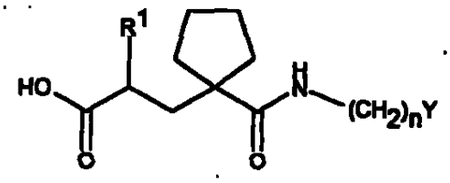
35. Un compuesto con se define en la reivindicación 2 y reivindicaciones dependientes de las mismas para uso en medicina.

36. Una formulación farmacéutica que incluye un compuesto como se define en la reivindicación 2 y reivindicaciones dependientes de las mismas con un excipiente farmacéuticamente aceptable.

37. Un método para el tratamiento o profilaxis de la disfunción sexual, que incluye administrarle a un paciente una cantidad efectiva de un compuesto como se define en cualquiera de las reivindicaciones 1 a 28.

38. Una formulación farmacéutica para disfunción sexual, que incluye una cantidad terapéuticamente eficaz de un compuesto según cualquiera de las reivindicaciones 1 a 28 junto con un excipiente farmacéuticamente aceptable.

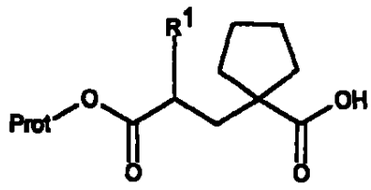
39. Un procedimiento para la preparación de un compuesto de fórmula I o sales de las mismas.



(I)

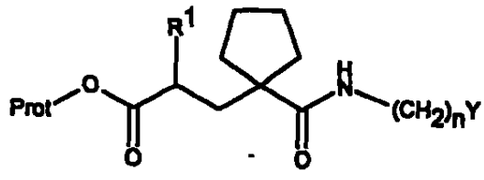
en donde R^1, n e Y como se define en la reivindicación 2, que comprende las etapas de:

a) reaccionar un compuesto de fórmula II



(II)

en donde Prot es un grupo protector adecuado, con un compuesto de fórmula $Y(CH_2)_nNH_2$ (III), para dar un compuesto de fórmula IV,



(IV)

y luego,

c) reaccionar el compuesto de fórmula IV bajo condiciones adecuadas de desprotección para dar el compuesto de fórmula I; y luego

d) opcionalmente formar una sal.

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gersey

Dated 10 May 2001

326

Official use

07JUL00 E551040-1 001298
P01/7700 0.00-0016684.3

Your reference
PCS10910WPM-PROV

06 JUL 2000

0016684.3

06 JUL 2000

Notes
Please type, or write in dark ink using CAPITAL letters. A prescribed fee is payable for a request for grant of a patent. For details, please contact the Patent Office (telephone 071-438 4700).

Rule 16 of the Patents Rules 1990 is the main rule governing the completion and filing of this form.

2 Do not give trading styles, for example, 'Trading as XYZ company', nationality or former names, for example, 'formerly (known as) ABC Ltd' as these are not required.

Warning
After an application for a Patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977 and will inform the applicant if such prohibition or restriction is necessary. Applicants resident in the United Kingdom are also reminded that under Section 23, applications may not be filed abroad without written permission unless an application has been filed not less than 6 weeks previously in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction revoked.

The Patent Office

Request for grant of a Patent

Form 1/77

Patents Act 1977

1 Title of invention

1 Please give the title of the invention

PHARMACEUTICAL COMPOSITION

2 Applicant's details

☐ First or only applicant

2a If you are applying as a corporate body please give:

Corporate name
PFIZER LIMITED

Country *(and State of incorporation, if appropriate)*
UNITED KINGDOM

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c In all cases, please give the following details:

Address
RAMSGATE ROAD
SANDWICH
KENT

UK postcode CT13 9NJ
(if applicable)

Country UNITED KINGDOM

ADP number *(if known)* 68926 73001

2d, and 2f:
If there are further applicants please provide details on a separate sheet of paper.

☐ **Second applicant (if any)**

2d If you are applying as a corporate body please give:

Corporate name

Country *(and State of incorporation, if appropriate)*

2e If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2f In all cases, please give the following details:

Address

UK postcode
(if applicable)

Country

ADP number
(if known)

3
An address for service in the United Kingdom must be supplied.
Please mark correct box

3 Address for service details

3a Have you appointed an agent to deal with your application?

Yes [X] No [ ] ➡ *go to 3b*

⬇ *Please give details below*

Agent's name
DR. RICHARD C. SEWELL
Agent's address
PFIZER LIMITED
RAMSGATE ROAD
SANDWICH
KENT
Postcode CT13 9NJ

Agent's ADP number 6892673001

3b:
If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.

3b If you have not appointed an agent please give a name and address in the United Kingdom to which all correspondence will be sent:

Name

Address

Postcode
ADP number
(if known)

Daytime telephone number *(if available)*

327

4 Reference number

4 Agent's or applicant's reference number *(if applicable)* PCS10910WPM-PROV

5 Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No ☒ ➡ *go to 6*

⬇ *please give details below*

☐ number of earlier application or patent number

☐ filing date *(day month year)*

☐ and the Section of the Patents Act 1977 under which you are claiming:

Please mark correct box

15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

6 Declaration of priority

6 If you are declaring priority from previous application(s), please give:

Country of filing	Priority application number *(if known)*	Filing date *(day,month,year)*

3 *If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number.*

Please give the date in all number format, for example, 31/05/90 for 31 May 1990.

he answer must be 'No' if:
any applicant is not an inventor
there is an inventor who is not an applicant, or
any applicant is a corporate body.

7 Inventorship

7 Are you (the applicant or applicants) the sole inventor or the joint inventors?

Please mark the correct box

Yes ☐ No ☒ ➡

A statement of inventorship on Patents Form 7/77 will need to be filed (see Rule 15).

lease supply duplicates of
laim(s), abstract, description
nd drawing(s).

8 Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77			
Claim(s)	11	Description	102
Abstract		Drawing(s)	

8b Which of the following documents also accompanies the application?

lease mark correct box(es)

Priority documents *(please state how many)*	
Translation(s) of Priority document *(please state how many)*	
Patents Form 7/77 - Statement of Inventorship and Right to Grant *(please state how many)*	
Patents Form 9/77 - Preliminary Examination/Search	
Patents Form 10/77 - Request for Substantive Examination	

ou or your appointed agent
see Rule 90 of the Patents
Rules 1990) must sign this
equest.

9 Request

I/We request the grant of a patent on the basis of this application.

Please sign here ➡

Signed [signature] Date 06/07/2000
(day month year)

completed fee sheet should
referably accompany the fee.

Please return the completed form, attachments and duplicates where requested, together with the prescribed fee to:

☐ ***The Comptroller***
The Patent Office
Cardiff Road
NEWPORT
Gwent
NP9 1RH

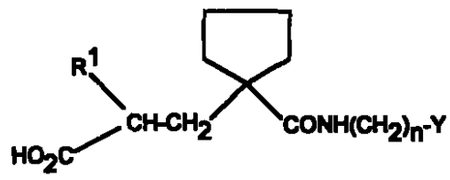
The Comptroller
The Patent Office
25 Southampton Buildings
London
WC2A 1AY

Pharmaceutical Composition

This invention relates to inhibitors of neutral endopeptidase enzyme (NEP) and derivatives thereof and to processes for the preparation of, intermediates used in the preparation of, compositions containing and the uses of said inhibitors. These inhibitors have utility in a variety of therapeutic areas including the treatment of sexual disorders in particular female sexual dysfunction, especially wherein the female sexual dysfunction treated includes female sexual arousal disorder.

NEP inhibitors are disclosed in WO 91/07386 and WO 91/10644.

According to a first aspect, the present invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, in the preparation of a medicament for the treatment of sexual dysfunction:



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6} alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

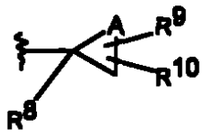
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or -NR^2R^3 wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

the -$(CH_2)_n$- linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

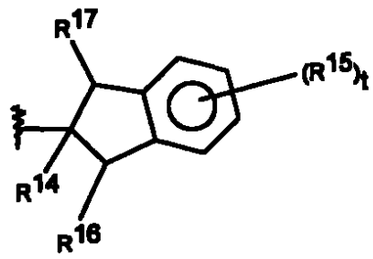
Y is the group



wherein A is -$(CH_2)_q$- where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, -CH_2OH, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, -CH_2OH, -$C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl, or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

Y is the group, -$C(O)$ NR^{11} R^{12} wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

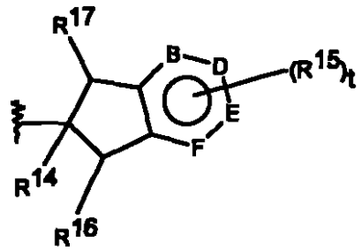
Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to

any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-8}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-8}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl (preferably pyridyl); and

u is 0, 1, 2 or 3.

Some of the compounds of formula I are disclosed in WO 91/10664 and WO 91/07386, but there is no teaching that they could be useful in the treatment of sexual dysfunction. The remaining compounds of formula I are novel. Therefore according to a second aspect, the invention provides a (novel) compound of formula

(I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, wherein R^1, n and Y are as defined in the first aspect with the proviso that Y is not the group $-C(O)NR^{11}R^{12}$ and R^{14} is not H or $-CH_2OH$.

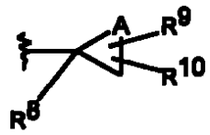
Unless otherwise specified, the compounds of the first and second aspects are hereinafter defined as compounds of the invention.

Where it is necessary to distinguish between the known and novel compounds of the invention, the novel compounds will be referred to as the compounds of the second aspect of the invention.

The following are preferred compounds of the invention, i.e. in accordance with the first and second aspects of the invention.

Preferably R^1 is C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl, C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl or C_{1-6}alkyl substituted with aryl. Particularly preferred R^1 substituents are C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl (especially methoxyethyl) or C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl (especially methoxyethoxymethyl). It is especially preferred that R^1 is C_{1-4}alkyl (preferably propyl).

When Y is the group



and the carbocyclic ring is fully saturated, then preferably one of R^9 or R^{10} is $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl. More, preferably the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl. More preferably, R^9 and R^{10} are attached to adjacent carbon atoms in the ring. More preferably, R^8 is CH_2OH.

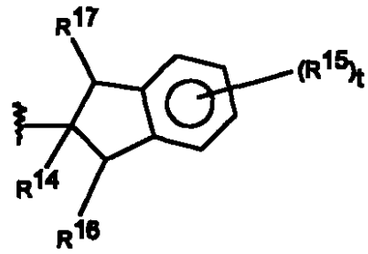
When Y is the group $-NR^{18}S(O)_uR^{19}$, preferably R^{18} is H. More preferably, R^{19} is benzyl or phenyl. More preferably u is 2.

330

Preferably Y is the optionally substituted 5-7 membered heterocyclic ring. More preferably the ring is an optionally substituted aromatic ring, particularly pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazolyl, thiazolyl, thiaiazolyl, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, pyridonyl, quinoxalinyl or quinazolinyl [especially oxadiazole (preferably 1,2,5- or 1,3,4-oxadiazole), pyridone (preferably 2-pyridone) or thiadiazole (preferably 1,3,4-thiadiazole) each of which may be substituted as defined in the first aspect. Preferably the heterocyclic ring is substituted by one or more C_{1-6}alkyl, phenyl or phenylC_{1-4}alkyl, more preferably by C_{1-4}alkyl or benzyl. Preferably Y is an *N*-substituted pyridone, preferably by benzyl or C_{1-4}alkyl.

Preferably Y is a lactam linked at the nitrogen.

Preferably Y is



wherein R^{14} is preferably CH_2OH or $C(O)NR^{11}R^{12}$, especially $C(O)NR^{11}R^{12}$. Preferably R^{16} and R^{17} are hydrogen. Preferably t is 0.

The chiral carbon attached to R^1 is preferably the R-enantiomer.

Particularly preferred compounds of the invention (referred to hereinafter as the list of 10 preferred compounds) are:

2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-4-methoxybutanoic acid (Example 35),

2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (Example 40),

(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (Example 44),

2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid (Example 43),
cis-3-(2-methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}cyclohexyl)-amino]carbonyl}cyclopentyl)methyl]propanoic acid (Example 38),
(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid (Example 31),
(+)-2-[(1-{[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid (Example 30),
2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21),
2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-pentanoic acid (Example 22), and
2-{[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 9).

In the above definition, unless otherwise indicated, alkyl groups having three or more carbon atoms may be straight or branched-chain. The term aryl as used herein means an aromatic hydrocarbon group such as phenyl or naphthyl which may optionally be substituted with, for example, one or more of OH, CN, CF_3, C_1-C_4 alkyl, C_1-C_4 alkoxy, halo, carbamoyl, aminosulphonyl, amino, mono or di(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Halo means fluoro, chloro, bromo or iodo.

In the above definition, unless otherwise indicated the term heterocyclyl means a 5 or 6 membered nitrogen, oxygen or sulphur containing heterocyclic group which, unless otherwise stated, may be saturated, unsaturated or aromatic and which may optionally include a further oxygen or one to three nitrogen atoms in the ring and which may optionally be benzofused or substituted with for example, one or more halo, C_1-C_4 alkyl, hydroxy, carbamoyl, benzyl, oxo, amino or mono or di-(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Particular examples of heterocycles include pyridyl, pyridonyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, furanyl, tetrahydrofuranyl, tetrahydropyranyl, dioxanyl, thienyl, oxazolyl, isoxazolyl, thiazolyl, oxadiazolyl, thiadiazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, quinoxalinyl, quinazolinyl and benzimidazolyl, each being optionally substituted as previously defined.

The compounds of the invention are inhibitors of the zinc-dependent, neutral endopeptidase EC.3.4.24.11., and it is proposed that the compounds of the invention will treat the disease states listed below. This enzyme is involved in the breakdown of several bioactive oligopeptides, cleaving peptide bonds on the amino side of hydrophobic amino acid residues. The peptides metabolised include atrial natriuretic peptides (ANP), bombesin, bradykinin, calcitonin gene-related peptide, endothelins, enkephalins, neurotensin, substance P and vasoactive intestinal peptide. Some of these peptides have potent vasodilatory and neurohormone functions, diuretic and natriuretic activity or mediate behaviour effects. Thus, the compounds of the invention, by inhibiting the neutral endopeptidase EC.3.4.24.11, can potentiate the biological effects of bioactive peptides. Thus, in particular the compounds have utility in the treatment of a number of disorders, including hypertension, heart failure, angina, renal insufficiency, cyclical oedema, Menières disease, hyperaldosteroneism (primary and secondary) and hypercalciuria. In addition, because of their ability to potentiate the effects of ANF the compounds have utility in the treatment of glaucoma. As a further result of their ability to inhibit the neutral endopeptidase E.C.3.4.24.11 the compounds of the invention may have activity in other therapeutic areas including for example the treatment of menstrual disorders, preterm labour, pre-eclampsia, endometriosis, and reproductive disorders (especially male and female infertility, polycystic ovarian syndrome, implantation failure). Also the compounds of the invention should treat asthma, inflammation, leukemia, pain, epilepsy, affective disorders, dementia and geriatric confusion, obesity and gastrointestinal disorders (especially diarrhoea and irritable bowel syndrome), wound healing (especially diabetic and venous ulcers and pressure sores), septic shock, the modulation of gastric acid secretion and the treatment of hyperreninaemia. In a preferred embodiment the compounds of the invention are useful in the treatment of male and female sexual dysfunction.

We have found that the compounds of the invention inhibit the enzyme neutral endopeptidase. Therefore, according to a further aspect, the invention provides the use of a compound as defined in the second aspect In the preparation of a medicament for the treatment or prophylaxis of a condition for which a beneficial therapeutic response can be obtained by the inhibition of neutral endopeptidase.

The compounds of the invention are particularly beneficial for the treatment of sexual dysfunction in the male (e.g. male erectile dysfunction), more particularly in the female - female sexual dysfunction (FSD).

In accordance with the invention, FSD can be defined as the difficulty or inability of a woman to find satisfaction in sexual expression. FSD is a collective term for several diverse female sexual disorders (Leiblum, S.R. (1998). Definition and classification of female sexual disorders. *Int. J. Impotence Res.*, 10, S104-S106; , Berman, J.R., Berman, L. & Goldstein, I. (1999). Female sexual dysfunction: Incidence, pathophysiology, evaluations and treatment options. *Urology*, 54, 385-391). The woman may have lack of desire, difficulty with arousal or orgasm, pain with intercourse or a combination of these problems. Several types of disease, medications, injuries or psychological problems can cause FSD. Treatments in development are targeted to treat specific subtypes of FSD, predominantly desire and arousal disorders.

The categories of FSD are best defined by contrasting them to the phases of normal female sexual response: desire, arousal and orgasm (Leiblum, S.R. (1998). Definition and classification of female sexual disorders. *Int. J. Impotence Res.*, 10, S104-S106). Desire or libido is the drive for sexual expression. Its manifestations often include sexual thoughts either when in the company of an interested partner or when exposed to other erotic stimuli. Arousal is the vascular response to sexual stimulation, an important component of which is genital engorgement and includes increased vaginal lubrication, elongation of the vagina and increased genital sensation/sensitivity. Orgasm is the release of sexual tension that has culminated during arousal.

Hence, FSD occurs when a woman has an inadequate or unsatisfactory response in any of these phases, usually desire, arousal or orgasm. FSD categories include hypoactive sexual desire disorder, sexual arousal disorder, orgasmic disorders and sexual pain disorders. Although the compounds of the invention will improve the genital response to sexual stimulation (as in female sexual arousal disorder), in doing so it may also improve the associated pain, distress and discomfort associated with intercourse and so treat other female sexual disorders.

Thus, in accordance with a preferred aspect of the invention, there is provided use of a compound of the invention in the preparation of a medicament for the treatment or

prophylaxis of hypoactive sexual desire disorder , sexual arousal disorder, orgasmic disorder and sexual pain disorder, more preferably for the treatment or propylaxis of sexual arousal disorder, orgasmic disorder, and sexual pain disorder, and most preferably in the treatment or prophylaxis of sexual arousal disorder.

Hypoactive sexual desire disorder is present if a woman has no or little desire to be sexual, and has no or few sexual thoughts or fantasies. This type of FSD can be caused by low testosterone levels, due either to natural menopause or to surgical menopause. Other causes include illness, medications, fatigue, depression and anxiety.

Female sexual arousal disorder (FSAD) is characterised by inadequate genital response to sexual stimulation. The genitalia do not undergo the engorgement that characterises normal sexual arousal. The vaginal walls are poorly lubricated, so that intercourse is painful. Orgasms may be impeded. Arousal disorder can be caused by reduced oestrogen at menopause or after childbirth and during lactation, as well as by illnesses, with vascular components such as diabetes and atherosclerosis. Other causes result from treatment with diuretics, antihistamines, antidepressants eg SSRIs or antihypertensive agents.

Sexual pain disorders (includes dyspareunia and vaginismus) is characterised by pain resulting from penetration and may be caused by medications which reduce lubrication, endometriosis, pelvic inflammatory disease, inflammatory bowel disease or urinary tract problems.

The prevalence of FSD is difficult to gauge because the term covers several types of problem, some of which are difficult to measure, and because the interest in treating FSD is relatively recent. Many women's sexual problems are associated either directly with the female ageing process or with chronic illnesses such as diabetes and hypertension.

Because FSD consists of several subtypes that express symptoms in separate phases of the sexual response cycle, there is not a single therapy. Current treatment of FSD focuses principally on psychological or relationship issues. Treatment of FSD is gradually evolving as more clinical and basic science studies are dedicated to the

investigation of this medical problem. Female sexual complaints are not all psychological in pathophysiology, especially for those individuals who may have a component of vasculogenic dysfunction (eg FSAD) contributing to the overall female sexual complaint. There are at present no drugs licensed for the treatment of FSD. Empirical drug therapy includes oestrogen administration (topically or as hormone replacement therapy), androgens or mood-altering drugs such as buspirone or trazodone. These treatment options are often unsatisfactory due to low efficacy or unacceptable side effects.

Since interest is relatively recent in treating FSD pharmacologically, therapy consists of the following:- psychological counselling, over-the-counter sexual lubricants, and investigational candidates, including drugs approved for other conditions. These medications consist of hormonal agents, either testosterone or combinations of oestrogen and testosterone and more recently vascular drugs, that have proved effective in male erectile dysfunction. None of these agents has been demonstrated to be very effective in treating FSD.

As discussed, the compounds of the invention are particularly useful for the treatment of female sexual arousal disorder (FSAD).

The Diagnostic and Statistical Manual (DSM) IV of the American Psychiatric Association defines Female Sexual Arousal Disorder (FSAD) as being:

> *"a persistent or recurrent inability to attain or to maintain until completion of the sexual activity adequate lubrication-swelling response of sexual excitement. The disturbance must cause marked distress or interpersonal difficulty."*

The arousal response consists of vasocongestion in the pelvis, vaginal lubrication and expansion and swelling of the external genitalia. The disturbance causes marked distress and/or interpersonal difficulty.

FSAD is a highly prevalent sexual disorder affecting pre-, peri- and post menopausal (±HRT) women. It is associated with concomitant disorders such as depression, cardiovascular diseases, diabetes and UG disorders.

The primary consequences of FSAD are lack of engorgement/swelling, lack of lubrication and lack of pleasurable genital sensation. The secondary consequences of FSAD are reduced sexual desire, pain during intercourse and difficulty in achieving an orgasm.

It has recently been hypothesised that there is a vascular basis for at least a proportion of patients with symptoms of FSAD (Goldstein *et al.*, Int. J. Impot. Res., 10, S84-S90,1998) with animal data supporting this view (Park *et al.*, Int. J. Impot. Res., 9, 27-37, 1997).

Drug candidates for treating FSAD, which are under investigation for efficacy, are primarily erectile dysfunction therapies that promote circulation to the male genitalia. They consist of two types of formulation, oral or sublingual medications (Apomorphine, Phentolamine, phosphodiesterase type 5 (PDE5) inhibitors e.g. Sildenafil), and prostaglandin (PGE_1) that are injected or administered transurethrally in men, and topically to the genitalia in women.

The present invention is advantageous as it provides a means for restoring a normal sexual arousal response - namely increased genital blood flow leading to- vaginal, clitoral and labial engorgement. This will result in increased vaginal lubrication *via* plasma transudation, increased vaginal compliance and increased genital sensitivity. Hence, the present invention provides a means to restore, or potentiate, the normal sexual arousal response.

Without being bound by theory, we believe that neuropeptides such as vasoactive intestinal peptide (VIP) are major neurotransmitter candidates in the control of the female sexual arousal response, especially in the control of genital blood flow. VIP and other neuropeptides are degraded/ metabolised by NEP EC3.4.24.11. Thus, NEP inhibitors will potentiate the endogenous vasorelaxant effect of VIP released during arousal. This will lead to a treatment of FSAD, such as through enhanced genital blood flow and hence genital engorgement. We have shown that selective inhibitors of NEP EC 3.4.24.11 enhance pelvic nerve-stimulated and VIP-induced increases in vaginal and clitoral blood flow. In addition, selective NEP inhibitors enhance VIP and nerve-mediated relaxations of isolated vagina wall.

Thus the present invention is advantageous as it helps provide a means for restoring a normal sexual arousal response - namely increased genital blood flow leading to vaginal, clitoral and labial engorgement. This will result in increased vaginal lubrication *via* plasma transudation, increased vaginal compliance and increased vaginal sensitivity. Hence, the present invention provides a means to restore, or potentiate the normal sexual arousal response.

Background teachings on NEP have been presented by Victor A. McKusick et al on http://www3.ncbi.nlm.nih.gov/Omim/searchomim.htm. The following information concerning NEP has been extracted from that source.

> "Common acute lymphocytic leukemia antigen is an important cell surface marker in the diagnosis of human acute lymphocytic leukemia (ALL). It is present on leukemic cells of pre-B phenotype, which represent 85% of cases of ALL. CALLA is not restricted to leukemic cells, however, and is found on a variety of normal tissues. CALLA is a glycoprotein that is particularly abundant in kidney, where it is present on the brush border of proximal tubules and on glomerular epithelium. Letarte et al. (1988) cloned a cDNA coding for CALLA and showed that the amino acid sequence deduced from the cDNA sequence is identical to that of human membrane-associated neutral endopeptidase (NEP; EC 3.4.24.11), also known as enkephalinase. NEP cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin. By cDNA transfection analysis, Shipp et al. (1989) confirmed that CALLA is a functional neutral endopeptidase of the type that has previously been called enkephalinase. Barker et al. (1989) demonstrated that the CALLA gene, which encodes a 100-kD type II transmembrane glycoprotein, exists in a single copy of greater than 45 kb which is not rearranged in malignancies expressing cell surface CALLA. The gene was located to human chromosome 3 by study of somatic cell hybrids and in situ hybridization regionalized the location to 3q21-q27. Tran-Paterson et al. (1989) also assigned the gene to chromosome 3 by Southern blot analysis of DNA from human-rodent somatic cell hybrids. D'Adamio et al. (1989) demonstrated that the CALLA gene spans more than 80 kb and is composed of 24 exons."

1. Barker, P. E.; Shipp, M. A.; D'Adamio, L.; Masteller, E. L.; Reinherz, E. L. The common acute lymphoblastic leukemia antigen gene maps to chromosomal region 3(q21-q27). J. Immun. 142: 283-287, 1989.

2. D'Adamio, L.; Shipp, M. A.; Masteller, E. L.; Reinherz, E. L. : Organization of the gene encoding common acute lymphoblastic leukemia antigen (neutral endopeptidase 24.11): multiple miniexons and separate 5-prime untranslated regions. Proc. Nat. Acad. Sci. 86: 7103-7107, 1989.

3. Letarte, M.; Vera, S.; Tran, R.; Addis, J. B. L.; Onizuka, R. J.; Quackenbush, E. J.; Jongeneel, C. V.; McInnes, R. R. : Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. J. Exp. Med. 168: 1247-1253, 1988.

4. Shipp, M. A.; Vijayaraghavan, J.; Schmidt, E. V.; Masteller, E. L.; D'Adamio, L.; Hersh, L. B.; Reinherz, E. L. : Common acute lymphoblastic leukemia antigen (CALLA) is active neutral endopeptidase 24.11 ('enkephalinase'): direct evidence by cDNA transfection analysis. Proc. Nat. Acad. Sci. 86: 297-301, 1989.

5. Tran-Paterson, R.; Willard, H. F.; Letarte, M. : The common acute lymphoblastic leukemia antigen (neutral endopeptidase--3.4.24.11) gene is located on human chromosome 3. Cancer Genet. Cytogenet. 42: 129-134, 1989.

The compounds of formula (I) may contain several asymmetric centres and thus they can exist an enantiomers and diastereomers. The invention includes both the separated individual isomers as well as mixutres of isomers.

The pharmaceutically or veterinarily acceptable salts of the compounds of the invention which contain a basic centre are, for example, non-toxic acid addition salts formed with inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulphuric and phosphoric acid, with carboxylic acids or with organo-sulphonic acids. Examples include the HCl, HBr, HI, sulphate or bisulphate, nitrate, phosphate or hydrogen phosphate, acetate, benzoate, succinate, saccarate, fumarate, maleate, lactate, citrate, tartrate, gluconate, camsylate, methanesulphonate, ethanesulphonate, benzenesulphonate, p-toluenesulphonate and pamoate salts. The pharmaceutically or veterinarily acceptable salts of the compounds of the invention which contain an acidic

centre are those formed with bases which form non-toxic salts. Examples include the alkali metal salts such as the sodium, potassium or calcium salts or salts with amines such as diethylamine and dicyclohexylamine. For a review on suitable pharmaceutical salts see Berge et al, J. Pharm, Sci., 66, 1-19, 1977.

The pharmaceutically acceptable solvates of the compounds of the invention include the hydrates thereof. All polymorphs of the compounds of the invention are also included within the scope of the invention.

In cases where the compounds of the invention exist as the E and Z isomers, the invention includes individual isomers as well as mixtures thereof.

In cases where compounds of the invention exist as tautomeric isomers, the invention includes individual tautomers as well as mixtures thereof.

In cases where the compounds of the invention exist as optical isomers, the invention includes individual isomers as well as mixtures thereof.

In cases where the compounds of the invention exist as diastereoisomers, the invention includes individual diastereoisomers as well as mixtures thereof.

Separation of diastereoisomers or E and Z isomers may be achieved by conventional techniques, e.g. by fractional crystallisation, chromatography or H.P.L.C. An individual enantiomer of a compound of the invention may also be prepared from a corresponding optically pure intermediate or by resolution, such as by H.P.L.C. of the corresponding racemate using a suitable chiral support or by fractional crystallisation of the diastereoisomeric salts formed by reaction of the corresponding racemate with a suitable optically active acid or base, as appropriate.

The present invention also includes all suitable isotopic variations of compounds of the invention or pharmaceutically acceptable salt thereof. An isotopic variation of compounds of the invention or a pharmaceutically acceptable salt thereof is defined as one in which at least one atom is replaced by an atom having the same atomic number but an atomic mass different from the atomic mass usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention and pharmaceutically acceptable salts thereof include isotopes of hydrogen, carbon,

335

nitrogen, oxygen, phosphorus, sulphur, fluorine and chlorine such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F and ^{36}Cl, respectively. Certain isotopic variations of the compounds of the invention and pharmaceutically acceptable salts thereof, for example, those in which a radioactive isotope such as ^{3}H or ^{14}C is incorporated, are useful in drug and/or substrate tissue distribution studies. Tritiated, i.e., ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with isotopes such as deuterium, i.e., ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements and hence may be preferred in some circumstances. Isotopic variations in compounds of the invention and pharmaceutically acceptable salts thereof can generally be prepared by conventional procedures such as by the illustrative methods or by the preparations described in the Examples and Preparations hereafter using appropriate isotopic variations of suitable reagents.

It will be appreciated by those skilled in the art that certain protected derivatives of the compounds of the invention, which may be made prior to a final deprotection stage, may not possess pharmacological activity as such, but may, in certain instances, be administered orally or parenterally and thereafter metabolised in the body to form compounds of the invention which are pharmacologically active. Such derivatives may therefore be described as "prodrugs". Further, certain compounds of the invention may act as prodrugs of other compounds of the invention.

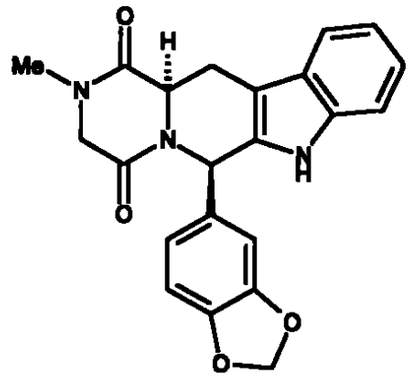
All protected derivatives, and prodrugs, of compounds of the invention are included within the scope of the invention. Examples of suitable pro-drugs for the compounds of the present invention are described in Drugs of Today, Volume 19, Number 9, 1983, pp 499 – 538 and in Topics in Chemistry, Chapter 31, pp 306 – 316 and in "Design of Prodrugs" by H. Bundgaard, Elsevier, 1985, Chapter 1 (the disclosures in which documents are incorporated herein by reference).

It will further be appreciated by those skilled in the art, that certain moieties, known to those skilled in the art as "pro-moieties", for example as described by H. Bundgaard in "Design of Prodrugs" (the disclosure in which document is incorporated herein by reference) may be placed on appropriate functionalities when such functionalities are present within compounds of the invention.

Preferred prodrugs for compounds of the invention include: esters, carbonate esters, hemi-esters, phosphate esters, nitro esters, sulfate esters, sulphoxides, amides, carbamates, azo-compounds, phosphamides, glycosides, ethers, acetals and ketals.

The compounds of the invention may also be combined with the following for the treatment of FSD (in particular FSAD):

Potentiators of cGMP (such as Sildenafil) and/or a centrally acting pharmaceutical (e.g. a dopamine agonist, such as apomorphine). Teachings on the use of apomorphine as a pharmaceutical may be found in US-A-5945117. In that particular document, apomorphine is delivered sub-lingually. In addition, or in the alternative, the agent may be used in combination with one or more of: one or more of a PDE inhibitor such as PDE2 (e.g. EHNA, and example 100 of EP 0771799-incorporated herein by reference) and such as a PDE5 inhibitor (eg sildenafil, 1-[[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f]-as-trazin-2-yl)-4-ethoxyphenyl]sulfonyl]-4-ethylpiperazine i.e. vardenafil / Bayer BA 38-9456) and IC351 (see structure below, Icos Lilly), one or more of a dopamine receptor agonist (eg apomorphine), one or more of a melanocortin receptor agonist (eg Melanotan II), one or more of a potassium channel opener (eg a KATP channel opener and/or a calcium activated potassium channel opener (eg minoxidil, nicorandil)), one or more of a hormone replacement therapy (eg HRT) agent, one or more of a testosterone replacement agent (inc DHEA (dehydroandrostendione)), one or more of an estrogen agonists , one or more of a serotonin receptor agonist, one or more of a prostinoid receptor agonist (eg alprostadil), one or more of an NPY (e.g. NPYY1) antagonist, one or more of a VIP agonist .



IC351 (Icos Lilly)

If a combination of active agents are administered, then they may be administered simultaneously, separately or sequentially.

The compounds of the invention, their pharmaceutically acceptable salts, and pharmaceutically acceptable solvates of either entity can be administered alone but, in human therapy will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

The compositions of the present invention may be administered by direct injection. The composition may be formulated for parenteral, mucosal, intramuscular, intravenous, subcutaneous, ocular, intraocular or transdermal administration. Depending upon the need, the agent may be administered at a dose of from 0.01 to 30 mg/kg body weight, such as from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight.

The term "administered" includes delivery by viral or non-viral techniques. Viral delivery mechanisms include but are not limited to adenoviral vectors, adeno-associated viral (AAV) vectos, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery mechanisms include lipid mediated transfection, liposomes, immunolipoosomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by direct injection. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered topically. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by inhalation. In addition or in the alternative the compositions (or component parts thereof) of the present invention may also be administered by one or more of: a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution such as by an oral route, or by a parenteral route where delivery is by an injectable form, such as, for example, by a rectal, ophthalmic (including intravitreal or intracameral), nasal, topical (including buccal and sublingual), intrauterine, vaginal or parenteral (including subcutaneous, intraperitoneal, intramuscular, intravenous, intradermal,

intracranial, intratracheal, and epidural) transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, or parenteral (e.g., intravenous, intraspinal, subcutaneous, transdermal or intramuscular) route.

By way of further example, the pharmaceutical composition of the present invention may be administered in accordance with a regimen of 1 to 10 times per day, such as once or twice per day. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy.

Hence, the term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular or subcutaneous route.

For example, the compounds of the invention or salts or solvates thereof can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed-, modified-, or controlled-release applications. The compounds of the invention may also be administered via intracavernosal injection.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethyl cellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk

337

sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

The compounds of the invention can also be administered parenterally, for example, intracavernosally, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. For such parenteral administration they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

For oral and parenteral administration to human patients, the daily dosage level of the compounds of the invention or salts or solvates thereof will usually be from 10 to 1000 mg (in single or divided doses).

Thus, for example, tablets or capsules of the compounds of the invention or salts or solvates thereof may contain from 5 to 1000mg, such as 5 mg to 500 mg of active compound for administration singly or two or more at a time, as appropriate. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention. The skilled person will also appreciate that, in the treatment of certain conditions (including MED and FSD), compounds of the invention may be taken as a single dose on an "as required" basis (i.e. as needed or desired).

The compounds of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray

presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A [trade mark] or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA [trade mark]), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 1 to 50 mg of a compound of the invention for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 1 to 50 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, the compounds of the invention or salts or solvates thereof can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a gel, hydrogel, lotion, solution, cream, ointment or dusting powder. The compounds of the invention or salts or solvates thereof may also be dermally administered. The compounds of the invention or salts or solvates thereof may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the ocular, pulmonary or rectal routes.

For ophthalmic use, the compounds can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds of the invention or salts or solvates thereof can be formulated as a suitable ointment containing the active compound

suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The compounds of the invention may also be used in combination with a cyclodextrin. Cyclodextrins are known to form inclusion and non-inclusion complexes with drug molecules. Formation of a drug-cyclodextrin complex may modify the solubility, dissolution rate, bioavailability and/or stability property of a drug molecule. Drug-cyclodextrin complexes are generally useful for most dosage forms and administration routes. As an alternative to direct complexation with the drug the cyclodextrin may be used as an auxiliary additive, e.g. as a carrier, diluent or solubiliser. Alpha-, beta- and gamma-cyclodextrins are most commonly used and suitable examples are described in WO-A-91/11172, WO-A-94/02518 and WO-A-98/55148.

In a preferred embodiment, the compounds of the invention are delivered systemically (such as orally, buccally, sublingually), more preferably orally. Preferably such systemic (most preferably oral) administration is used to treat sexual dysfunction in a male or female, more preferably FSD and more preferably still FSAD.

Thus in a particularly preferred embodiment, there is provided the use of the compounds of the invention in the manufacture of a systemically delivered (preferably orally delivered) medicament for the treatment or prophylaxis of FSD, more preferably FSAD.

Since NEP is present throughout the body, it is very unexpected that the compounds of the invention can be administered systemically and achieve a therapeutic response in the genitalia (preferably the female genitalia) without provoking intolerable (adverse) side effects. Thus in the in vivo (rabbit) results hereafter, the compounds of the invention administered systemically increased genital blood flow, upon sexual arousal (mimiced by pelvic nerve stimulation) without adversely affecting cardiovascular parameters, such as causing a significant hypotensive or hypertensive.

Preferably the compounds of the invention are administered for the treatment of sexual dysfunction (more preferably FSD) in the sexually stimulated patient (by sexual stimulation we mean to include visual, auditory or tactile stimulation).The stimulation can be before, after or during said administration.

Thus the compounds of the invention enhance the pathways/mechanisms that underlie sexual aroual in the female gentialia restoring or improving the sexual arousal response to sexual stimulation.

Thus a preferred embodiment provides the use of a compound of the invention in the preparation of a medicament for the treatment or prophyaxis of sexual dysfunction (more preferably FSD) in the stimulated patient.

For veterinary use, a compound of the invention or a veterinarily acceptable salt thereof, or a veterinarily acceptable solvate or pro-drug thereof, is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

Compounds of the invention may be prepared, in known manner, in a variety of ways.

Throughout the specification, general formulae are designated by Roman numerals I, II, III, IV etc. Subsets of these general formulae are defined as Ia, Ib, Ic etc, ... IVa, IVb, IVc etc.

According to a further aspect of the invention, compounds of general formula I may be prepared according to reaction scheme 1, by reacting a compound of formula II (where Prot is a suitable protecting group) with a primary amine of formula III to give a compound of formula IV. Deprotection gives compounds of formula I.

Compounds of formula II and III are novel and form a further aspect of the invention.

Preferred reaction conditions for the acid/amine coupling step comprise reacting II with III (or its amine salt) in the presence of an activating agent, optionally a catalyst, and an excess of an acid acceptor, in a suitable solvent. Particularly preferred reaction

339

conditions comprise reacting II (1-1.5 equivalents), III (or its salt 1-1.5 equivalents), in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCDI) or N,N'-dicyclohexylcarbodiimide (DCC) (1.1-1.3 equivalents), 1-hydroxybenzotrazole hydrate (HOBT) or dimethylaminopyridine (DMAP) (1.05-1.2 equivalents), *N*-methyl morpholine (NMM) or triethyamine (2.3-3 equivalents), in dimethylformamide or dichloromethane at between room temperature and 90°C for 16-18 hours.

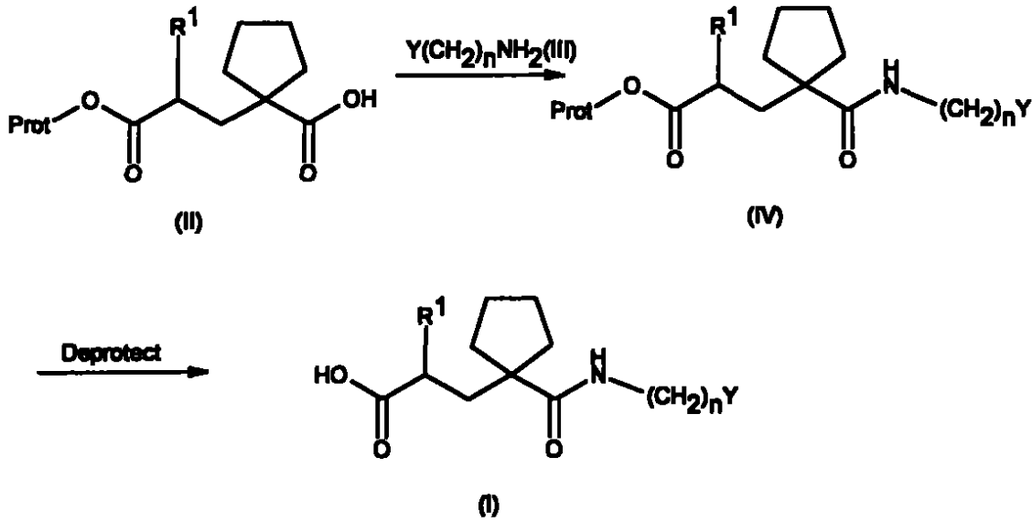
Alternatively, the acid/amine coupling step may be prepared via the acid chloride in the presence of an excess of acid acceptor, in a suitable solvent. The acid chloride may be isolated or it may be generated in situ. Preferred reaction conditions comprise reacting the acid chloride of II (1-1.1 equivalents), III (or its salt, 1 to 1.5 equivalents), triethyamine or *N*-methyl morpholine (1.4-10 equivalents), in dichloromethane at room temperature for 24 hours. Compounds of formula II can be converted to the acid chloride *in situ* by treatment with oxalyl chloride in dichloromethane in the presence of a catalytic amount of dimethylformamide for 2 hours at room temperature.

Methods for deprotection of an acid group depend on the protecting group. For examples of protection/deprotection methodology see "Protective groups in Organic synthesis", TW Greene and PGM Wutz.

For example, when Prot is a *tert*-butyl, deprotection conditions comprise reacting IV with trifluoroacetic acid/dichloromethane (1:1-1.5 by volume), at room temperature for 2-18 hours, optionally in the presence of a carbocation scavenger, e.g. anisole (10 equivalents). When Y contains a hydroxy group, base hydrolysis of the intermediate trifluoroacetic acid ester may be necessary. Alternative methodology for deprotection when Prot is *tert*-butyl comprises treating II with hydrochloric acid in dichloromethane at room temperature for 3 hours. For the avoidance of doubt, Prot as *tert*-butyl is given by way of Example and is not intended to be limited to *tert*-Butyl.

When Prot is benzyl, deprotection conditions comprise reacting IV with palladium on charcoal (5-10%) in aqueous ethanol (40-95%) at 15-60 psi at room temperature for 2hrs to 3 days.

Scheme 1



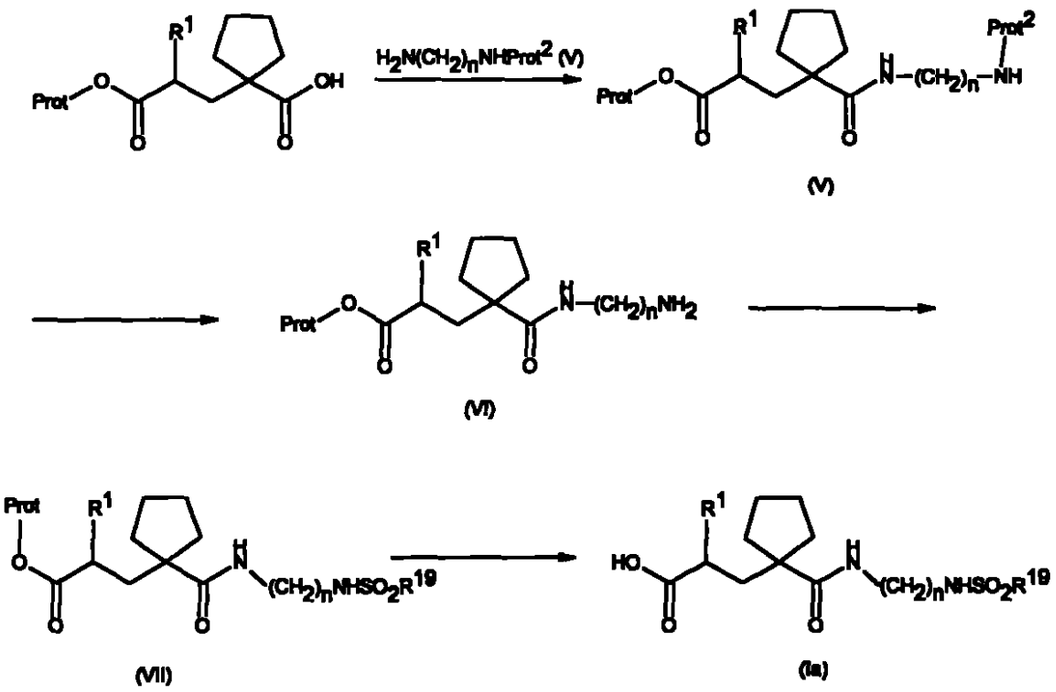
Compounds of formula Ia, i.e. compounds of general formula I where Y is - $NHSO_2R^{19}$, may be prepared according to reaction scheme 2. Compounds of formula V are first prepared by reacting compounds of formula II with compounds of formula V where $Prot^2$ is a suitable amine protecting group. Preferred reaction conditions are analogous to those described the acid/amine coupling step for Scheme 1 above. Selective amine deprotection of compounds of formula V gives compounds of formula VI. Compounds of formula VI are reacted with $R^{19}SO_2Cl$ in the presence of an acid acceptor in a suitable solvent to form compounds of formula VII. Deprotection of compounds of formula VII under analogous conditions to those described for the deprotection step of Scheme 1 gives compounds of formula Ia.

Methods for deprotection of an amine group depend on the protecting group. For examples of protection/deprotection methodology see "Protective groups in Organic Synthesis", TW Greene and PGM Wutz. For example, when $Prot^2$ is benzoyloxycarbonyl, deprotection conditions comprise reacting V with palladium on charcoal (10%) in ethanol at room temperature for 18 hours.

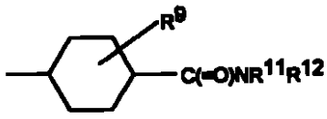
Preferred methods for preparation of the compounds of formula VII comprise reaction of VI with $R^{19}SO_2Cl$ (1 equivalent) in the presence of triethyamine (1.5-2.5 equivalents) in dichloromethane at room temperature for 2 to 3 days.

340

Scheme 2



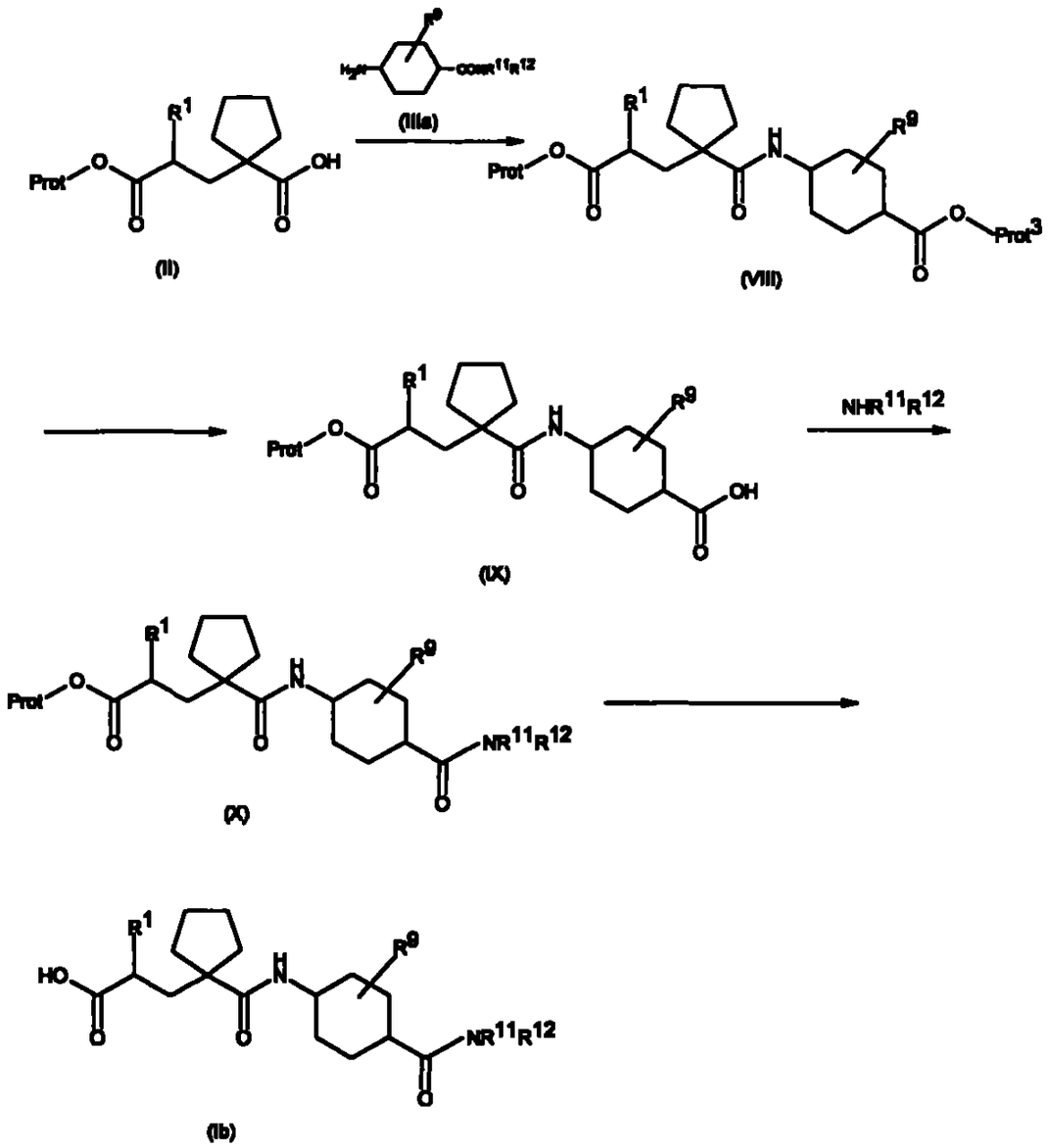
Compounds of formula Ib, i.e. compounds of formula I where n is 0 and Y is



, may be prepared according to reaction scheme 3. Compounds of formula II are reacted with compounds of formula IIIa under analogous conditions to acid/amine coupling conditions of Scheme 1 to give compounds of formula VIII, where $Prot^3$ is a protecting group which can be selectively removed in the presence of protecting group Prot. A preferred protecting group $Prot^3$ is a base labile ester group. Consequently, treatment of compound of formula VIII under basic conditions gives compounds of formula IX. Compounds of formula IX are reacted with compounds of formula $NHR^{11}R^{12}$ under analogous conditions to acid/amine coupling conditions of Scheme 1 to form compounds of formula X. Deprotection of compounds of formula X under analogous conditions to the deprotection step in Scheme 1 gives compounds of formula Ib.

Preferred conditions for removal of protecting group $Prot^3$ from VIII comprise treatment of VIII with sodium hydroxide (1N) in methanol at room temperature for 22 hours.

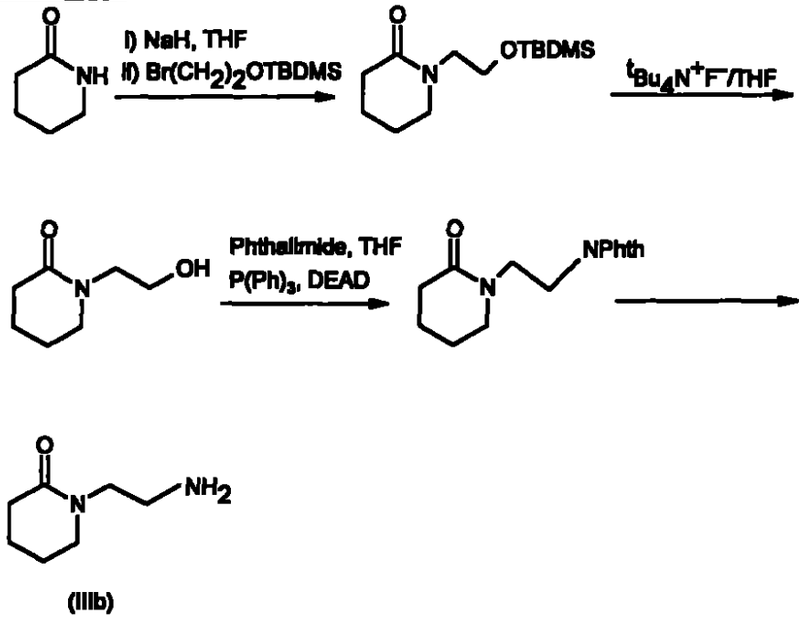
Scheme 3



Compounds of formula IIIb, i.e. compounds of general formula III where n is 2, Y is 2-oxopiperidino, may be prepared according to reaction scheme 4.

341

Scheme 4



(IIIb)

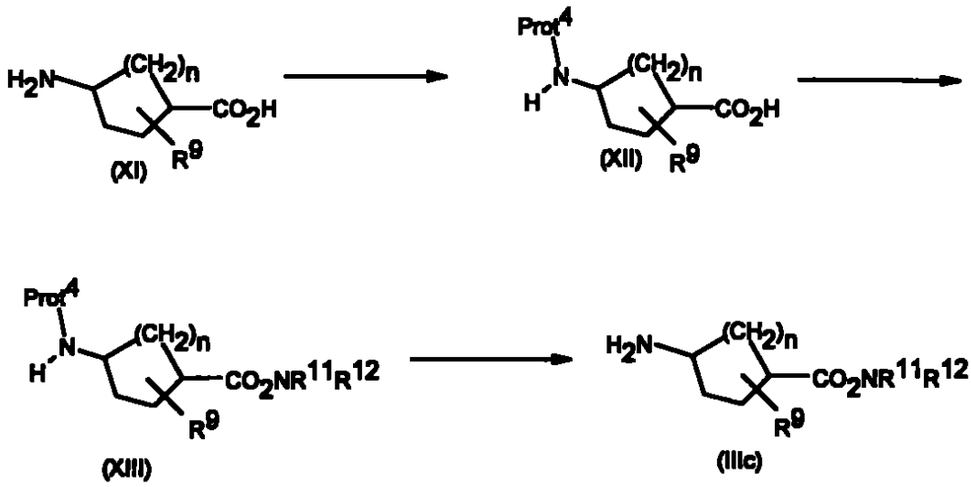
Compounds of formula IIIc where n is 1 or 2, may be prepared according to reaction scheme 5. Compounds of formula XI are protected at the amine moiety with a suitable protecting group $Prot^4$ to form compounds of formula XII. A preferred protecting group is *tert*-butyloxycarbonyl. Compounds of formula XII are reacted under typical acid/amine coupling conditions with $NHR^{11}R^{12}$ to form compounds of formula XIII, which on deprotection form compounds of formula IIIb.

Typical reaction conditions for introducing the *tert*-butyloxycarbonyl protecting group comprise treating XI with (*tert*-butyloxycarbonyl)$_2$O in dioxan and 2N sodium hydroxide at room temperature for 18 hrs.

Typical acid/amine coupling conditions comprise treating XII and $NHR^{11}R^{12}$ with benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PYBOP), 1-hydroxybenzotrazole hydrate (HOBT), Hünigs base, an amine (eg triethylamine), in dimethylformamide at room temperature for 2hrs. Alternatively, XII and $NHR^{11}R^{12}$ may be treated with1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOBT, N-methyl morpholine (NMM), in dimethylformamide at room temperature for 18 hrs.

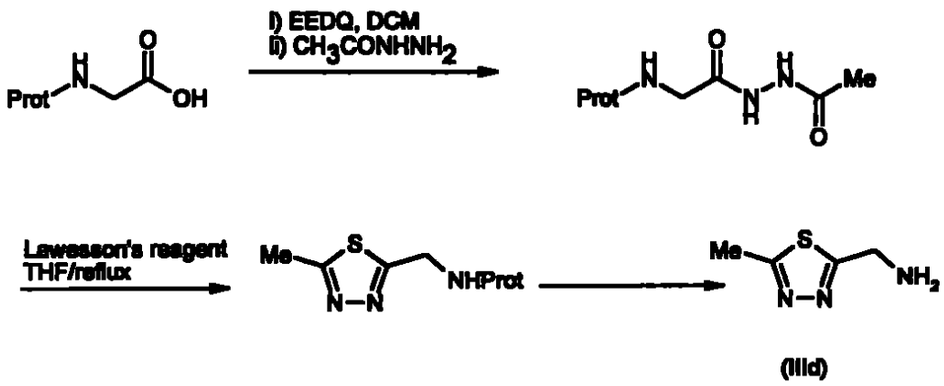
Typical reaction conditions for deprotection when $Prot^4$ is *tert*-butyloxycarbonyl comprise reacting XIII with hydrochloric acid or trifluoroacetic acid in dichloromethane at room temperature for 2 to 4 hrs

Scheme 5



Compounds of formula IIId can be prepared according to reaction scheme 6. The protecting group is preferably *tert*-butyloxycarbonyl, which is removed under standard conditions, as previously described.

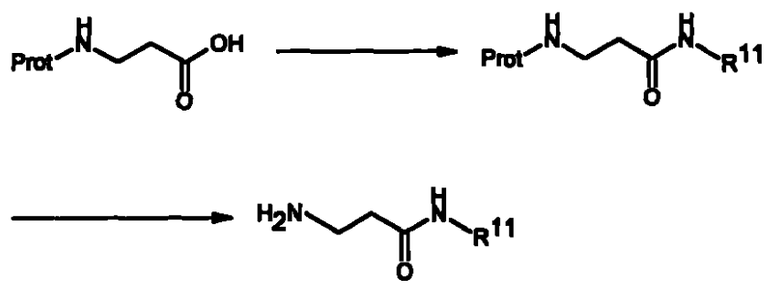
Scheme 6



Compounds of formula IIIe are prepared according to reaction scheme 7 using standard acid/amine coupling reactions, as previously described. The protecting group is preferably benzyloxycarbonyl which may be removed under standard conditions, typically palladium on charcoal (5-10%) in ethanol at room temperature and 50 psi for 4 hrs.

342

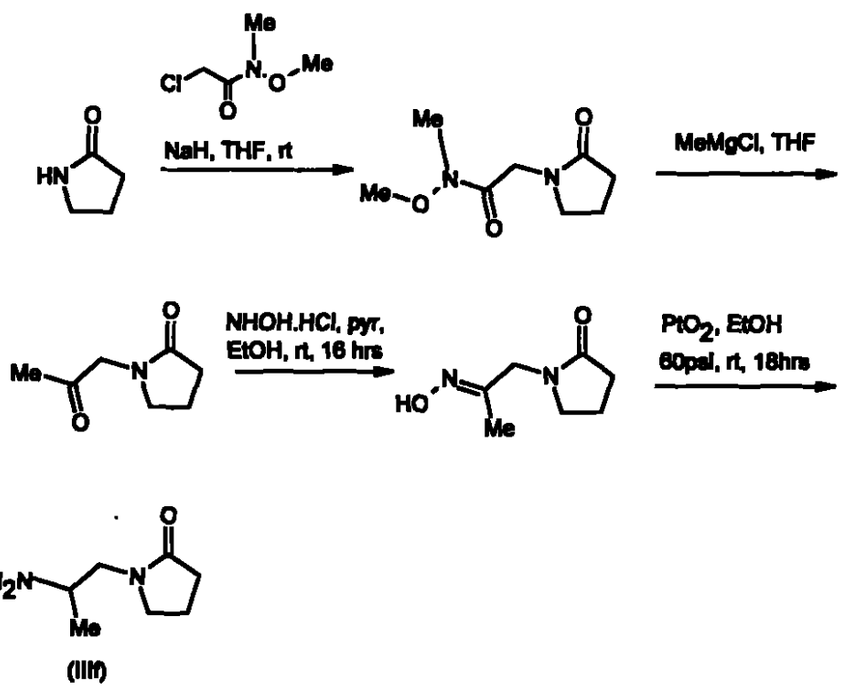
Scheme 7



(IIIe)

Compounds of formula IIIf may be prepared according to reaction scheme 8.

Scheme 8

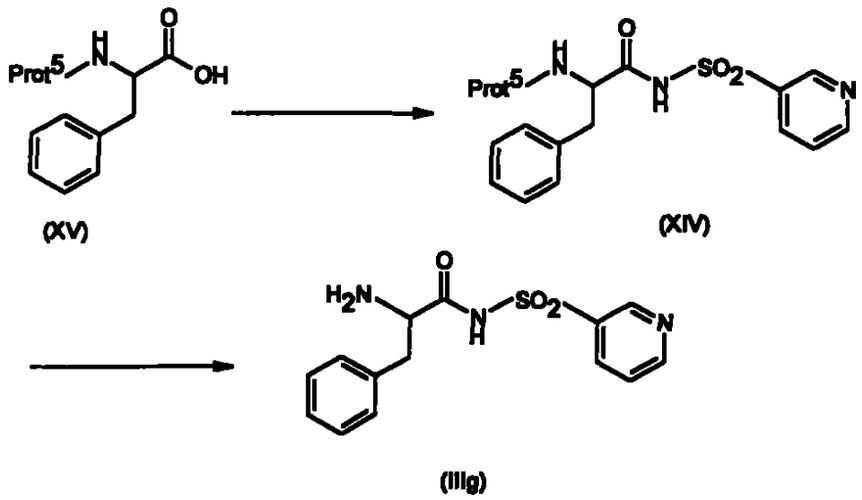


(IIIf)

Compounds of formula IIIg may be prepared in two steps according to reaction scheme 9. As a first step, compounds of formula XIV are prepared from compounds of formula XV using standard acid/amine coupling methodology analogous to the acid/amine coupling conditions described for reaction scheme 1. Prot^5 represents a suitable leaving group, preferably *tert*-butyloxycarbonyl. The second step comprises removal of Prot^5. When Prot^5 is *tert*-butyloxycarbonyl then preferred reaction

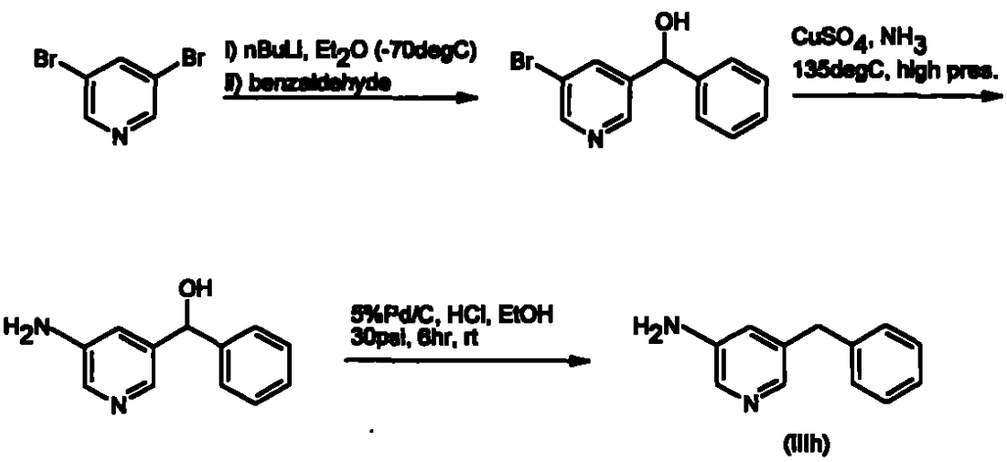
conditions comprise treatment with hydrochloric acid in diethyl ether/ethyl acetate at room temperature for 18 hrs.

Scheme 9



Compounds of formula IIIh may be prepared in three steps according to reaction scheme 10.

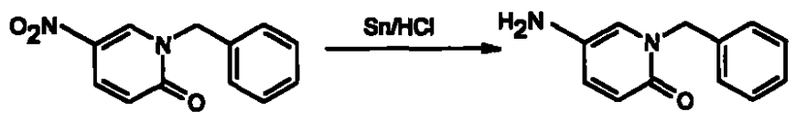
Scheme 10



Compounds of formula IIIj may be prepared by reduction of a nitro group according to reaction scheme 11.

343

Scheme 11

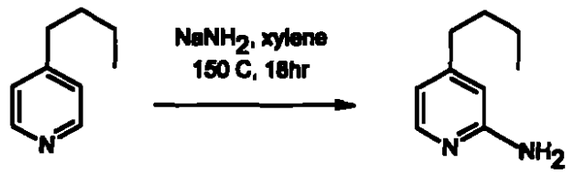


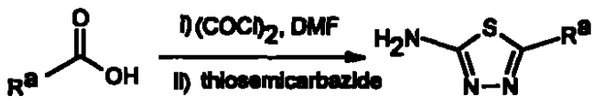
(III)

Further methods for preparing compounds of formula III are give in Scheme 12 below, where R^a is C_{1-6}alkyl or alkoxy.

Scheme 12







All of the above reactions and the preparations of novel starting materials used in the preceding methods are conventional and appropriate reagents and reaction conditions for their performance or preparation as well as procedures for isolating the desired products will be well-known to those skilled in the art with reference to literature precedents and the Examples and Preparations hereto.

A pharmaceutically acceptable salt of a compound of the formula (I) may be readily prepared by mixing together solutions of a compound of the formula (I) and the desired acid or base, as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent.

It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention . "Active Ingredient" means a compound according to formula 1 or a pharmaceutically acceptable salt thereof.

Formulation 1: A tablet is prepared using the following ingredients:

	weight/mg
Active ingredient	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	5
Total	665

the components are blended and compressed to form tablets each weighing 665mg.

Formulation 2: An intravenous formulation may be prepared as follows:

Active ingredient	100mg
Isotonic saline	1,000ml

The invention additionally includes:

(i) a pharmaceutical composition including a compound of the second aspect of the invention, together with a pharmaceutically acceptable excipient, diluent or carrier;

(ii) a compound of the second aspect of the invention for use as a medicament;

(iii) a method of treating sexual dysfunction (preferably FSD) in a mammal including treating said mammal with an effective amount of a compound of the invention;

(iv) A sexual dysfunction treating pharmaceutical composition comprising a compound of the invention together with a pharmaceutically acceptable excipient, diluent or carrier.

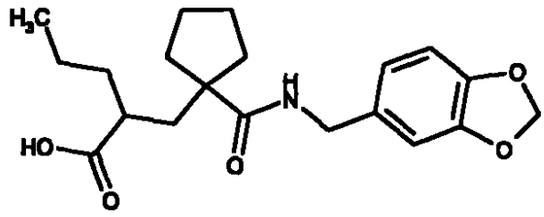
3414

The following Examples illustrate the preparation of the compounds of general formula (I):

Examples

Example 1

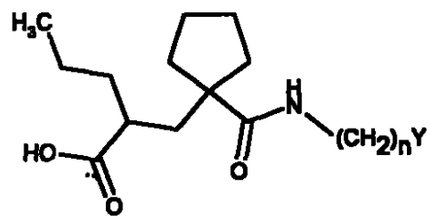
2-({1-[(1,3-Benzodioxol-5-ylamino)carbonyl]cyclopentyl}methyl)pentanoic acid



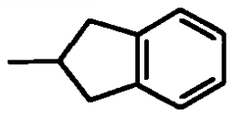
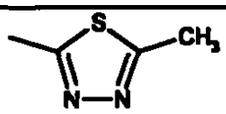
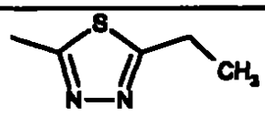
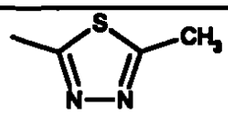
Trifluoroacetic acid (5ml) was added to a solution of the *tert*-butyl ester from preparation 34 (130mg, 0.31mmol) in dichloromethane (5ml), and the solution stirred at room temperature for 4 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with toluene and dichloromethane to afford the title compound as a clear oil, 112 mg, ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.83 (t, 3H), 1.22-1.40 (m, 3H), 1.50-1.72 (m, 8H), 1.95 (m, 1H), 2.10 (m, 2H), 2.19 (m, 1H), 4.30 (m, 2H), 5.93 (s, 2H), 5.99 (bs, 1H), 6.74 (m, 3H); LRMS: m/z 380 (MH^-).

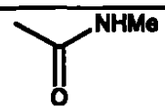
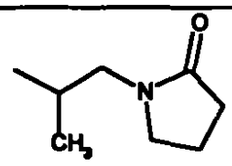
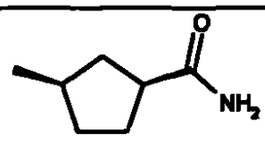
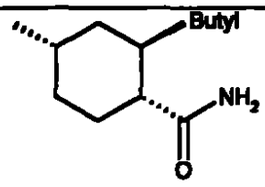
Examples 2 to 9

Compounds of formula Ic, I.e. Compounds of general formula I where R^1 is propyl, where prepared from the corresponding *tert*-butyl ester, following a similar procedure to that described in Example 1.



(Ic)

Ex	n	R	Yield	Data
2[1]	0		78	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (s, 3H), 1.17-2.04 (m, 14H), 2.27-2.38 (m, 1H), 2.64-2.80 (m, 2H), 3.20-3.31 (m, 2H), 4.60-4.72 (m, 1H), 5.97 (d, 1H), 7.03-7.18 (m, 4H). LRMS : m/z 343.8 (M^+).
3[2,3]	0		81	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.30-1.42 (m, 4H), 1.59-1.81 (m, 7H), 2.18 (m, 1H), 2.30 (m, 1H), 2.42 (m, 1H), 2.55 (m, 1H), 2.61 (s, 3H). LRMS : m/z 324 (MH^+). Mp 184-186°C Anal. Found: C, 55.50; H, 7.22; N, 12.61. $C_{15}H_{23}N_3O_3S$ requires C, 55.36; H, 7.14; N, 12.91%.
4[3]	0		86	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.92 (t, 3H), 1.35 (t, 3H), 1.25-1.80 (m, 11H), 2.20-2.50 (m, 4H), 2.95 (q, 2H), 12.10 (bs, 1H). LRMS : m/z 339.8 (MH^+) Anal. Found: C, 56.46; H, 7.46; N, 12.36. $C_{16}H_{25}N_3O_3S$ requires C, 56.62; H, 7.44; N, 12.37%.
5[2]	1		81	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 1.20-1.70 (m, 11H), 1.90-2.20 (m, 3H), 2.25 (m, 1H), 2.70 (s, 3H), 4.75 (m, 2H), 7.10 (bs, 1H). LRMS : m/z 340.6 (MH^+)

Ex	n	R	Yield	Data
6[2]	2		45	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.25-1.40 (m, 3H), 1.41-1.70 (m, 8H), 1.92 (m, 1H), 2.00-2.18 (m, 2H), 2.38 (m, 1H), 2.42 (t, 2H), 2.80 (d, 3H), 3.40-3.60 (m, 2H), 6.50 (bs, 1H), 6.74 (bs, 1H). LRMS : m/z 313.2 (MH^+)
7	0		93	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.19 (d, 3H), 1.21-1.69 (m, 11H), 1.89-2.10 (m, 5H), 2.30 (m, 1H), 2.41 (m, 2H), 2.95 (m, 1H), 3.35 (m, 1H), 3.63 (m, 2H), 4.20 (m, 1H), 6.58-6.70 (m, 1H). LRMS : m/z 353.1 (MH^+)
8	0		99	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.39 (m, 3H), 1.41-2.10 (m, 1H), 2.80 (m, 1H), 4.35 (m, 17H), 5.81 (d, 1H), 6.30 (bs, 0.5H), 6.43 (bs, 0.5H), 7.40 (bd, 0.5H), 7.61 (bd, 0.5H). LRMS : m/z 339.8 (MH^+)
9	0			^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (m, 6H), 1.08-2.08 (m, 29H), 4.29 (m, 1H), 5.95 (d, 1H), 6.43 (s, 1H), 7.80 (d, 1H). LRMS : m/z 409.5 (MH^+)

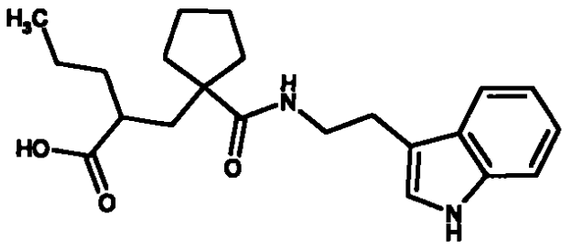
1 = additionally purified by column chromatography on silica gel using ethyl acetate:pentane as eluant.

2 = additionally purified by column chromatography on silica gel using dichloromethane:methanol as eluant.

3 = recrystallised from ether

Example 10

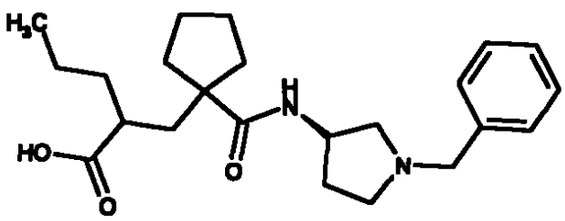
2-{[1-({[2-(1*H*-Indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



Trifluoroacetic acid (2.61ml, 33.9mmol) was added to a solution of the *tert*-butyl ester from preparation 44 (482mg, 1.13mmol) and anisole (1.23ml, 11.3mmol) in dichloromethane (4ml), and the reaction stirred at room temperature for 4 hours. The mixture was washed with water, then brine, dried ($MgSO_4$), concentrated under reduced pressure and the residue azeotroped with toluene. The residual brown oil was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant, and re-columned using an elution gradient of ethyl acetate:pentane (30:70 to 50:50) to afford the title compound as a clear foam, 136mg, 32%; ^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.82 (s, 3H), 1.16-1.77 (m, 12H), 1.78-2.03 (m, 2H), 2.36 (m, 1H), 2.97 (m, 2H), 3.61 (m, 2H), 5.83 (m, 1H), 7.04 (s, 1H), 7.09-7.23 (m, 2H), 7.39 (d, 1H), 7.61 (d, 1H), 8.15 (m, 1H); LRMS : m/z 371.8 (MH^+.

Example 11

2-{[1-({[(3*S*)-1-Benzylpyrrolidinyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid

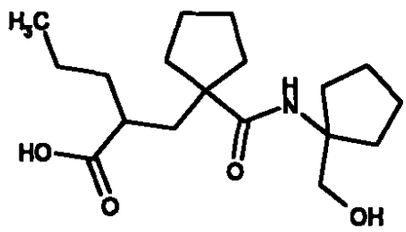


A solution of the *tert*-butyl ester from preparation 45 (70mg, 0.16mmol) in trifluoroacetic acid (1ml) and dichloromethane (1ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with dichloromethane. The residue was partitioned between water (1ml) and ethyl acetate (5ml), and the pH of the aqueous layer adjusted to 6 using sodium bicarbonate solution. The layers were separated, the organic phase dried (Na_2SO_4), evaporated under reduced pressure and the residue azeotroped with dichloromethane, to give the title compound as a beige foam, 45mg, 73%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-2.95 (m, 19H), 3.52 (m, 1H), 3.75 (m, 1H), 3.95 (m, 1H), 4.25 (m,

1H), 4.45 (m, 1H), 6.96 (bs, 1H), 7.39 (m, 5H); LRMS : m/z 387 (MH^+); Anal. Found: C, 61.11; H, 7.69; N, 6.00. $C_{23}H_{34}N_2O_3;CH_2Cl_2$ requires C, 61.14; H, 7.70; N, 5.94%.

Example 12

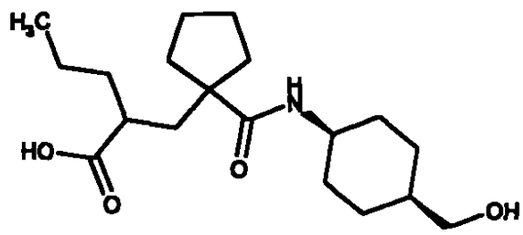
2-{[1-({[1-(Hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



A solution of the *tert*-butyl ester from preparation 33 (38mg, 0.1mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with toluene and then dichloromethane to give a colourless gum. This was suspended in a solution of potassium carbonate (50mg, 0.3mmol) in methanol, and the mixture stirred for 2 hours at room temperature. The methanol was removed under reduced pressure, the residual aqueous mixture diluted with water (20ml), and acidifed to pH 2 using 2N hydrochloric acid. This solution was extracted with ethyl acetate (2x20ml), and the combined organic solutions dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil, 32mg, 97%; ^1H NMR ($CDCl_3$, 400MHz) δ:: 0.88 (t, 3H), 1.20-1.40 (m, 3H), 1.41-1.90 (m, 17H), 2.01-2.20 (m, 2H), 2.40 (m, 1H), 3.71 (dd, 2H), 5.80 (bs, 1H); LRMS : m/z 326.1 (MH^+)

Example 13

Cis-2-{[1-({[4-(Hydroxymethyl)cyclohexyl]amino}carbonyl)cyclopentyl]methyl} pentanoic acid

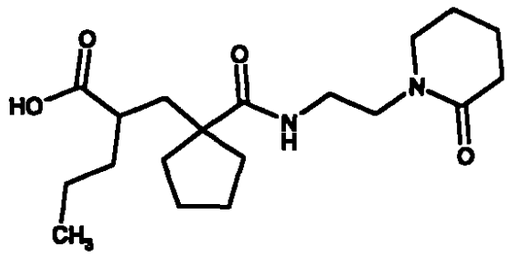


The title compound was obtained as a colourless gum in 68%, from the *tert*-butyl ester from preparation 43, following the procedure described in example 12, except the

product was additionally purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.21-1.40 (m, 6H), 1.52-1.70 (m, 15H), 1.92-2.11 (m, 3H), 2.39 (m, 1H), 3.55 (d, 2H), 4.01 (m, 1H), 5.90 (m, 1H); LRMS : m/z 340.3 (MH^+).

Example 14

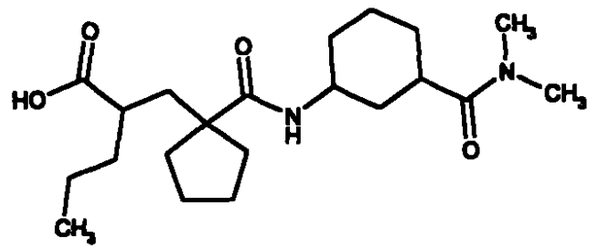
2-{[1-({[2-(2-Oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



Hydrogen chloride gas was bubbled through an ice-cold solution of the *tert*-butyl ester from preparation 47 (43mg, 0.105mmol) in dichloromethane (10ml), for 20 minutes. The solution was then stirred at room temperature for 3 hours. The mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give a glass-like solid. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound, 6mg; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.36 (m, 4H), 1.41-1.69 (m, 7H), 1.79 (m, 4H), 1.90-2.10 (m, 3H), 2.30 (m, 1H), 2.38 (t, 2H), 3.30-3.60 (m, 6H), 7.00 (bs, 1H); LRMS : m/z 351 $(M-H)^-$.

Example 15

2-({1-[({3-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]cyclopentyl}methyl) pentanoic acid

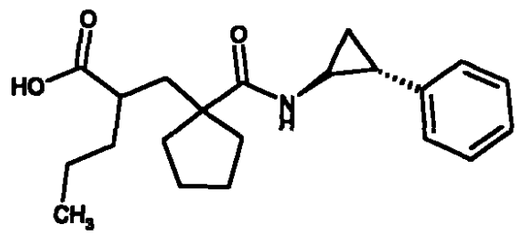


The title compound was obtained as a solid in 85% yield, from the *tert*-butyl ester from preparation 42, following a similar method to that described in example 14, except that dichloromethane:methanol:acetic acid (95:3:2) was used as the chromatographic

eluant; ^{1}H NMR ($CDCl_3$, 400MHz) d: 0.89 (t, 3H), 1.09-1.76 (m, 12H), 1.80-2.17 (m, 10H), 2.37 (m, 1H), 2.68 (m, 1H), 2.95 (s, 3H), 3.04 (s, 3H), 3.83 (m, 1H), 6.06 (m, 1H); LRMS : m/z 381 (MH^+); Anal. Found: C, 63.31; H, 9.17; N, 6.53. $C_{21}H_{36}N_2O_4.H_2O$ requires C, 63.29; H, 9.61; N, 7.03%.

Example 16

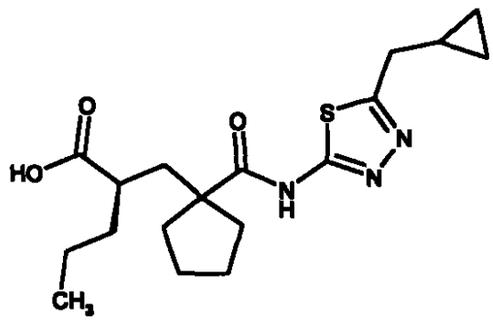
2-{[1-({[(1*R*,2*R*)-2-Phenylcyclopropyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



The title compound was obtained quantitatively as an orange gum from the *tert*-butyl ester from preparation 46, following a similar procedure to that described in example 14; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.12-2.14 (m, 17H), 2.38 (m, 1H), 2.87 (m, 1H), 6.10 (s, 1H), 7.13 (m, 3H), 7.25 (m, 2H); LRMS : m/z 344.3 (MH^+).

Example 17

(2*R*)-2-{[1-({[5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid

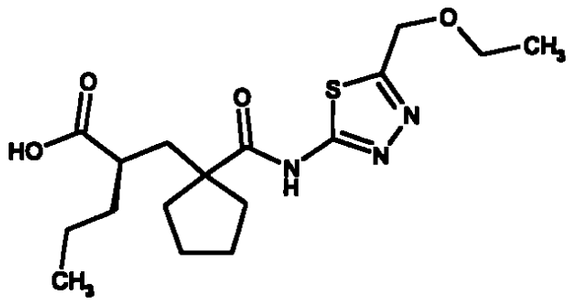


A solution of the *tert*-butyl ester from preparation 50 (63mg, 0.15mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml), was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant , to give the title compound as a white foam, 46mg, 83%; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.38 (m, 2H), 0.62 (m, 2H), 0.82 (t, 3H), 1.12 (m, 1H), 1.26 (m,

2H), 1.38 (m, 1H), 1.52 (m, 1H), 1.78-1.78 (m, 6H), 1.90 (m, 1H), 2.23 (m, 4H), 2.92 (d, 2H); LRMS : m/z 366.0 (MH^+); $[\alpha]_D$ = -7.75° (c = 0.08, methanol).

Example 18

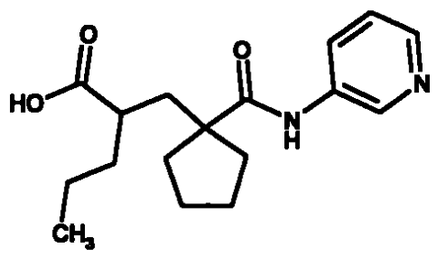
(2*R*)-2-{[1-({[5-(Ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid



The title compound was obtained as a white foam in 62% yield, from the *tert*-butyl ester from preparation 51, following a similar procedure to that described in example 17; ^1H NMR (CD_3OD, 400MHz) δ: 0.82 (t, 3H), 1.21-1.40 (m, 7H), 1.50 (m, 1H), 1.60-1.77 (m, 7H), 1.88 (m, 1H), 2.23 (m, 4H), 3.62 (q, 2H); $[\alpha]_D$ = -6.08° (c = 0.25, methanol).

Example 19

2-({1-[(3-Pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoic acid

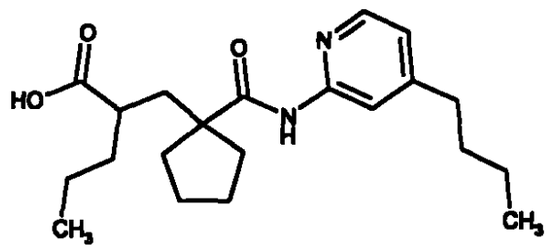


A mixture of the benzyl ester from preparation 52 (130mg, 0.33mmol) and 10% palladium on charcoal (20mg) in 95% aqueous ethanol (3ml) was hydrogenated at 15psi and room temperature for 2 hours. The reaction was filtered through Arbocel , washing through with ethanol, and the filtrate evaporated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 103mg, 83%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 2H), 1.44 (m, 1H), 1.58-1.82 (m, 8H), 2.19 (m, 1H), 2.39 (m, 2H), 2.52 (m, 1H), 6.88 (m, 1H), 7.67 (m, 1H), 7.82 (d, 1H), 8.38 (d, 1H), 9.78 (s, 1H); LRMS : m/z 305 (MH^+).

348

Example 20

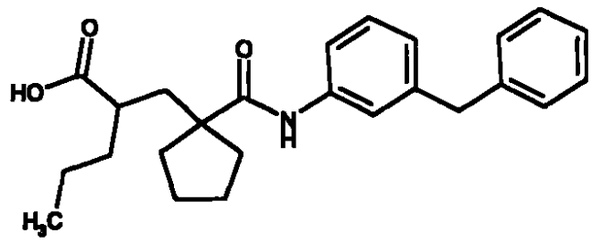
2-[(1-[(4-Butyl-2-pyridinyl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid



The title compound was obtained in 92% yield from the benzyl ester from preparation 55, following a similar procedure to that described in example 19; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (m, 6H), 1.28-1.50 (m, 5H), 1.58-1.81 (m, 10H), 2.20 (m, 1H), 2.40 (m, 2H), 2.58 (m, 3H), 6.70 (d, 1H), 7.68 (d, 1H), 8.22 (s, 1H), 9.90 (bs, 1H).

Example 21

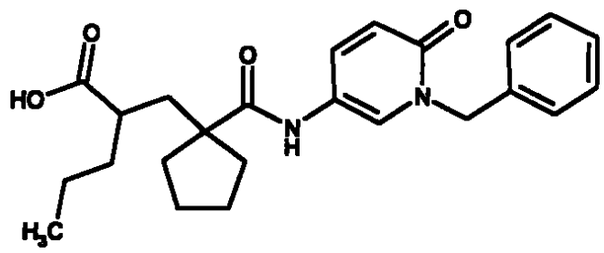
2-({1-[(3-Benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 53 (1.3mg, 2.47mmol) and 5% palladium on charcoal (130mg) in water (10ml) and ethanol (40ml) was hydrogenated at 30 psi and room temperature for 2 hours. The reaction mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure, and the residue triturated with dichloromethane. The residual gum was triturated with ether, then hexane, and dried at 50°C, to give the title compound as a solid, 0.79g, 81%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.95 (t, 3H), 1.24-1.51 (m, 3H), 1.58-1.80 (m, 7H), 1.88 (dd, 1H), 2.15 (m, 2H), 2.24 (m, 1H), 2.48 (m, 1H), 4.00 (s, 2H), 6.98 (d, 1H), 7.24 (m, 6H), 7.40 (m, 3H); Anal. Found: C, 75.48; H, 7.76; N, 3.59. $C_{25}H_{31}NO_3$;0.25H_2O requires C, 75.44; H, 7.98; N, 3.51%.

Example 22

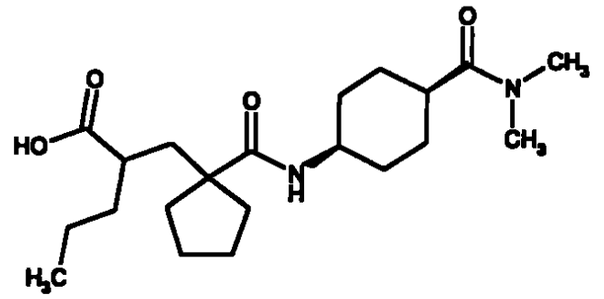
2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-pentanoic acid.



The title compound was obtained as a white foam in 51% yield from the benzyl ester from preparation 56, following a similar procedure to that described in example 21, except, the product was purified by column chromatography on silica gel, using ethyl acetate as eluant; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.28-1.80 (m, 12H), 2.01 (m, 1H), 2.30-2.52 (m, 2H), 5.02 (dd, 2H), 6.60 (d, 1H), 7.27 (m, 5H), 7.70 (s, 1H), 8.34 (s, 1H); Anal. Found: C, 69.52; H, 7.41; N, 6.51. $C_{24}H_{30}N_2O_4$;0.25H_2O requires C, 69.45; H, 7.41; N, 6.75.

Example 23

Cis-2-({1-[({4-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoic acid



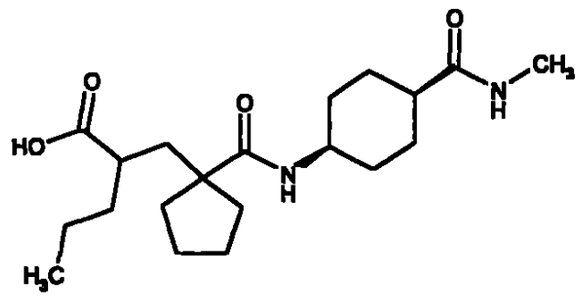
A mixture of the benzyl ester from preparation 58 (150mg, 0.33mmol) and 10% palladium on charcoal (20mg) in water (0.3ml) and ethanol (3.5ml) was hydrogenated at 15 psi and room temperature for 3 days. The reaction mixture was filtered through Arbocel®, and the filtrate concentrated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 85mg, 65%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.29-1.96 (m, 18H), 2.01-2.23 (m, 4H), 2.37 (m, 1H), 2.62 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.96 (m, 1H), 5.98 (m, 1H); LRMS : m/z 381.8 (MH^+); Anal.

3419

Found: C, 63.81; H, 9.58; N, 6.99. $C_{21}H_{38}N_2O_4$;0.2CH_2Cl_2 requires C, 64.06; H, 9.23; N, 7.05%.

Example 24

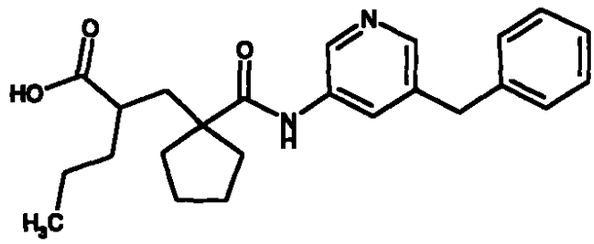
Cis-2-({1-[({4-[(Methylamino)carbonyl]cyclohexyl}amino)carbonyl]cyclopentyl}-methyl)pentanoic acid



The title compound was obtained as a white solid in 34% yield from the benzyl ester from preparation 59, following the procedure described in example 23; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.90 (t, 3H), 1.26-2.02 (m, 20H), 2.19 (m, 3H), 2.39 (m, 1H), 2.82 (d, 3H), 4.00 (m, 1H), 5.69 (m, 1H), 6.00 (d, 1H); LRMS : m/z 365 (M-H$^+$).

Example 25

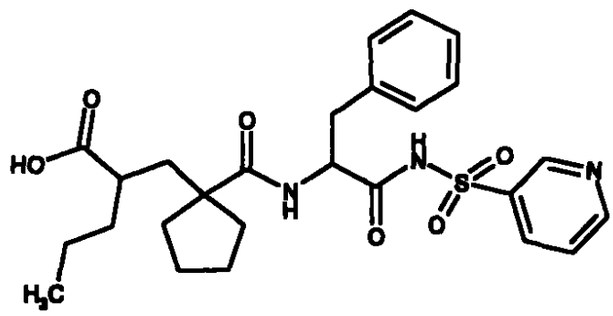
2-[(1-{[(5-Benzyl-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-pentanoic acid.



A mixture of the benzyl ester from preparation 54 (850mg, 1.76mmol) and 5% palladium on charcoal (100mg) in 20% aqueous ethanol (30ml) was hydrogenated at 30 psi and room temperature for 2 hours. The mixture was filtered through Arbocel , the filtrate evaporated under reduced pressure, and the residue azeotroped with dichloromethane to give the title compound as a foam, 0.63g; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.92 (t, 3H), 1.30-1.83 (m, 11H), 2.07 (m, 1H), 2.42 (m, 3H), 3.82 (s, 2H), 7.15-7.38 (5H), 7.80 (s, 1H), 8.48 (s, 1H), 8.59 (s, 1H), 8.62 (s, 1H); Anal. Found: C, 72.29; H, 7.70; N, 6.90. $C_{24}H_{30}N_2O_3$;0.25H_2O requires C, 72.24; H, 7.70; N, 7.02%.

Example 26

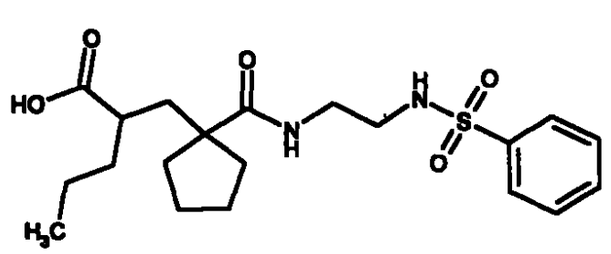
2-({1-[({1-Benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoic acid.



A mixture of the benzyl ester from preparation 57 (918mg, 1.52mmol) and 10% palladium on charcoal (90mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 4 ½ hours. Tlc analysis showed starting material remaining, so additional catalyst (70mg) was added, and the mixture hydrogenated for a further 18 hours. Tlc analysis, again showed starting material remaining, so further catalyst (70mg) was added, and hydrogenation continued for an additional 6 hours. The reaction mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:acetic acid:ethanol (99:1:0 to 79.1:0.9:20) to afford the title compound as a white foam, 271mg, 35%; ^{1}H NMR (DMSOd_6, 300MHz) δ: 0.75 (m, 3H), 0.96-1.42 (m, 11H), 1.61-1.99 (m, 4H), 2.75-3.02 (m, 2H), 4.45 (m, 1H), 7.20 (m, 6H), 7.62 (m, 1H), 8.24 (m, 1H), 8.83 (s, 1H), 9.01 (s, 1H), 11.98 (bs, 1H), 12.70 (bs, 1H); IR (KBr disc) 1185, 1195 (m), 1455, 1515, 1640, 1704, 2870, 2930, 2960 (s).

Example 27

2-({1-[({2-[(Phenylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}methyl)pentanoic acid



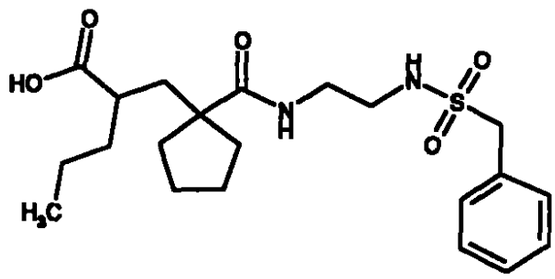
A mixture of the amine from preparation 61 (235mg, 0.72mmol), benzenesulphonyl chloride (127mg, 0.72mmol) and triethylamine (150µl, 1.08mmol) in dichloromethane

350

(6ml) was stirred at room temperature for 2 days. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to give a clear oil. This was then dissolved in trifluoroacetic acid (3ml) and dichloromethane (3ml) and the solution stirred at room temperature for 6 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) to afford the title compound as a clear oil, 204mg, 69%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.22-1.43 (m, 4H), 1.43-2.18 (m, 10H), 2.36 (m, 1H), 3.11 (m, 2H), 3.20-3.31 (m, 1H), 3.42-3.53 (m, 1H), 6.13-6.24 (m, 1H), 7.42-7.59 (m, 3H), 7.84 (m, 2H); LRMS : m/z 411.8 (MH^+); Anal. Found: C, 57.26; H, 7.40; N, 6.61. $C_{20}H_{30}N_2O_5S$ requires C, 57.18; H, 7.22; N, 6.62%.

Example 28

2-({1-[({2-[(Benzylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}methyl)pentanoic acid

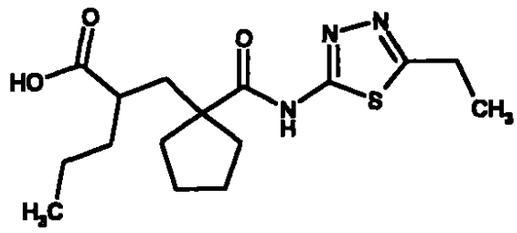


The title compound was obtained as a clear oil in 97% yield, from the amine from preparation 61, following the procedure described in example 27, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.87 (t, 3H), 1.19-1.72 (m, 11H), 1.80-1.96 (m, 1H), 2.00-2.16 (m, 2H), 2.27-2.38 (m, 1H), 2.92-3.21 (m, 3H), 3.23-3.39 (m, 1H), 4.25 (s, 2H), 5.80-6.06 (m, 1H), 6.38 (m, 1H), 7.29-7.43 (m, 5H); LRMS : m/z 425.8 (MH^+).

Example 29
(-)-2-[(1-[[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)methyl]pentanoic acid

and

Example 30
(+)-2-[(1-[[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)methyl]pentanoic acid



The acid from Example 4 (824mg) was further purified by HPLC using an AD column and using hexane:*iso*-propanol:trifluoroacetic acid (85:15:0.2) as eluant to give the title compound of example 29 as a white foam, 400mg, 99.5% ee, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.36 (m, 6H), 1.50-1.80 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.30 (bs, 1H), LRMS : m/z 338 (MH^+), $[\alpha]_D$ = -9.0° (c = 0.1, methanol), and the title compound of example 30 as a white foam, 386mg, 99% ee, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.36 (m, 6H), 1.50-1.79 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.27 (bs, 1H); LRMS: m/z 338 (MH^+); and $[\alpha]_D$ = +3.8° (c = 0.1, methanol)

351

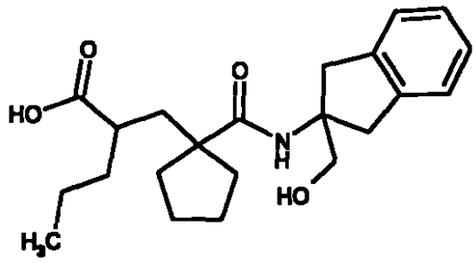
Example 31

(+)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid

and

Example 32

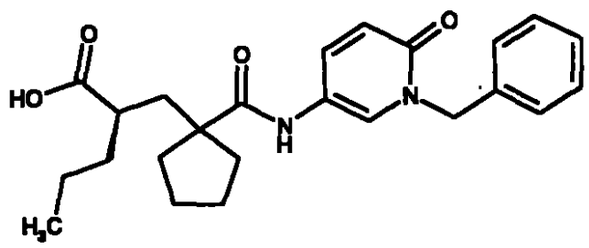
(-)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1H-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid



2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid (WO 9110644) was further purified by HPLC using an AD column and hexane:isopropanol:trifluoroacetic acid (90:10:0.1) as eluant, to give the title compound of example 31, 99% ee, $[\alpha]_D$ = +10.4° (c = 0.067, ethanol) and the title compound of example 32, 99% ee, $[\alpha]_D$ = -10.9° (c = 0.046, ethanol).

Example 33

(2*R*)-2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-pentanoic acid.

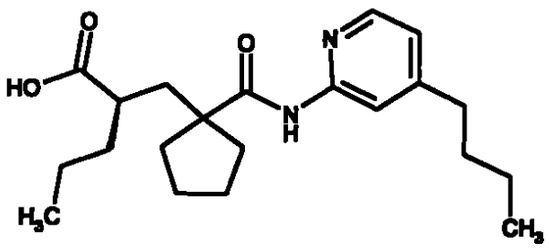


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (191mg, 1.0mmol), 1-hydroxybenzotriazole hydrate (135mg, 01.0mmol), N-methylmorpholine (165µl, 1.5mmol) and finally the amine from preparation 28 (150mg, 0.69mmol) were added to a solution of the acid from preparation 2 (284mg, 1.0mmol) in N,N-dimethylformamide (8ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (4x50ml), and brine (50ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using ethyl acetate:pentane (30:70) to give a yellow oil,

191mg. This intermediate was dissolved in dichloromethane (3ml) and trifluoroacetic acid (3ml) and the solution stirred at room temperature for 5 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to give the title compound as a foam, 77mg, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (t, 3H), 1.20-1.76 (m, 12H), 1.93-2.02 (m, 1H), 2.20-2.46 (m, 3H), 4.95 (d, 1H), 5.04 (d, 1H), 6.61 (d, 1H), 7.21 (m, 1H), 7.50 (s, 1H), 8.23 (s, 1H); LRMS : m/z 411.6 $(MH)^+$; $[\alpha]_D$ = -3.8° (c = 0.052, ethanol).

Example 34

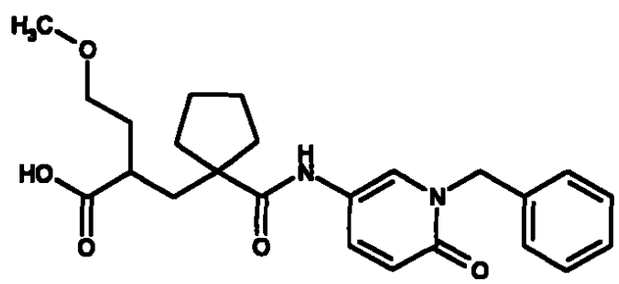
(2*R*)-2-[(1-[[(4-Butyl-2-pyridinyl)amino]carbonyl]cyclopentyl)methyl]pentanoic acid



The title compound was obtained in 43% yield from the acid from preparation 2 and the amine from preparation 30, following a similar procedure to that described in example 33, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80-1.00 (m, 6H), 1.22-1.84 (m, 18H), 2.03-2.56 (m, 3H), 2.77 (m, 1H), 7.14 (d, 1H), 8.08 (d, 1H), 8.23 (s, 1H), 11.71 (brs, 1H). LRMS : m/z 361.7 $(MH)^+$, $[\alpha]_D$ = -1.4° (c = 0.14, ethanol).

Example 35

2-[(1-[[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]cyclopentyl)methyl]-4-methoxybutanoic acid



A mixture of the benzyl ester from preparation 62 (850mg, 1.64mmol), and 5% palladium on charcoal (250mg) in 40% aqueous ethanol (21ml), was hydrogenated at 30 psi and room temperature for 30 minutes. The reaction mixture was filtered through Hyflo®, and the filtrate evaporated under reduced pressure. The residual foam was

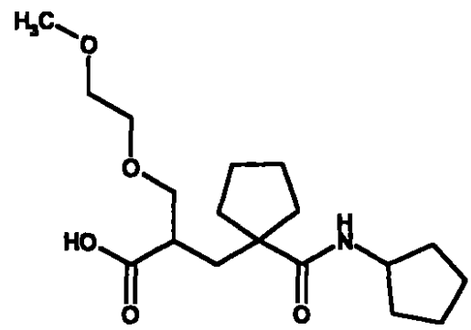
352

purified by column chromatography on silica gel using dichloromethane:methanol (97:3) as eluant to give the title compound as a white foam, 550mg, 79%; ^{1}H NMR (DMSO-d_6, 300MHz) δ: 1.24-2.17 (m, 12H), 2.18-2.31 (m, 1H), 3.07 (s, 3H), 3.21 (t, 2H), 5.08 (s, 2H), 6.63 (d, 1H), 7.23-7.41 (m, 5H), 7.72 (d, 1H), 8.24 (s, 1H).

Anal. Found: C, 67.46; H, 7.18; N, 6.24. $C_{24}H_{35}N_2O_5$ requires C, 67.58; H, 7.09; N, 6.57%.

Example 36

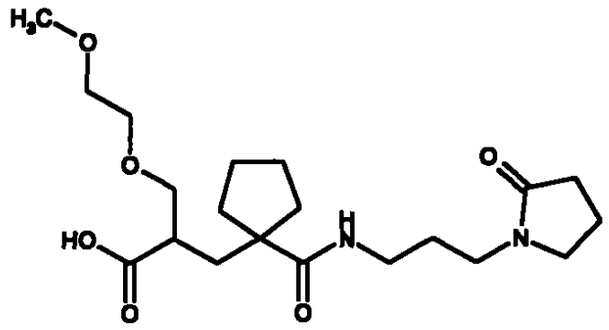
3-{1-[(Cyclopentylamino)carbonyl]cyclopentyl}-2-[(2-methoxyethoxy)methyl]propanoic acid



A solution of the *tert*-butyl ester from preparation 64 (320mg, 0.80mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room temperature for 8 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) to give the title compound as a clear oil, 171mg, 62%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.29-1.40 (m, 2H), 1.42-1.69 (m, 10H), 1.75 (dd, 1H), 1.87-2.03 (m, 5H), 2.64 (m, 1H), 3.34 (s, 3H), 3.43-3.52 (m, 3H), 3.57 (m, 2H), 3.61 (m, 1H), 4.08-4.20 (m, 1H), 5.89 (d, 1H); LRMS : m/z 340 (MH^+).

Example 37

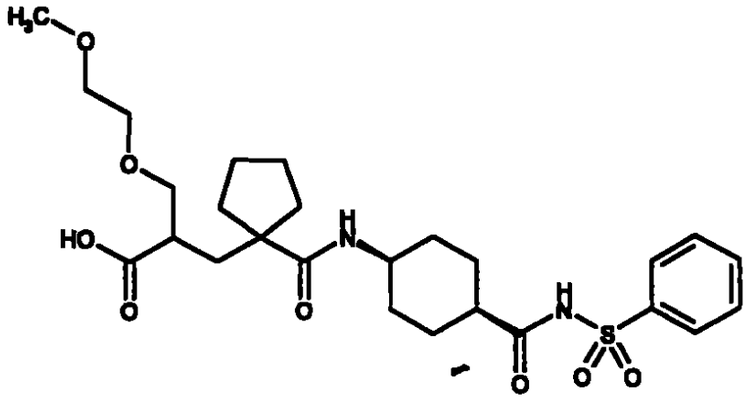
3-(2-Methoxyethoxy)-2-[[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)-cyclopentyl]methyl]propanoic acid



The title compound was obtained as a clear oil in 57% yield from the *tert*-butyl ester of preparation 65, following the procedure described in example 36, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.56-1.78 (m, 8H), 1.94-2.17 (m, 6H), 2.44 (m, 2H), 2.68-2.76 (m, 1H), 3.10-3.21 (m, 1H), 3.22-3.31 (m, 1H), 3.37 (s, 3H), 3.40 (m, 2H), 3.44-3.56 (m, 5H), 3.60 (m, 2H), 3.68 (m, 1H), 6.91-7.01 (m, 1H); LRMS : m/z 398.7 (M^+)

Example 38

Cis-3-(2-Methoxyethoxy)-2-[(1-[[(4-[[(phenylsulfonyl)amino]carbonyl]cyclohexyl)-amino]carbonyl]cyclopentyl)methyl]propanoic acid



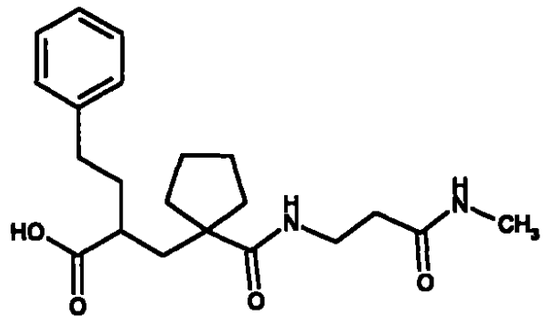
A solution of the *tert*-butyl ester from preparation 66 (446mg, 0.75mmol) in dichloromethane (5ml) and trifluoroacetic acid (5ml) was stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and the residue azeotroped with dichloromethane, then toluene, and finally ether, to afford the title compound as a white foam, 385mg, 95%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.48-2.17 (m, 18H), 2.40 (s, 1H), 2.66 (s, 1H), 3.37 (s, 3H), 3.50-3.70 (m, 6H), 3.94 (s, 1H), 6.10 (d, 1H), 6.59 (s, 1H), 7.55 (t, 2H), 7.61 (m, 1H), 8.02 (d, 2H), 9.11 (s, 1H); Anal. Found: C, 54.88; H, 6.90; N, 5.04. $C_{28}H_{38}N_2O_8S;1.7H_2O$ requires C, 57.97; H, 7.11; N,

353

5.20%.

Example 39

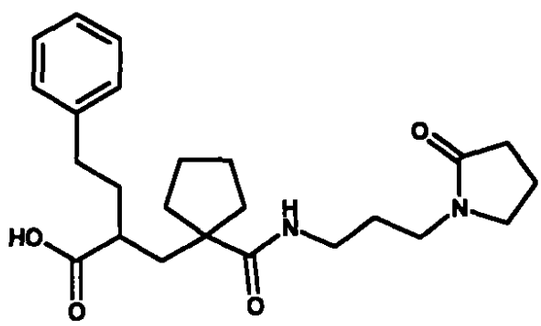
2-[[1-({[3-(Methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl]-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 68 (160mg, 0.34mmol) and 10% palladium on charcoal (100mg) in ethanol (30ml) was hydrogenated at room temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel® and the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:acetic acid (95:5:0 to 95:5:0.5) to afford the title compound as a white foam, 100mg, 79%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.40-1.70 (m, 8H), 1.95 (m, 3H), 2.10 (m, 1H), 2.35 (d, 3H), 2.59 (m, 2H), 2.75 (t, 3H), 3.42 (m, 2H), 6.25 (bs, 1H), 6.70 (bs, 1H), 7.13-7.25 (m, 5H); and LRMS: m/z 375.0 (MH^+).

Example 40

2-[[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]-methyl]-4-phenylbutanoic acid.

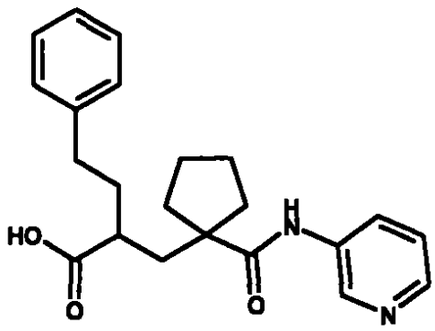


A mixture of the benzyl ester from preparation 67 (780mg, 1.55mmol) and 10% palladium on charcoal (100mg) in ethanol:water (90:10 by volume), (30ml) was

hydrogenated at room temperature under 60psi H_2 pressure for 1.5 hours. The catalyst was filtered off, and the filtrate evaporated under reduced pressure to provide the title compound as a white foam, 473mg, 74%; ^{1}H NMR ($CDCl_3$, 300MHz) d : 1.26-1.77 (m, 10H), 1.78-2.46 (m, 11H), 2.49-2.70 (m, 2H), 2.95-3.36 (m, 4H), 6.92-7.38 (m, 5H); Anal. Found: C, 64.05; H, 7.73; N, 6.22. $C_{24}H_{34}N_2O_4$;0.75H_2O requires C, 65.88; H, 7.83; N, 6.40%.

Example 41

4-Phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoic acid

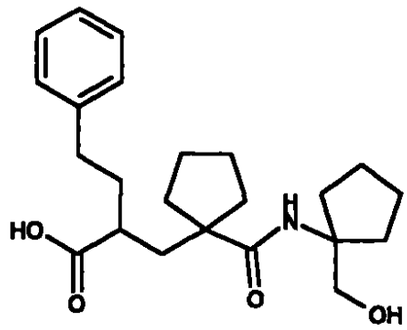


A mixture of the benzyl ester from preparation 71 (700mg, 1.53mmol) and 5% palladium on charcoal (70mg) in ethanol:water (90:10 by volume, 50ml) was hydrogenated at room temperature under 30 psi H_2 pressure for 5 hours. The catalyst was filtered through Arbocel®, washing well with ethanol, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant to provide the title compound as a white foam, 510mg, 91%; mp 80-85°C (collapses to a gum); ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.40-2.78 (m, 15H), 6.93-7.39 (m, 5H), 7.93 (m, 1H), 8.59 (d, 1H), 9.17 (d, 1H), 9.41 (s, 1H); Anal. Found: C, 70.83; H, 7.10; N, 7.64. $C_{22}H_{26}N_2O_3$;0.3H_2O requires C, 70.94; H, 7.22; N, 7.52%.

354

Example 42

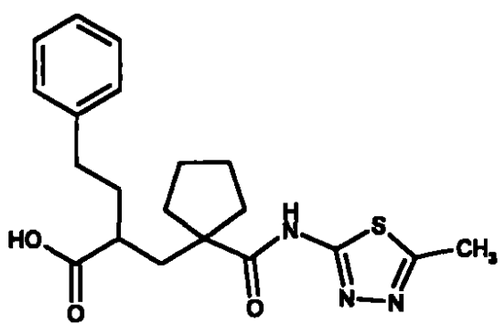
2-{[1-({[1-(Hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 69 (118mg, 0.25mmol) and 10% palladium on charcoal (100mg) in ethanol (20ml) was hydrogenated at room temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane to give the title compound as a colourless gum, 95mg, 98%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.41-1.80 (m, 17H), 1.90 (m, 1H), 1.92-2.20 (m, 3H), 2.40 (m, 1H), 2.60 (m, 2H), 3.60 (d, 1H), 3.71 (d, 1H), 5.80 (bs, 1H), 7.15-7.30 (m, 5H); LRMS : m/z 388.1 (MH^+)

Example 43

2-[(1-{[(5-Methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 70 (187mg, 0.39mmol) and 10% palladium on charcoal (80mg) in ethanol (20ml) was hydrogenated at 60 psi for 18 hours. Tlc analysis showed starting material remaining, so additional 10% palladium on charcoal (100mg) was added, and the reaction continued for a further 5 hours. Tlc analysis again showed starting material remaining, so additional catalyst (100mg) was added, and hydrogenation continued for 18 hours. The mixture was filtered through Arbocel , and the filtrate concentrated under reduced pressure, and azeotroped with

dichloromethane. The crude product was purified by chromatography on silica gel using a Biotage® column, and dichloromethane:methanol (95:5) as eluant to afford the title compound as a clear oil, 80mg, 53%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.51-1.89 (m, 9H), 2.03 (m, 1H), 2.20 (m, 1H), 2.40 (m, 2H), 2.60 (m, 5H), 7.15-7.30 (m, 5H); LRMS : m/z 387.8 (MH^+).

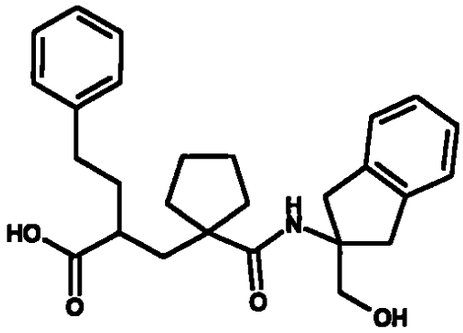
Example 44

(+)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid

and

Example 45

(-)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid



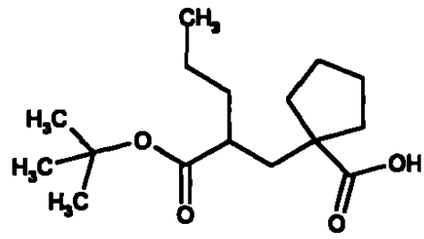
2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (WO 9110644) may be purified by standard HPLC procedures using an AD column and hexane:isopropanol:trifluoroacetic acid (70:30:0.2) as eluant, to give the title compound of example 44, 99.5% ee; $[\alpha]_D$ = +9.1° (c = 1.76 in ethanol); and the title compound of example 45, 99.5% ee; $[\alpha]_D$ = -10.5° (c = 2.2 in ethanol).

The following Preparations describe the preparation of certain intermediates used in the preceding Examples.

Preparations

Preparation 1

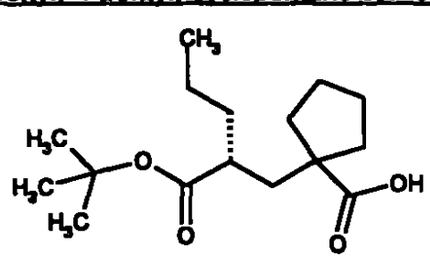
1-[2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid



A mixture of 1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (EP 274234) (23g, 81.5mmol) and 10% palladium on charcoal (2g) in dry ethanol (200ml) was hydrogenated at 30psi and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel, using ethyl acetate:pentane (40:60) as the eluant, to provide the desired product as a clear oil, 21g, 91%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); LRMS : m/z 283 $(M-H)^-$

Preparation 2

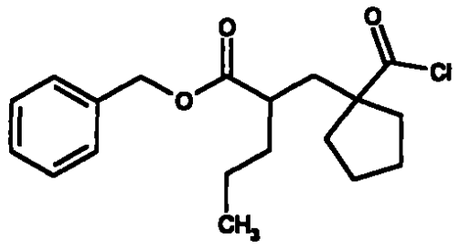
1-[(2*R*)-2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid



A mixture of (R)-1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (WO 9113054) (10g, 35.4mmol) and 10% palladium on charcoal (600mg) in dry ethanol (25ml) was hydrogenated at 1 atm. and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give the title compound as a yellow oil, 9.6g, 95%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); $[\alpha]_D$ = -3.3° (c = 0.09, ethanol).

Preparation 3

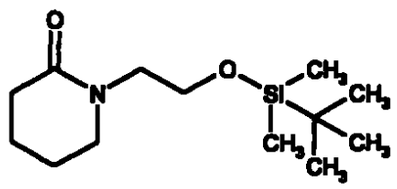
Benzyl 2-{[1-(chlorocarbonyl)cyclopentyl]methyl}pentanoate



Oxalyl chloride (1.15ml, 13.2mmol) was added to an ice-cooled solution of 1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentanecarboxylic acid (EP 274234) (2.0g, 6.3mmol) in dry dichloromethane (20ml), and the solution stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give the title compound as a golden oil, 2.1g; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.28 (m, 2H), 1.43 (m, 2H), 1.63 (m, 6H), 2.00 (m, 1H), 2.08-2.35 (m, 3H), 2.44 (m, 1H), 5.15 (s, 2H), 7.28 (m, 5H).

Preparation 4

1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-2-piperidinone

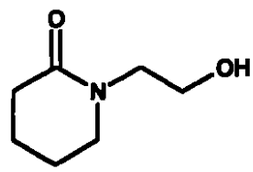


Sodium hydride (807mg, 60% dispersion in mineral oil, 20.18mmol) was added portionwise to a solution of δ-valerolactam (2.0g, 20.2mmol) in tetrahydrofuran (100ml) under nitrogen. (2-Bromoethoxy)(*tert*-butyl)dimethylsilane (4.33ml, 20.2mmol) was added portionwise, and the reaction heated at 70°C for 18 hours. Water (50ml) was added to the cooled reaction, the mixture concentrated *in vacuo*, to remove the tetrahydrofuran, and extracted with ethyl acetate (200ml). The organic solution was dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 97:3) to give the title compound, 3.25g; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.00 (s, 6H), 0.83 (s, 9H), 1.75 (m, 4H), 2.35 (m, 2H), 3.39 (m, 4H), 3.75 (t, 2H); LRMS : *m/z* 257.9 (M^+)

356

Preparation 5

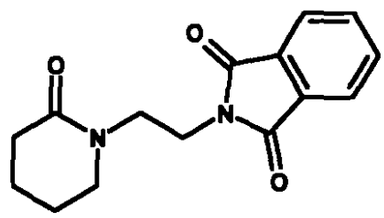
1-(2-Hydroxyethyl)-2-piperidinone



Tetra-n-butylammonium fluoride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added to a solution of the lactam from preparation 4 (3.3g, 12.8mmol) in tetrahydrofuran (50ml), and the reaction stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure, the residue azeotroped with dichloromethane, and purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 95:5) to give the title compound as an oil; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.80 (m, 4H), 2.40 (t, 2H), 3.38 (t, 2H), 3.42 (t, 1H), 3.56 (t, 2H), 3.80 (t, 2H).

Preparation 6

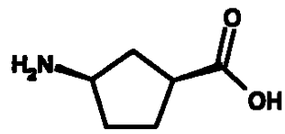
2-[2-(2-Oxo-1-piperidinyl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione



Pthalimide (952mg, 6.47mmol) was added to a solution of the alcohol from preparation 5 (842mg, 5.88mmol) in tetrahydrofuran (30ml), and the mixture sonicated until a solution was obtained. Polymer supported triphenyl phosphine (2.5g, 7.5mmol) and diethyl azodicarboxylate (1.15ml, 7.31mmol) were added, and the reaction stirred at room temperature for 18 hours. The mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 100:0), to give the title compound as a white foam, 1.6g (containing some impurities); ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.60-1.80 (m, 4H), 2.17 (m, 2H), 3.30 (m, 2H), 3.60 (m, 2H), 3.83 (m, 2H), 7.62 (m, 2H), 7.79 (m, 2H); LRMS : m/z 273.2 (MH^+).

Preparation 7

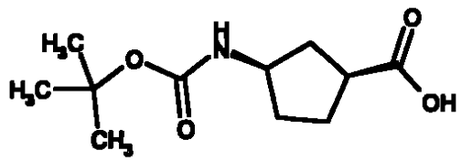
(1*S*,3*R*)-3-Aminocyclopentanecarboxylic acid



Platinum oxide (1g) was added to a solution of (1*R*,4*S*)-4-amino-cyclopent-2-ene carboxylic acid (5.3g, 41.7mmol) in water (70ml), and the mixture was hydrogenated at 45 psi and room temperature for 18 hours. The mixture was filtered through Arbocel , the filtrate evaporated under reduced pressure, and the residue azeotroped with toluene, to afford the title compound as an off-white solid; ^{1}H NMR (D_2O, 400MHz) δ: 1.70-1.92 (m, 3H), 2.00 (m, 2H), 2.18 (m, 1H), 2.77 (m, 1H), 3.68 (m, 1H); LRMS : m/z 129.8 (MH^+).

Preparation 8

(1*S*,3*R*)-3-[(*tert*-Butoxycarbonyl)amino]cyclopentanecarboxylic acid

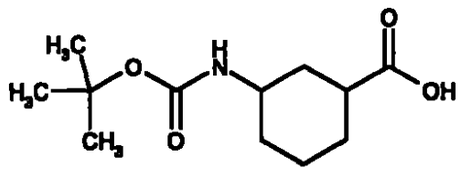


Di-*tert*-butyl dicarbonate (10g, 45.8mmol) was added to an ice-cooled solution of the amino acid from preparation 7 (5.4g, 41.8mmol) in dioxan (42.5ml) and sodium hydroxide solution (42.5ml, 1N, 42.5mmol), and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure to remove the dioxan, then acidifed to pH 2 using 2N hydrochloric acid. The aqueous solution was extracted with ethyl acetate (5x100ml), the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure to give a white solid. This was triturated with hexane, to give the desired compound as a crystalline solid, 8.0g, 83%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.58-2.06 (m, 5H), 2.21 (m, 1H), 2.84 (m, 1H), 4.01 (m, 1H), 4.84 (m, 1H); LRMS: m/z 228 $(M-H)^-$.

357

Preparation 9

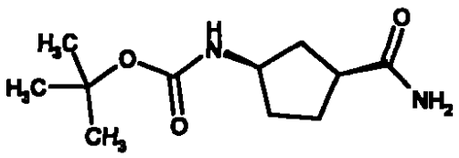
3-[(*tert*-Butoxycarbonyl)amino]cyclohexanecarboxylic acid



The title compound was obtained as a white solid in 81% yield, from 3-aminocyclohexanecarboxylic acid, following the procedure described in preparation 8; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.04 (m, 1H), 1.19-1.50 (m, 13H), 1.83 (m, 1H), 1.97 (m, 2H), 2.24 (m, 1H), 2.40 (m, 1H), 3.44 (bs, 1H), 4.42 (bs, 1H).

Preparation 10

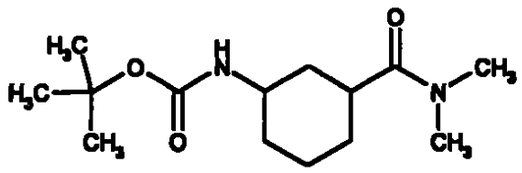
tert-Butyl (1*R*,3*S*)-3-(aminocarbonyl)cyclopentylcarbamate



Benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (3.4g, 6.54mmol), 1-hydroxybenzotriazole hydrate (883mg, 6.54mmol), ammonium chloride (467mg, 8.72mmol) and N-ethyldiisopropylamine (3.04ml, 17.5mmol) were added sequentially to a solution of the acid from preparation 8 (1.0g, 4.37mmol) in N,N-dimethylformamide (16ml), and the reaction stirred at room temperature for 2 hours. The mixture was diluted with ethyl acetate (100ml), washed with water (3x), and brine, then dried ($MgSO_4$) and evaporated under reduced pressure. The residual gum was purified by chromatography on silica gel using a Biotage® column, and an elution gradient of dichloromethane:methanol (98:2 to 95:5). The product was triturated with ether to afford the title compound as a white solid, 438mg, 44%; ^{1}H NMR ($DMSOd_6$, 400MHz) δ: 1.34 (s, 9H), 1.40 (m, 2H), 1.64 (m, 3H), 1.90 (m, 1H), 2.55 (m, 1H), 3.70 (m, 1H), 6.70 (bs, 1H), 6.80 (d, 1H), 7.22 (bs, 1H).

Preparation 11

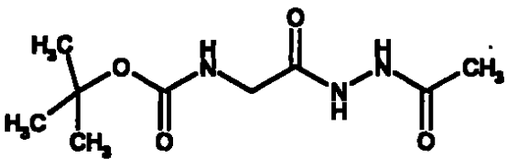
tert-Butyl 3-[(dimethylamino)carbonyl]cyclohexylcarbamate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.19g, 6.19mmol), 1-hydroxybenzotriazole hydrate (840mg, 6.19mmol), N-methylmorpholine (1.1ml, 10.1mmol) and finally 33% ethanolic dimethylamine (1.5ml) were added to a solution of the acid from preparation 9 (1.37g, 5.6mmol) in N,N-dimethylformamide (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure, the residue diluted with ethyl acetate and washed with water (2x). The mixture was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of methanol:dichloromethane (5:95 to 10:90), to give the title compound, 998mg, 66%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.12 (m, 1H), 1.40 (m, 11H), 1.70 (m, 2H), 1.85 (m, 1H), 2.00 (m, 2H), 2.62 (m, 1H), 2.96 (s, 3H), 3.05 (s, 3H), 3.50 (m, 1H), 4.50 (m, 1H).

Preparation 12

tert-Butyl 2-(2-acetylhydrazino)-2-oxoethylcarbamate

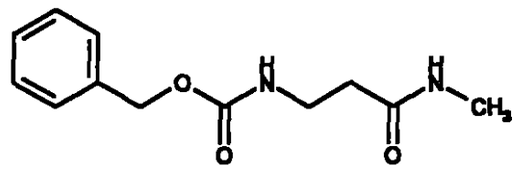


2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (7.06g, 28.5mmol) was added to a solution of N-*tert*-butoxycarbonylglycine (5.0g, 28.6mmol) in dichloromethane (75ml), and the solution stirred for 15 minutes. Acetic hydrazide (2.6g, 35.1mmol) was added, and the reaction stirred at room temperature for 18 hours. The resulting precipitate was filtered off, and dried *in vacuo*, to afford a white crystalline solid, 2.42g. The filtrate was concentrated under reduced pressure, diluted with ether, and the resulting precipitate filtered and dried *in vacuo*, to afford additional product as a white solid, 4.4g, 67% in total; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 2.02 (s, 3H), 3.87 (d, 2H), 5.22 (bs, 1H), 8.27 (bs, 1H), 8.84 (bs, 1H); LRMS : m/z 249.2 (MNH_4^+); Anal. Found: C, 46.41; H, 7.36; N, 17.98, $C_9H_{17}N_3O_4$ requires C, 46.66; H, 7.41; N, 18.13%.

358

Preparation 13

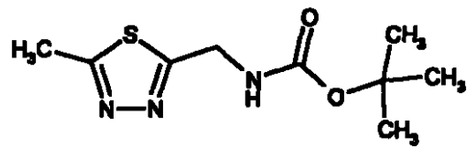
Benzyl 3-(methylamino)-3-oxopropylcarbamate



A mixture of N-[(benzyloxy)carbonyl]-β-alanine (10g, 44.8mmol), methylamine hydrochloride (3.33g, 49.28mmol), 1-hydroxybenzotriazole hydrate (6.05g, 44.8mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.3g, 53.76mmol) and N-methylmorpholine (11.33ml, 103mmol) in dichloromethane (200ml) was stirred at room temperature for 16 hours. The resulting precipitate was filtered off to give the desired product as a colourless foam, and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:hexane (90:10 to 100:0) to give additional product, 7.96g, 75% in total; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 2.42 (t, 2H), 2.80 (s, 3H), 3.50 (m, 2H), 5.21 (s, 2H), 5.49 (bs, 1H), 5.63 (bs, 1H), 7.36 (m, 5H); Anal. Found: C, 60.68; H, 7.00; N, 11.95. $C_{12}H_{16}N_2O_3$ requires C, 61.00; H, 6.83; N, 11.86%.

Preparation 14

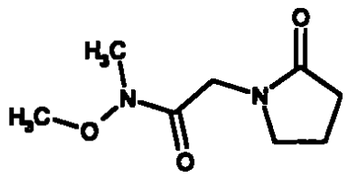
tert-Butyl (5-methyl-1,3,4-thiadiazol-2-yl)methylcarbamate



Lawesson's reagent (960mg, 2.38mmol) was added to a solution of the hydrazide from preparation 12 (500mg, 2.16mmol) in tetrahydrofuran (40ml), and the reaction heated under reflux for 3 hours, then stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 80:20) to give an oil. This was dissolved in ethyl acetate (100ml), charcoal (2g) added, the mixture stirred for 10 minutes then filtered. The filtrate was concentrated under reduced pressure, and the residue azeotroped with dichloromethane to afford the title compound as a crystalline solid, 441mg, 89%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.45 (s, 9H), 2.77 (s, 3H), 4.66 (d, 2H), 5.22 (bs, 1H); LRMS : m/z 230.1 (MH^+).

Preparation 15

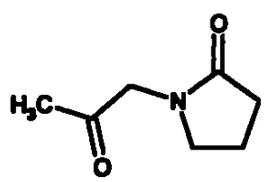
N-Methoxy-*N*-methyl-2-(2-oxo-1-pyrrolidinyl)acetamide



2-Chloro-N-methoxy-N-methylacetamide (3.2g, 23.3mmol) was added to a suspension of 2-pyrrolidinone (2.0g, 23.5mmol) and sodium hydride (940mg, 60% dispersion in mineral oil, 23.5mmol) in tetrahydrofuran (60ml), and the reaction stirred at room temperature for 48 hours. The mixture was quenched with water (150ml), and extracted with ethyl acetate (200ml) and dichloromethane (200ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was triturated with hexane, then ether to afford the title compound as white crystals, 1.8g, 41%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.02 (m, 2H), 2.40 (t, 2H), 3.17 (s, 3H), 3.48 (t, 2H), 3.72 (s, 3H), 4.19 (s, 2H); LRMS : m/z 186.9 (MH^+).

Preparation 16

1-(2-Oxopropyl)-2-pyrrolidinone

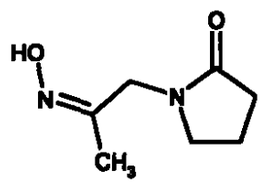


Methylmagnesium chloride (2.7ml, 3M in tetrahydrofuran, 8.1mmol) was added to a cooled (-20°C) solution of the amide from preparation 15 (1.5g, 8.1mmol) in tetrahydrofuran (50ml), and the reaction allowed to warm to room temperature, then stirred for an hour. The mixture was quenched by the addition of aqueous ammonium chloride solution, then extracted with ethyl acetate (3x50ml). The combined organic solutions were dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as an oil, 645mg, 56%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.07 (m, 2H), 2.17 (s, 3H), 2.42 (t, 2H), 3.42 (t, 2H), 4.10 (s, 2H).

359

Preparation 17

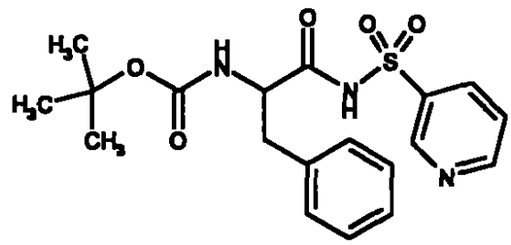
1-[2-(Hydroxyimino)propyl]-2-pyrrolidinone



Hydroxylamine hydrochloride (316mg, 4.55mmol) and then pyridine (370µl, 4.58mmol) were added to a solution of the amide from preparation 16 (643mg, 4.55mmol) in ethanol (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 90:10). The product was triturated with ether to give the title compound as a white solid, 375mg, 53%; ^{1}Hmr (DMSOd_6, 400MHz) δ: 1.60 (s, 3H), 1.87 (m, 2H), 2.20 (t, 2H), 3.19 (t, 2H), 3.78 (s, 2H), 10.77 (s, 1H); LRMS : m/z 157.4 (MH^+).

Preparation 18

tert-Butyl 1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethylcarbamate

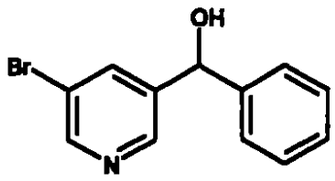


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (939mg, 4.9mmol), 1-hydroxybenzotriazole hydrate (562mg, 4.15mmol), and N-methylmorpholine (952mg, 9.42mmol) were added to an ice-cold solution of N-*tert*-butoxycarbonyl-L-phenylalanine (1.0g, 3.77mmol) in dichloromethane (20ml), and the mixture stirred for 15 minutes. 3-Pyridinesulphonamide (Mon. für Chemie; 72; 77; 1938) (596mg, 3.77mmol) was added, and the reaction stirred at room temperature for 24 hours. The mixture was evaporated under reduced pressure and the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The aqueous layer was extracted well with ethyl acetate, then dichloromethane, the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified twice by column chromatography on silica gel, using an elution gradient of ethyl acetate:ethanol (100:0 to 90:10) to give the desired product as a white foam, 1.01g, 66; ^{1}H NMR (DMSOd_6, 300MHz) δ: 1.30 (s, 9H), 2.77 (m, 1H), 2.97 (m, 1H),

3.84 (m, 1H), 5.95 (bs, 1H), 6.96 (m, 2H), 7.08 (m, 3H), 7.42 (m, 1H), 8.05 (d, 1H), 8.60 (d, 1H), 8.84 (m, 1H); $[\alpha]_D$ = -10° (0.1% solution in methanol).

Preparation 19

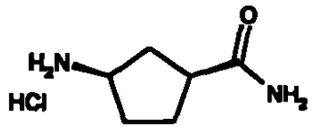
(5-Bromo-3-pyridinyl)(phenyl)methanol



n-Butyl lithium (17ml, 2.5M in hexanes, 42.5mmol) was added dropwise to cooled (-78 °C) solution of 3,5-dibromopyridine (10g, 42.2mmol) in ether (200ml), so as to maintain an internal temperature <-70°C. The mixture was then stirred for 15 minutes, and a solution of benzaldehyde (4.5g, 42.5mmol) in ether (20ml) was added dropwise, again maintaining the temperature <-70°C. The mixture was stirred for 15 minutes, then allowed to warm to room temperature over an hour. The reaction was quenched by the addition of 0.9M ammonium chloride solution (200ml), the layers separated, and the aqueous phase extracted with ether. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:ether (95:5 to 80:20) to give the title compound as a yellow oil, 7.6g, 68%; ^{1}H NMR (D_2O, 300MHz) δ: 5.80 (s, 1H), 7.37 (m, 5H), 7.90 (s, 1H), 8.40 (s, 1H), 8.44 (s, 1H).

Preparation 20

(1*S*,3*R*)-3-Aminocyclopentanecarboxamide hydrochloride

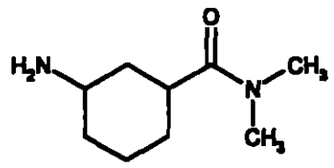


Hydrogen chloride gas was bubbled through an ice-cooled solution of the amide from preparation 10 (438mg, 1.92mmol) in dichloromethane (50ml) for 10 minutes, and the resulting suspension stirred at room temperature for 2 hours. The mixture was purged with nitrogen, then evaporated under reduced pressure. The residue was triturated with ether, to afford the title compound as a solid; ^{1}H NMR (D_2O, 400MHz) δ: 1.63-1.82 (m, 3H), 1.92-2.07 (m, 2H), 2.19 (m, 1H), 2.82 (m, 1H), 3.62 (m, 1H).

360

Preparation 21

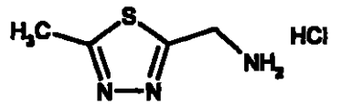
3-Amino-*N,N*-dimethylcyclohexanecarboxamide



A solution of the amide from preparation 11 (997mg, 3.69mmol) in trifluoroacetic acid (8ml) and dichloromethane (8ml) was stirred at room temperature for 4 hours. The mixture was concentrated under reduced pressure and the residue partitioned between dichloromethane (25ml) and sodium bicarbonate solution (25ml). The pH was adjusted to 9 using sodium hydroxide solution, the layers separated, and the aqueous phase evaporated under reduced pressure. The resulting solid was triturated with hot ethyl acetate, the suspension filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (84:14:2) to afford the title compound as a colourless oil, 346mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.08 (m, 1H), 1.25-1.54 (m, 6H), 1.72 (m, 1H), 1.86 (m, 2H), 2.53-2.75 (m, 2H), 2.96 (s, 3H), 3.03 (s, 3H);

Preparation 22

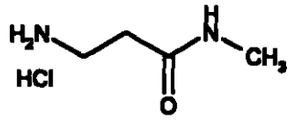
(5-Methyl-1,3,4-thiadiazol-2-yl)methylamine hydrochloride



Hydrogen chloride gas was bubbled through an ice-cooled solution of the thiadiazole from preparation 14 (425mg, 1.85mmol) in dichloromethane (50ml) for 15 minutes, and the reaction stirred at room temperature for 1 hour. The mixture was purged with nitrogen, then evaporated under reduced pressure to afford the title compound as a white solid; ^{1}H NMR ($DMSOd_6$, 400MHz) δ: 2.75 (s, 3H), 4.48 (m, 2H), 8.80 (bs, 3H).

Preparation 23

3-Amino-N-methylpropanamide hydrochloride

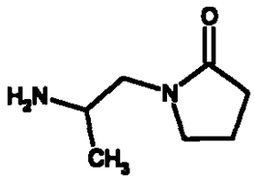


A mixture of the benzyl carbamate from preparation 13 (7.92g, 33.5mmol) and 5% palladium on charcoal (800mg) in ethanol (300ml) was hydrogenated at 50 psi and

room temperature for 4 hours. The reaction mixture was filtered through Arbocel , washing through with ethanol, and 1N hydrochloric acid (36.9ml, 36.9mmol) was added to the combined filtrate. This solution was evaporated under reduced pressure and the residue azeotroped with dichloromethane to afford the title compound as a colourless foam, 4.66g, ^{1}H NMR (DMSOd_6, 300MHz) δ: 2.46 (t, 2H), 2.60 (s, 3H), 2.95 (m, 2H), 7.98-8.16 (m, 2H).

Preparation 24

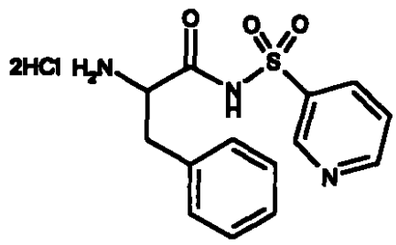
1-(2-Aminopropyl)-2-pyrrolidinone



A mixture of the oxime from preparation 17 (375mg, 2.40mmol) and platinum oxide (300mg) in ethanol (20ml) was hydrogenated at 60psi and room temperature for 18 hours. Tlc analysis showed starting material remaining, so additional platinum oxide (100mg) was added and the reaction continued for a further 4 hours. The mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:0.88 ammonia (95:5:0.5 to 90:10:1) to give the title compound as a clear oil, 170mg, 50%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.02 (d, 3H), 1.36 (bs, 2H), 2.00 (m, 2H), 2.38 (t, 2H), 3.00-3.16 (m, 2H), 3.21 (m, 1H), 3.35-3.45 (m, 2H); LRMS : m/z 143 (MH^+).

Preparation 25

N-(2-Amino-3-phenylpropanoyl)-3-pyridinesulphonamide dihydrochloride



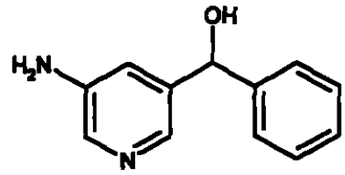
Saturated ethereal hydrochloric acid (40ml) was added to an ice-cold solution of the sulphonamide from preparation 18 (959mg, 2.37mmol) in ethyl acetate (30ml) and ether (10ml), and the solution stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with

361

dichloromethane (3x) to afford the title compound as a white solid, 959mg; ^{1}H NMR (DMSOd_6, 300MHz) δ: 3.23-3.50 (m, 1H), 3.70-3.98 (m, 1H), 4.13 (m, 1H), 7.05 (m, 2H), 7.20 (m, 3H), 7.78 (m, 1H), 8.36 (d, 1H), 8.44 (bs, 2H), 8.95 (d, 1H), 9.02 (s, 1H);[$\alpha]_D$ = +138° (0.5% solution in methanol).

Preparation 26

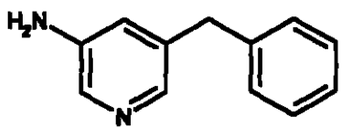
(5-Amino-3-pyridinyl)(phenyl)methanol



A mixture of the bromide from preparation 19 (2.0g, 7.60mmol) and copper (II) sulphate pentahydrate (350mg, 1.40mmol) in 0.88 ammonia (18ml) was heated at 135 °C in a sealed vessel for 24 hours. Sodium hydroxide solution (1N, 10ml) was added to the cooled solution, and the mixture was then extracted with ether (6x). The combined organic extracts were dried ($MgSO_4$), and concentrated under reduced pressure to a low volume. The resulting precipitate was filtered, washed with ether and dried to give the title compound as a solid, 1.25g, 83%; mp 92-94°C; ^{1}H NMR (DMSOd_6, 300MHz) δ: 5.22 (s, 2H), 5.59 (d, 1H), 5.86 (d, 1H), 6.83 (s, 1H), 7.20 (m, 1H), 7.34 (m, 4H), 7.78 (m, 2H).

Preparation 27

5-Benzyl-3-pyridinylamine

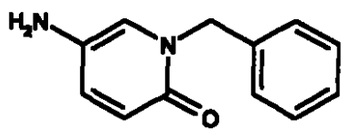


A mixture of the alcohol from preparation 26 (700mg, 3.5mmol) and 5% palladium on charcoal (70mg) in hydrochloric acid (5ml, 1N) and ethanol (20ml) was hydrogenated at 30 psi and room temperature for 6 hours. The mixture was filtered through Arbocel ®, and the filtrate concentrated under reduced pressure. The residue was basified using aqueous sodium bicarbonate solution, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (92:8:0.4) as eluant, to give the title compound as a solid, 500mg, 78%; mp 107-109°C; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 3.61

(bs, 2H), 3.94 (s, 2H), 6.78 (s, 1H), 7.24 (m, 5H), 7.98 (s, 2H).

Preparation 28

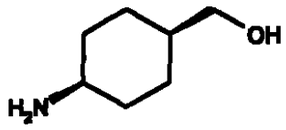
5-Amino-1-benzyl-2(1*H*)-pyridinone



A mixture of 1-benzyl-5-nitro-1H-pyridin-2-one (Justus Liebigs Ann. Chem. 484; 1930; 52) (1.0g, 4.35mmol), and granulated tin (3.5g, 29.5mmol) in concentrated hydrochloric acid (14ml) was heated at 90°C for 1.5 hours. The cooled solution was diluted with water, neutralised using sodium carbonate solution, and extracted with ethyl acetate (250ml in total). The combined organic extracts were filtered, dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as a pale green solid, (turned blue with time), 440mg, 51%; ^1H NMR ($CDCl_3$, 250MHz) δ: 4.12-4.47 (bs, 2H), 5.00 (s, 2H), 6.31 (d, 1H), 6.86 (s, 1H), 7.07 (m, 1H), 7.14-7.42 (m, 5H).

Preparation 29

Cis-(4-Aminocyclohexyl)methanol

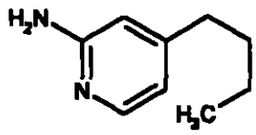


Lithium aluminium hydride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added dropwise to an ice-cooled solution of cis-4-aminocyclohexanecarboxylic acid (1.33g, 9.29mmol) in tetrahydrofuran (50ml), and once addition was complete, the reaction was heated under reflux for 6 hours. The resulting suspension was cooled to 5°C, and water (0.6ml), aqueous sodium hydroxide solution (1.1ml, 2M), then water (0.6ml) were added sequentially. The resulting suspension was filtered, and the filtrate evaporated under reduced pressure to give an oil, which was used without further purification; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.40-1.80 (m, 12H), 3.00 (m, 1H), 3.55 (d, 2H); LRMS : m/z 130.2 (MH^+).

362

Preparation 30

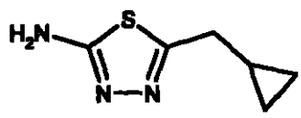
2-Amino-4-butylpyridine



A mixture of 4-butylpyridine (5.0g, 37.0mmol) and 95% sodium amide (1.7g, 40.7mmol) in xylene (10ml) was heated at 150°C for 18 hours. The cooled mixture was diluted with ether (100ml) and extracted with 2N hydrochloric acid (twice). The aqueous extracts were basified using sodium hydroxide solution, and re-extracted with ether. These combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (97:3:0.15) as eluant, to afford the title compound as a crystalline solid, 2.1g, 38%; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.38 (m, 2H), 1.60 (m, 2H), 2.52 (t, 2H), 4.38 (bs, 2H), 6.38 (s, 1H), 6.55 (d, 1H), 7.98 (d, 1H); Anal. Found: C, 72.01; H, 9.47; N, 18.53. $C_9H_{14}N_2$ requires C, 71.96; H, 9.39; N, 18.65%.

Preparation 31

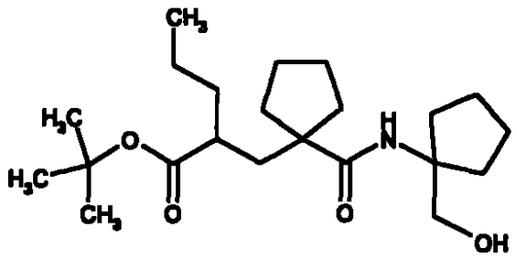
5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-amine



Oxalyl chloride (3.13ml, 35.9mmol) and N,N-dimethylformamide (1 drop) were added to a solution of cyclopropylacetic acid (3g, 29.9mmol) in dichloromethane (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure and azeotroped with dichloromethane to give a brown oil. A mixture of this intermediate acid chloride (887mg, 7.48mmol) and thiosemicarbazide (455mg, 4.99mmol) were heated at 70°C for 18 hours, then cooled. Water was added, the mixture basified to pH 9 using 50% aqueous sodium hydroxide solution, and the resulting precipitate filtered and dried, to give a cream solid, 410mg, 53%; ^1H NMR (CD_3OD, 400MHz) δ: 0.28 (m, 2H), 0.60 (m, 2H), 1.02 (m, 1H), 2.77 (d, 2H); LRMS : m/z 155.2 (MH^+).

Preparation 33

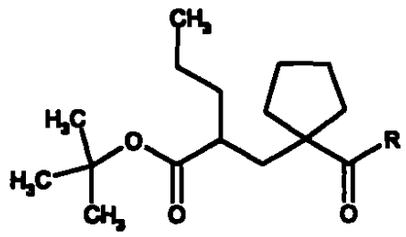
tert-Butyl 2-{[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (41mg, 0.21mmol), 1-hydroxybenzotriazole hydrate (27mg, 0.2mmol), N-methylmorpholine (35µl, 0.31mmol) and finally 1-amino-1-cyclopentanemethanol (25mg, 0.22mmol) were added to a solution of the acid from preparation 1 (150mg, 0.53mmol) in N,N-dimethylformamide (3ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (3x25ml), and brine (25ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using ethyl acetate:pentane (30:70) as the eluant to afford the title compound, 38mg, 57%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.29 (m, 3H), 1.41-1.78 (m, 26H), 1.78-1.98 (m, 4H), 2.04 (m, 1H), 2.26 (m, 1H), 3.59 (dd, 1H), 3.70 (dd, 1H), 4.80 (t, 1H), 5.81 (s, 1H); LRMS : m/z 380 (MH^+).

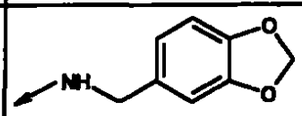
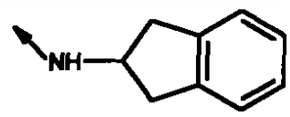
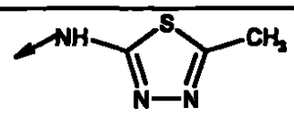
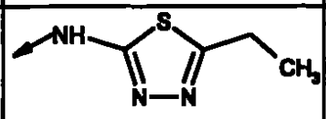
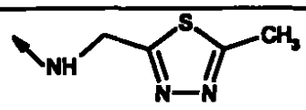
Preparations 34 to 43

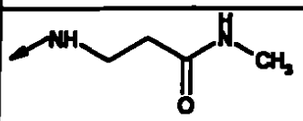
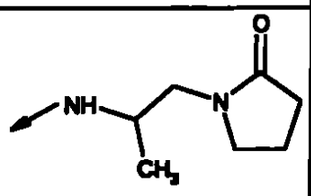
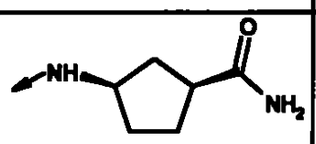
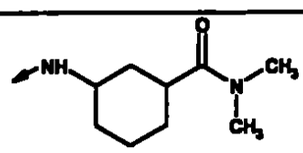
The following compounds of general structure:



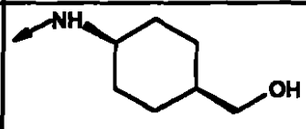
were prepared from the acid from preparation 1 and the appropriate amine compound, following a similar procedure to that described in preparation 33.

363

Prep	R	Starting amine	Yield (%)	Data
34		Piperonylamine	88	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.26 (m, 4H), 1.42 (s, 9H), 1.46 (m, 2H), 1.59-1.75 (m, 5H), 1.95 (m, 2H), 2.06 (m, 1H), 2.22 (m, 1H), 4.26 (dd, 1H), 4.39 (dd, 1H), 5.95 (m, 3H), 6.78 (m, 3H). LRMS : m/z 418.3 (MH^+)
35[1]		2-Aminoindan hydrochloride	40	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.25 (m, 3H), 4H), 1.42 (m, 12H), 1.56-1.70 (m, 4H), 1.90 (m, 2H), 2.02 (m, 1H), 2.22 (m, 1H), 2.80 (m, 2H), 3.35 (m, 2H), 4.76 (m, 1H), 5.86 (d, 1H), 7.19 (m, 4H). LRMS : m/z 400.3 (MH^+)
36[2]		2-Amino-5-methyl-1,3,4-thiadiazole	76	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.20-1.85 (m, 20H), 2.18 (m, 4H), 2.67 (s, 3H), 9.80 (bs, 1H). LRMS : m/z 382.3 (MH^+)
37[2]		2-Amino-5-ethyl-1,3,4-thiadiazole	92	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.20-1.80 (m, 22H), 1.84 (m, 1H), 2.20 (m, 4H), 3.04 (q, 2H), 9.10 (bs, 1H). LRMS : m/z 396.2 (MH^+)
38		Preparation 22	77	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 4H), 1.42 (s, 9H), 1.44-1.76 (m, 7H), 1.95-2.12 (m, 3H), 2.20 (m, 1H), 2.76 (s, 3H), 4.74 (dd, 1H), 4.82 (dd, 1H), 6.54 (bs, 1H). LRMS : m/z 396.2 (MH^+)

Prep	R	Starting amine	Yield (%)	Data
39[1,2]		Preparation 23	60	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.21-1.38 (m, 3H), 1.40-1.70 (m, 17H), 1.88-2.04 (m, 3H), 2.20 (m, 1H), 2.39 (t, 2H), 2.80 (d, 3H), 3.53 (m, 2H), 6.13 (bs, 1H), 6.40 (m, 1H). LRMS : m/z 369.5 (MH^+)
40[2]		Preparation 24	70	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (m, 3H), 1.16 (2xd, 3H), 1.20-1.72 (m, 21H), 1.83 (m, 1H), 1.98 (m, 3H), 2.17 (m, 1H), 2.38 (m, 2H), 1.96 (m, 1H), 3.34 (m, 1H), 3.54-3.62 (m, 2H), 4.15-4.20 (m, 1H), 6.21-6.35 (2xbd, 1H). LRMS : m/z 409.3 (MH^+).
41[2]		Preparation 20	94	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.19-1.38 (m, 4H), 1.42 (m, 12H), 1.60 (m, 3H), 1.74-2.02 (m, 10H), 2.18 (m, 1H), 2.78 (m, 1H), 4.38 (m, 1H), 5.32 (bs, 1H), 5.57 (bs, 1H), 7.28 (bs, 1H). LRMS : m/z 395 (MH^+)
42[2]		Preparation 21	91	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (t, 3H), 1.18-1.78 (m, 25H), 1.84-2.03 (m, 6H), 2.22 (m, 1H), 2.68 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.84 (m, 1H), 5.76 (m, 1H). LRMS : m/z 437.7 (MH^+)

364

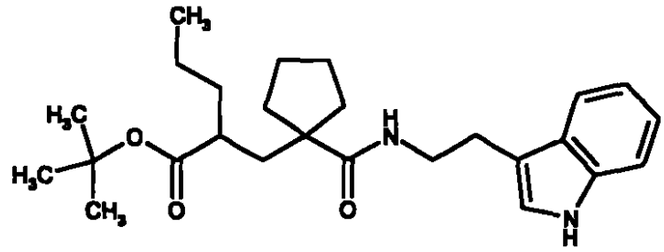
Prep	R	Starting amine	Yield (%)	Data
43[2]		Preparation 29	99	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.20-1.79 (m, 30H), 1.90 (m, 2H), 2.05 (m, 1H), 2.24 (m, 1H), 3.56 (m, 2H), 4.04 (m, 1H), 5.82 (bd, 1H). LRMS : m/z 396.4 (MH^+)

1 = reaction conducted at room temperature

2 = Methanol:dichloromethane was used as the column eluant

Preparation 44

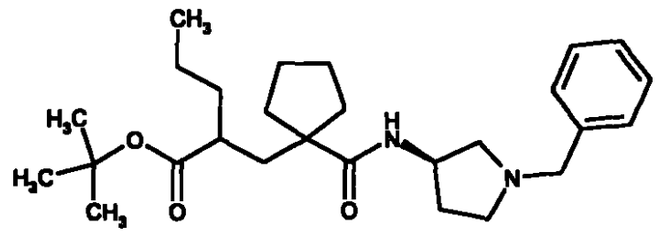
tert-Butyl 2-{[1-({[2-(1*H*-indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoate



The title compound was obtained as a pale yellow oil in 80% yield from the acid from preparation 1 and tryptamine, following a similar procedure to that described in preparation 33, except the reaction was performed in dichloromethane at room temperature; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.86 (t, 3H), 1.26 (m, 3H), 1.42 (m, 11H), 1.50-1.69 (m, 6H), 1.83 (m, 1H), 1.90-2.05 (m, 2H), 2.22 (m, 1H), 2.99 (t, 3H), 3.60 (m, 2H), 5.78 (m, 1H), 7.06 (s, 1H), 7.14 (m, 1H), 7.20 (m, 1H), 7.38 (d, 1H), 7.63 (d, 1H), 8.02 (bs, 1H); LRMS : m/z 427.5 (MH^+).

Preparation 45

tert-Butyl 2-{(1-{[(3*S*)-1-benzylpyrrolidinyl]amino}cyclopentyl)methyl]pentanoate

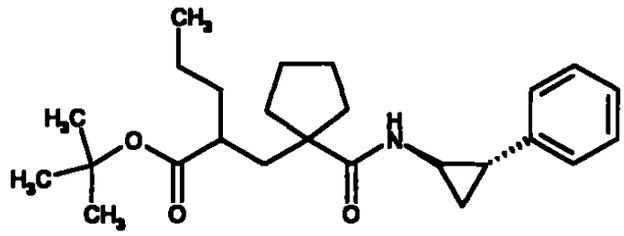


The title compound was obtained quantitatively from the acid from preparation 1 and (3S)-1-benzyl-3-aminopyrrolidine, following a similar procedure to that described in

preparation 44; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.10-1.76 (m, 21H), 1.90-2.05 (m, 3H), 2.20-2.38 (m, 3H), 2.58 (m, 2H), 2.84 (m, 1H), 3.62 (s, 2H), 4.45 (m, 1H), 6.02 (m, 1H), 7.33 (m, 5H).

Preparation 46

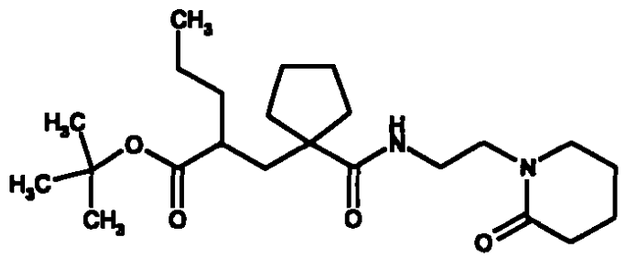
tert-Butyl 2-{[1-({[cis-2-phenylcyclopropyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (81mg, 0.42mmol), N-methylmorpholine (0.15ml, 1.06mmol) and tranylcypromine hydrochloride (60mg, 0.35mmol) were added to a solution of the acid from preparation 1 (100mg, 0.35mmol) in dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The reaction mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 95:5) to afford the title compound as a yellow oil, 85mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.16 (m, 1H), 1.20-1.58 (m, 16H), 1.63 (m, 5H), 1.90-2.14 (m, 4H), 2.23 (m, 1H), 2.90 (m, 1H), 6.00 (m, 1H), 7.19 (m, 3H), 7.24 (m, 2H); LRMS : m/z 400 (MH^+).

Preparation 47

tert-Butyl 2-{[1-({[2-(2-oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]-methyl}pentanoate



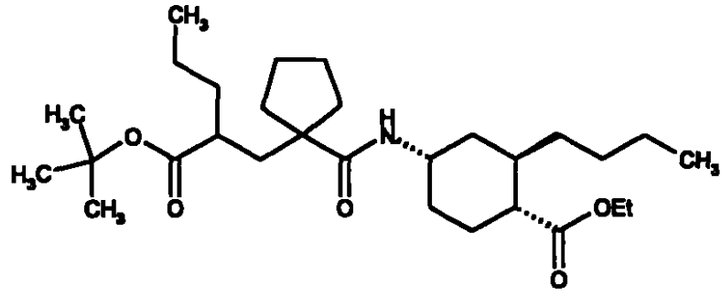
Hydrazine monohydrate (34μl, 0.70mmol) was added to a solution of the compound from preparation 6 (171mg, 0.63mmol) in ethanol (10ml), and the reaction heated under reflux for 5 hours. The cooled mixture was filtered, the filtrate concentrated under reduced pressure, the residue suspended in dichloromethane, and the

365

suspension re-filtered. The resulting filtrate was concentrated under reduced pressure, and the residue purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (90:10:1) as eluant to give the amine, 16mg. The acid from preparation 1 (32mg, 0.11mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (25mg, 0.13mmol), 1-hydroxybenzotriazole hydrate (17mg, 0.13mmol), and N-methylmorpholine (25µl, 0.23mmol) were added to a solution of this amine in N,N-dimethylformamide (2ml), and the reaction stirred at room temperature for 18 hours. The mixture was partitioned between ethyl acetate and water, and the layers separated. The organc phase was washed with water (2x), dried ($MgSO_4$), and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98.5:1.5 to 95:5) to afford the title compound as an oil, 43mg, 17%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.22 (m, 3H), 1.38-1.65 (m, 17H), 1.58 (m, 4H), 1.95 (m, 3H), 2.17 (m, 1H), 2.37 (m, 2H), 3.30 (m, 2H), 3.38 (m, 2H), 3.50 (m, 2H), 6.76 (m, 1H); LRMS : m/z 409.2 (MH^+)

Preparation 48

Ethyl (1*R*,2*R*,4*S*)-4-[({1-[2-(*tert*-butoxycarbonyl)pentyl]cyclopentyl}carbonyl)amino]-2-butylcyclohexanecarboxylate

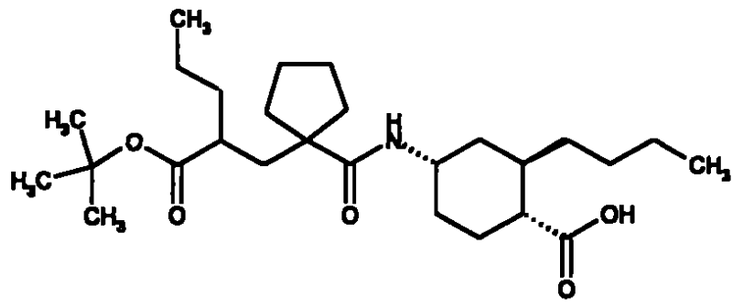


A mixture of the acid from preparation 1 (109mg, 0.38mmol), (1*R*,2*R*,4*S*)-4-amino-2-butyl-cyclohexanecarboxylic acid ethyl ester hydrochoride (WO, 9009374), (101mg, 0.38mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (95mg, 0.50mmol), 1-hydroxybenzotriazole hydrate (60mg, 0.40mmol) and triethylamine (0.12ml, 0.87mmol) in dichloromethane (3ml), was stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue treated with sodium bicarbonate solution and extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as eluant, and azeotroped with dichloromethane to afford the

title compound, 190mg; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (m, 6H), 1.20-1.40 (m, 13H), 1.40-2.10 (m, 25H), 2.16-2.30 (m, 2H), 4.18 (m, 3H), 5.83 (d, 1H).

Preparation 49

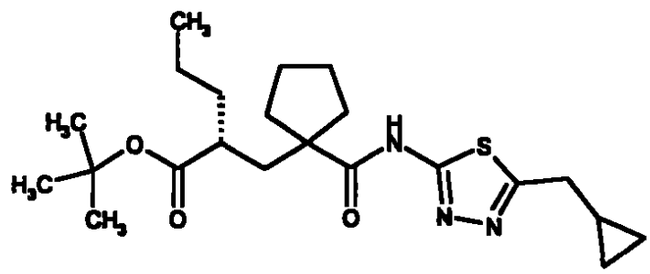
(1*R*, 2*R*,4*S*)-4-[({1-[2-(*tert*-Butoxycarbonyl)pentyl]cyclopentyl}carbonyl)amino]-2-butylcyclohexanecarboxylic acid



A mixture of the ethyl ester from preparation 48 (190mg, 0.39mmol) and 1N sodium hydroxide solution (0.85ml, 0.85mmol) in methanol (1.5ml) was stirred at room temperature for 22 hours. The reaction mixture was acidifed to pH 1 using hydrochloric acid (2N), then partitioned between ethyl acetate and water. The layers were separated, and the organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to afford the title compound, 130mg, 72%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (m, 6H), 1.20-2.12 (m, 36H), 2.24 (m, 2H), 4.18 (m, 1H), 5.82 (d, 1H); LRMS : m/z 464 $(M-H)^-$

Preparation 50

tert-Butyl (2*R*)-2-{[1-({[5-(cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]methyl}pentanoate



The title compound was prepared from the acid from preparation 2 and the amine from preparation 31, in 65% yield, following the procedure described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.35 (m, 2H), 0.63 (m, 2H), 0.80 (m, 3H), 1.10 (m, 1H), 1.20-1.94 (m, 20H), 2.19 (m, 4H), 2.93 (t, 2H), 3.50 (s, 1H); LRMS : m/z 422.4 (MH^+)

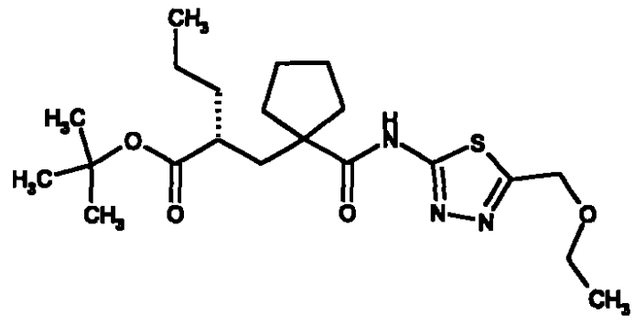
366

$[\alpha]_D$ = -14.15° (c = 0.082, methanol).

372

Preparation 51

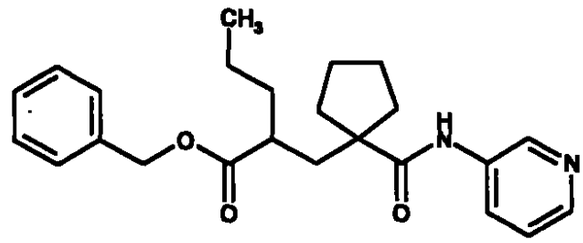
tert-Butyl (2*R*)-2-{[1-({[5-(ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



The title compound was prepared from the acid from preparation 2 and 5-(ethoxymethyl)-1,3,4-thiadiazol-2-amine, in 51% yield, following the procedure described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.10-1.78 (m, 25H), 1.82 (m, 1H), 2.19 (m, 5H), 3.48 (s, 1H), 4.82 (s, 2H), 10.16 (brs, 1H); LRMS : m/z 426.4 (MH^+); $[\alpha]_D$ = -12.50° (c = 0.08, methanol).

Preparation 52

Benzyl 2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoate

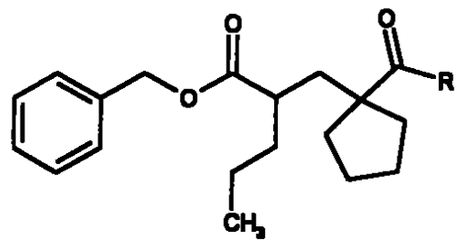


Triethylamine (0.11ml, 0.78mmol) was added to a mixture of the acid chloride from preparation 3 (200mg, 0.60mmol) and 2-aminopyridine (61mg, 0.65mmol) in dichloromethane (3ml), and the reaction stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue partitioned between sodium bicarbonate solution (5ml) and ethyl acetate (20ml), and the layers separated. The organic phase was dried ($MgSO_4$), and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant, to afford the title compound, 130mg; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.21 (m, 3H), 1.40 (m, 1H), 1.43-1.72 (m, 6H), 1.81 (d, 1H), 1.98 (*m*, 1H), 2.18 (*m*, 1H), 2.24 (*m*, 1H), 2.46 (*m*, 1H), 4.98 (*m*, 2H), 7.20-7.38 (*m*,

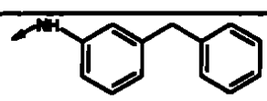
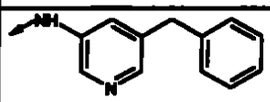
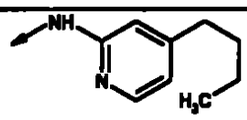
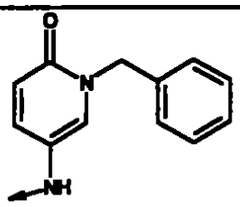
6H), 7.42 (s, 1H), 8.06 (d, 1H), 8.35 (d, 1H), 8.56 (s, 1H).

Preparations 53 to 56

The following compounds of general formula:



were prepared from the acid chloride from preparation 3 and the appropriate amine, following a similar procedure to that described in preparation 52.

Prep	R	Yield (%)	Data
53[1]		90	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.24 (m, 2H), 1.40-1.76 (m, 7H), 1.84 (dd, 1H), 1.98 (m, 1H), 2.19 (dd, 1H), 2.28 (m, 1H), 2.56 (m, 1H), 3.98 (s, 2H), 4.99 (dd, 2H), 6.98 (d, 1H), 7.19-7.42 (m, 15H).
54		65	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.24 (m, 3H), 1.39-1.78 (m, 6H), 1.82 (dd, 1H), 1.98 (m, 2H), 2.20 (dd, 1H), 2.25 (m, 1H), 2.50 (m, 1H), 3.98 (s, 2H), 4.98 (dd, 2H), 7.18-7.40 (m, 10H), 7.45 (s, 1H), 7.98 (s, 1H), 8.23 (s, 1H), 8.42 (s, 1H).
55		30	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 0.92 (t, 3H), 1.21 (m, 2H), 1.30-1.70 (m, 12H), 1.82 (dd, 1H), 2.04 (m, 1H), 2.20 (m, 2H), 2.50 (m, 1H), 2.58 (t, 2H), 4.98 (dd, 2H), 6.83 (d, 1H), 7.30 (m, 5H), 7.90 (s, 1H), 8.08 (s, 1H), 8.15 (d, 1H).
56[2]		53	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.25 (m, 2H), 1.27-1.99 (m, 10H), 2.07-2.30 (m, 2H), 2.47 (m, 1H), 4.99 (s, 2H), 5.10 (dd, 2H), 6.59 (d, 1H), 7.15 (d, 1H), 7.34 (m, 11H), 8.10 (s, 1H).

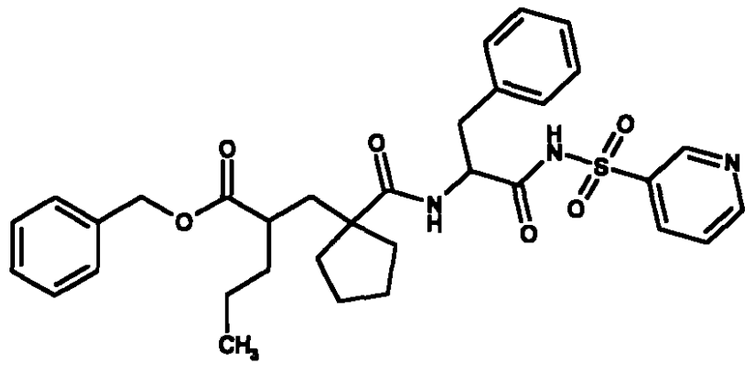
1 = dichloromethane used as the column eluant

2 = N-methylmorpholine was used as the base

367

Preparation 57

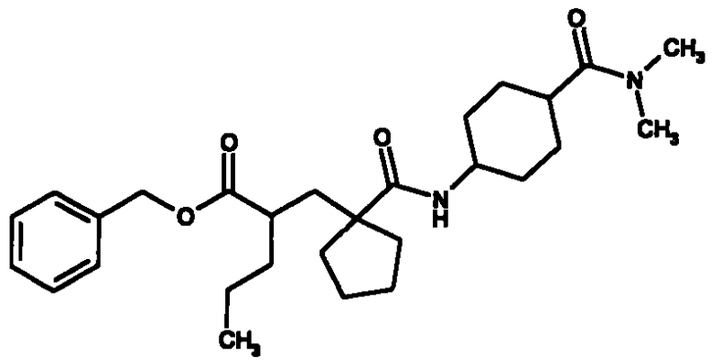
Benzyl 2-({1-[({1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoate



The amine hydrochloride from preparation 25 (828mg, 2.19mmol) and N-methylmorpholine (2.21g, 21.9mmol) was added to an ice-cold solution of the acid chloride from preparation 3 (737mg, 2.19mmol) in dichloromethane (50ml), and the reaction stirred at room temperature for 24 hours. The reaction mixture was evaporated under reduced pressure, the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The organic phase was washed with brine (25ml), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:methanol (100:0 to 95:5) to give the title compound as a cream foam, 975mg, 73%; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.72 (m, 3H), 0.94-2.20 (m, 17H), 2.84 (m, 1H), 3.00 (m, 1H), 4.18 (m, 1H), 5.00 (m, 2H), 6.95 (m, 2H), 7.02 (m, 3H), 7.38 (m, 6H), 8.06 (m, 1H), 8.60 (m, 1H), 8.87 (s, 1H).

Preparation 58

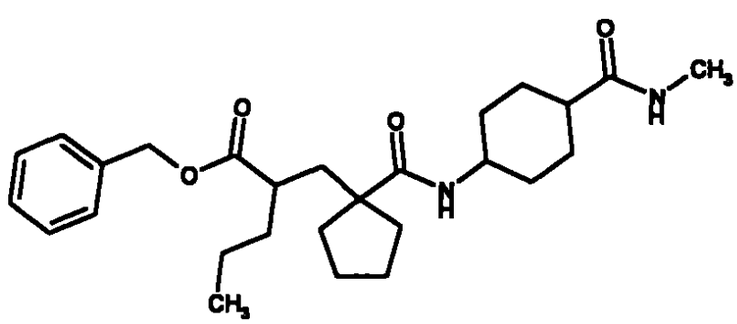
Cis-Benzyl 2-({1-[({4-[(dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate



A mixture of cis-4-[[(1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentyl)carbonyl]amino}-cyclohexanecarboxylic acid (EP 274234) (200mg, 0.45mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (112mg, 0.58mmol), 1-hydroxybenzotriazole hydrate (70mg, 0.46mmol) and dimethylamine (0.56ml, 2M in tetrahydrofuran, 1.12mmol) in dichloromethane (5ml) was stirred at room temperature for 16 hours. The mixture was concentrated under reduced pressure and the residue partitioned between sodium bicarbonate solution and ethyl acetate, and the layers separated. The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant to afford the title compound, 150mg; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.22 (m, 3H), 1.32-1.88 (m, H), 2.00 (m, 4H), 2.40 (m, 1H), 2.60 (m, 1H), 2.97 (s, 3H), 3.04 (s, 3H), 4.04 (m, 1H), 5.12 (s, 2H), 5.80 (bd, 1H), 7.37 (m, 5H).

Preparation 59

Cis-Benzyl 2-({1-[({4-[(methylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate



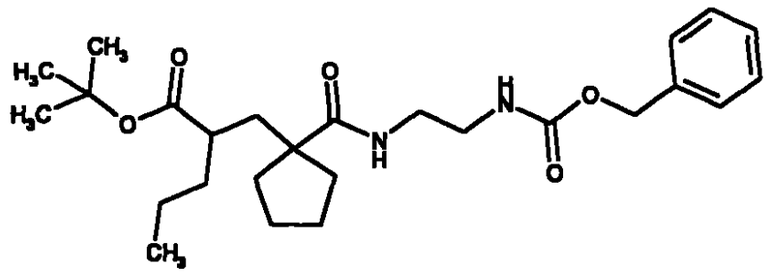
The title compound was prepared in 49% yield from cis-4-[[(1-{2-

368

[(benzyloxy)carbonyl]pentyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylic acid (EP 274234) and methylamine (2M in tetrahydrofuran), following the procedure described in preparation 58; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.17-2.12 (m, 22H), 2.21 (m, 1H), 2.41 (m, 1H), 2.80 (d, 3H), 4.00 (m, 1H), 5.12 (s, 2H), 5.61 (m, 1H), 5.79 (d, 1H), 7.38 (m, 5H).

Preparation 60

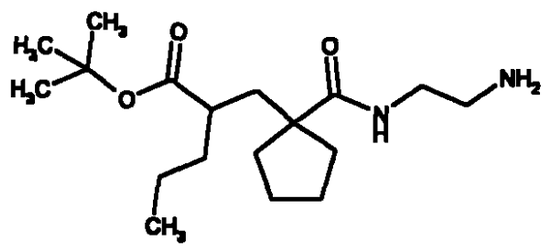
tert-Butyl 2-[(1-[[(2-[[(benzyloxy)carbonyl]amino]ethyl)amino]carbonyl]-cyclopentyl)methyl]pentanoate



The title compound was obtained as a yellow oil in 55% yield, from the acid from preparation 1 and N-benzyloxycarbonyl-1,2-diaminoethane, following a similar procedure to that described in preparation 44; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.74 (m, 17H), 1.90 (m, 2H), 2.04 (m, 1H), 2.20 (m, 1H), 3.32 (m, 3H), 3.44 (m, 1H), 5.10 (s, 2H), 5.61 (m, 1H), 6.20 (m, 1H), 7.38 (m, 5H).

Preparation 61

tert-Butyl 2-[(1-[[(2-aminoethyl)amino]carbonyl}cyclopentyl)methyl]pentanoate

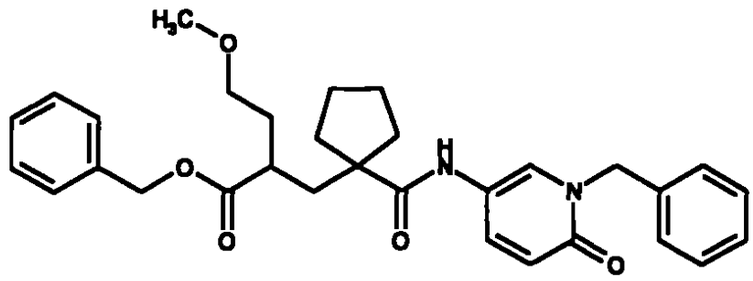


A mixture of the carbamate from preparation 60 (1.43g, 3.10mmol) and 10% palladium on charcoal (200mg) in ethanol (8ml) was hydrogenated at room temperature and 1 atm for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to afford the title compound, 920mg, 92%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.54 (m, 12H), 1.61 (m, 5H), 1.92-2.12 (m, 3H), 2.20 (m, 1H), 2.98 (m, 2H), 3.38 (m, 1H), 3.42 (m, 1H), 3.97

(m, 2H), 6.65 (m, 1H); LRMS : m/z 326.8 (M^+).

Preparation 62

Benzyl 2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]cyclopentyl)-methyl]-4-methoxybutanoate

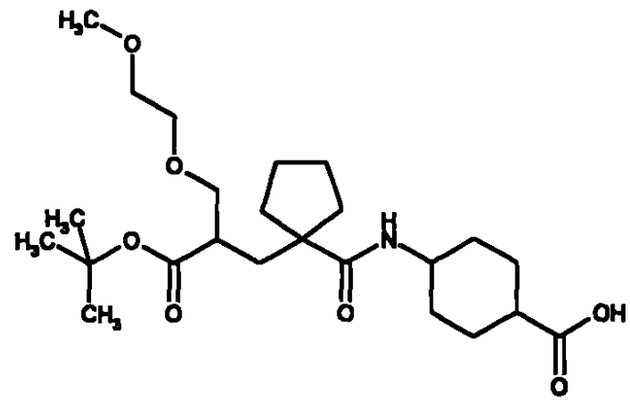


Oxalyl chloride (0.26ml, 3.0mmol) was added to an ice-cooled solution of 1-[2-[(benzyloxy)carbonyl]-4-methoxybutyl]cyclopentanecarboxylic acid (EP 274234) (1.0g, 3.0mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (20ml), and the reaction stirred at room temperature for 2 hours. The solution was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x10ml). The product was dissolved in dichloromethane (20ml), then cooled in an ice-bath. The amine from preparation 28 (600mg, 3mmol) and N-methylmorpholine (0.6ml, 5.45mmol) were added and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and partitioned between water and ether. The organic layer was washed with hydrochloric acid (2N), sodium bicarbonate solution, then water, dried ($MgSO_4$) and evaporated under reduced pressure. The residual green solid was purified by medium pressure column chromatography on silica gel using ethyl acetate:hexane (90:10) as eluant to afford the title compound, 880mg, 57%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.37-2.28 (m, 12H), 2.46-2.64 (m, 1H), 3.20 (s, 3H), 3.31 (m, 2H), 4.97 (dd, 2H), 5.08 (dd, 2H), 6.57 (d, 1H), 7.12 (m, 1H), 7.18-7.48 (m, 10H), 8.08 (d, 1H).

369

Preparation 63

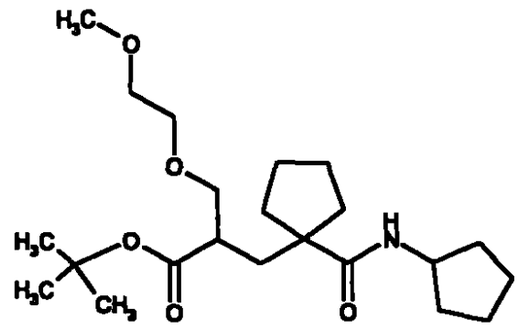
4-[[(1-{3-*tert*-Butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)-carbonyl]amino}cyclohexanecarboxylic acid



A mixture of benzyl 4-[[(1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylate (EP 274234), and 10% palladium on charcoal (250mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 18 hours. The reaction mixture was filtered through Solkafloc®, the filtrate concentrated under reduced pressure and the residue azeotroped with toluene (3x) and then dichloromethane (3x), to give the title compound, 2.0g, 96%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.48 (s, 9H), 1.53-1.84 (m, 14H), 1.94-2.10 (m, 5H), 2.60 (m, 2H), 3.40 (s, 3H), 3.41-3.63 (m, 5H), 3.96 (m, 1H), 5.90 (bd, 1H).

Preparation 64

tert-Butyl 3-{1-[(cyclopentylamino)carbonyl]cyclopentyl}-2-[(2-methoxyethoxy)methyl]-propanoate

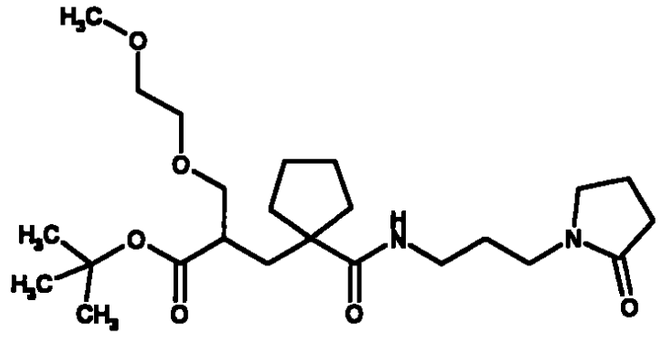


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (197mg, 1.07mmol), 1-hydroxybenzotriazole hydrate (139mg, 1.07mmol), N-methylmorpholine (0.18ml, 1.64mmol) and cyclopentylamine (101µl, 1.07mmol) were added to a solution of 1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}-cyclopentanecarboxylic acid (EP

274234) (400mg, 1.07mmol) in dichloromethane (5ml), and the reaction stirred at room temperature for 22 hours. The reaction was quenched by the addition of water, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to afford the title compound as a clear oil, 320mg, 78%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.22-2.02 (m, 27H), 2.58 (m, 1H), 3.36 (s, 3H), 3.40 (m, 1H), 3.46 (m, 2H), 3.57 (m, 3H), 4.10-4.20 (m, 1H), 5.80 (bs, 1H).

Preparation 65

tert-Butyl 3-(2-methoxyethoxy)-2-[[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]methyl]propanoate

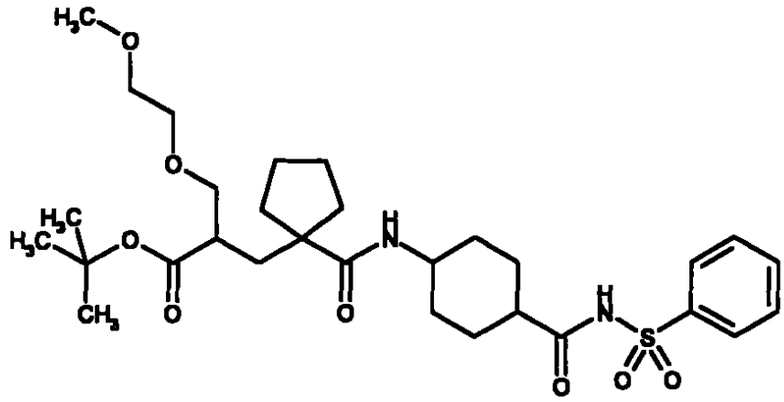


The title compound was obtained as a clear oil in 97% yield from 1-[3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl]-cyclopentanecarboxylic acid (EP 274234) and 1-(3-aminopropyl)-2-pyrrolidinone, following a similar procedure to that described in preparation 64, except dichloromethane:methanol (95:5) was used as the column eluant, ^1H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.50 (m, 2H), 1.60-1.70 (m, 7H), 1.78 (m, 1H), 1.90 (m, 1H), 2.20 (m, 4H), 2.40 (m, 2H), 2.58 (m, 1H), 3.14 (m, 1H), 3.20 (m, 1H), 3.38 (m, 6H), 3.42-3.60 (m, 6H), 7.00 (m, 1H).

370

Preparation 66

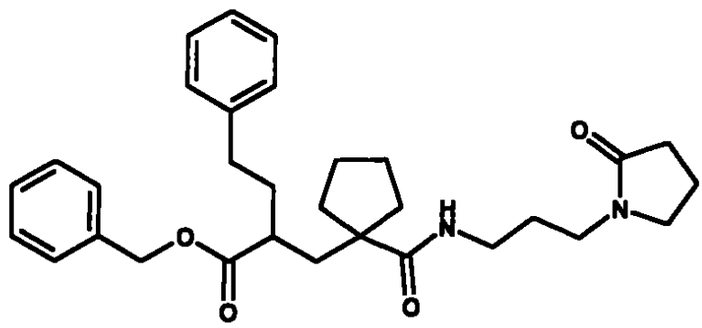
Cis-*tert*-Butyl 3-(2-methoxyethoxy)-2-[(1-[[(4-[[(phenylsulfonyl)amino]carbonyl]-cyclohexyl)amino]carbonyl]cyclopentyl)methyl]propanoate



N,N'-Dicyclohexylcarbodiimide (199mg, 0.97mmol), 4-dimethylaminopyridine (118mg, 0.97mmol) and benzenesulphonamide (152mg, 0.97mmol) were added to an ice-cooled solution of the acid from preparation 63 (400mg, 0.878mmol) in dichloromethane (12ml) and N,N-dimethylformamide (0.5ml), and the reaction stirred at room temperature for 20 hours. The mixture was concentrated under reduced pressure and the residue suspended in cold ethyl acetate. The resulting insoluble material was filtered off, the filtrate washed with hydrochloric acid (1N), and water, then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound as a white foam, 480mg, 92%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.44 (s, 9H), 1.63 (m, 13H), 1.80 (m, 2H), 1.88 (m, 1H), 1.98 (m, 2H), 2.36 (m, 1H), 2.57 (m, 1H), 3.38 (s, 3H), 3.40 (m, 1H), 3.51 (t, 2H), 3.58 (m, 3H), 3.95 (m, 1H), 5.92 (d, 1H), 7.56 (m, 2H), 7.62 (m, 1H), 8.05 (d, 2H), 8.75 (bs, 1H); LRMS : m/z 618 (MNa^+).

Preparation 67

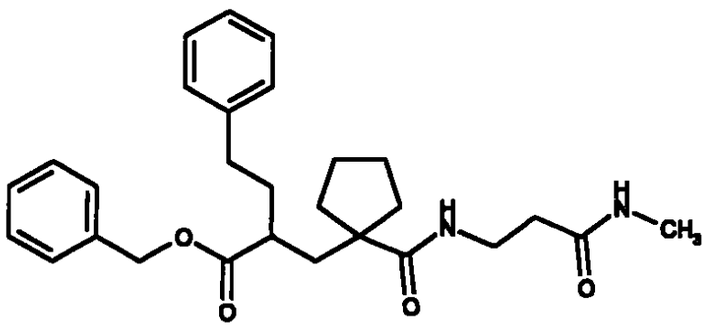
Benzyl 2-{[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.06g, 5.53mmol), 1-hydroxybenzotriazole hydrate (0.60g, 4.44mmol) and 4-methylmorpholine (0.56g, 5.54mmol) were added sequentially to a cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (1.5g, 3.94mmol) in dry dichloromethane (15ml) at room temperature, followed by N-(3-aminopropyl)-2-pyrrolidinone (0.56g, 3.94mmol), and the reaction stirred at room temperature for 18 hours. The mixture was washed with water, 2N hydrochloric acid, saturated aqueous sodium bicarbonate solution, and then dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as the eluant to provide the title compound as a clear gum, 800mg, 40%; ^1H NMR ($CDCl_3$, 300MHz) d : 1.37-2.20 (m, 16H), 2.34-2.58 (m, 5H), 2.92-3.46 (m, 6H), 5.07 (d, 1H), 5.18 (d, 1H), 6.98-7.47 (m, 10H).

Preparation 68

Benzyl 2-{[1-({[3-(methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoate

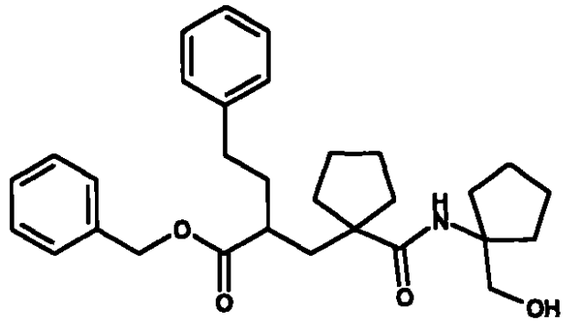


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (122mg, 0.64mmol), 1-hydroxybenzotriazole hydrate (86mg, 0.64mmol) and 4-methylmorpholine (173µl,

1.59mmol) were added sequentially to a cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (202mg, 0.53mmol) in N,N-dimethylformamide (5ml) at room temperature, followed by the amine hydrochloride from preparation 23 (146mg, 1.06mmol), and the reaction stirred at 90°C for 18 hours. The cooled solution was concentrated under reduced pressure and the residue partitioned between water (20ml) and ethyl acetate (100ml). The layers were separated, the organic phase washed with water (3x30ml), brine (25ml) dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (98:2) as eluant to afford the title compound as a colourless oil, 162mg, 67%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.38-1.53 (m, 2H), 1.53-1.96 (m, 8H), 2.02 (m, 2H), 2.27 (t, 2H), 2.46 (m, 3H), 2.76 (d, 3H), 3.44 (m, 2H), 5.13 (s, 2H), 5.79 (bs, 1H), 6.38 (m, 1H), 7.06 (d, 2H), 7.18 (m, 1H), 7.22 (m, 2H), 7.38 (m, 5H); LRMS : m/z 465.5 (MH^+).

Preparation 69

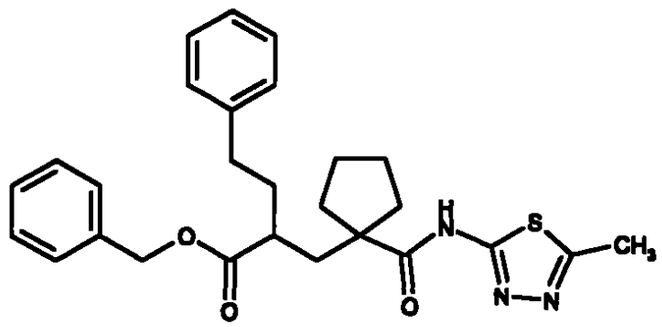
Benzyl 2-{[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoate



The title compound was obtained as a crystalline solid (48%) from 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) and 1-amino-1-cyclopentanemethanol, following a similar procedure to that described in preparation 68, except the reaction mixture was stirred at room temperature for 18 hours, and the crude product purified by column chromatography on silica gel using ethyl acetate:pentane as eluant; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.38 (m, 2H), 1.50-1.95 (m, 16H), 2.01 (m, 2H), 2.45 (m, 3H), 3.49 (dd, 1H), 3.60 (dd, 1H), 4.58 (m, 1H), 5.10 (s, 2H), 5.67 (s, 1H), 7.01 (d, 2H), 7.14 (m, 1H), 7.20 (m, 2H), 7.36 (m, 5H); LRMS : m/z 478.3 (MH^+).

Preparation 70

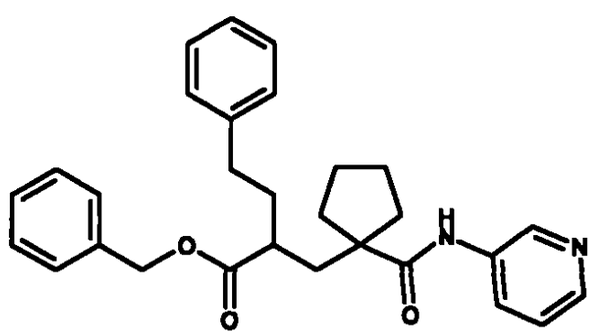
Benzyl 2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoate



The title compound was obtained as a clear oil in 74% yield from 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) and 2-amino-5-methyl-1,3,4-thiadiazole, following a similar procedure to that described in preparation 68; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.58-1.76 (m, 7H), 1.83-1.98 (m, 3H), 2.03 (m, 1H), 2.20 (m, 1H), 2.35 (m, 1H), 2.44 (m, 3H), 2.65 (s, 3H), 5.02 (dd, 2H), 7.00 (d, 2H), 7.15 (m, 1H), 7.19 (m, 2H), 7.35 (m, 5H); LRMS : m/z 478.7 (MH^+).

Preparation 71

Benzyl 4-phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoate



Oxalyl chloride (2.29ml, 26.3mmol) was added to a solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (5.0g, 13.14mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (25ml), and the solution stirred for 2.5 hours. The mixture was evaporated under reduced pressure, the residue azeotroped with dichloromethane to give a yellow oil. This was then dissolved in dichloromethane (50ml) and a solution of this acid chloride (10ml, 2.45mmol) was added to an ice-cooled solution of triethylamine (248mg, 2.45mmol) and 3-aminopyridine (253mg, 2.70mmol) in dry dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The solution was washed with water

372

(3x), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate:hexane (40:60) as eluant, and repeated using an elution gradient of ether:hexane (90:10 to 100:0). The product was crystallised from ethyl acetate:hexane to afford the title compound, 740mg, 66%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.38-2.07 (m, 10H), 2.10-2.37 (m, 2H), 2.42-2.63 (m, 3H), 5.02 (s, 2H), 6.94-7.44 (m, 10H), 7.50 (s, 1H), 8.03 (d, 1H), 8.36 (d, 1H), 8.52 (s, 1H).

NEP Assay:

The Preparation and Assay of Soluble Neutral Endopeptidase (NEP) from Canine, Rat, Rabbit and Human Kidney Cortex.

Soluble NEP is obtained from the kidney cortex and activity is assayed by measuring the rate of cleavage of the NEP substrate Abz-D-Arg-Arg-Leu-EDDnp to generate its fluorescent product, Abz-D-Arg-Arg.

Experimental Procedure:-

1 Materials

All water is double de ionised.

1.1 Tissues:

Human Kidney	IIAM (Pennsylvania. U.S.A.)
Rat Kidney	In house tissue supply
Rabbit Kidney	In house tissue supply
Canine Kidney	In house tissue supply

1.2 Homogenisation medium:

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.3 Tris buffer (NEP buffer):

50ml of 50mM Tris pH 7.4 (Sigma T2663) is diluted in 950ml of water.

1.4 Substrate (Abz-D-Arg-Arg-Leu-EDDnp):

Made to order from SNPE, and is stored as a powder at –20°C. A 2mM stock is made by gently re-suspending the substrate in Tris buffer, this should not be vortexed or sonicated. 600µl aliquots of the 2mM stock are stored at –20 for up to one month. (Medeiros, M.A.S., Franca,

M.S.F. et al., (1997), Brazilian Journal of Medical and Biological Research, 30, 1157-1162).

1.5 Total product:

Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined. The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.6 Stock solution:

A 300µM stock of Phosphoramidon (Sigma R7385) is made up in NEP buffer and stored in 50µl aliquots at -20.

1.7 Dimethyl sulphoxide (DMSO).

1.8 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.9 Black 96 well flat bottom assay plates (Costar 3915).

1.10 Topseal A (Packard 6005185).

1.11 Centrifuge tubes

2 Specific Equipment

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 Fluostar galaxy.

2.5 Wesbart 1589 shaking incubator.

3 Methods

3.1 Tissue Preparation

3.2 Dog, rat, rabbit, and human NEP is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approximately 10 volumes of homogenisation buffer (1.2) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.8) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a

Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer containing magnesium chloride (9mg MgCl in 5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,630rpm) for 12 minutes in a Beckman centrifuge (2.3) before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge (2.1) and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer containing magnesium chloride (0.9mg MgCl in 0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer (2.2). This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4 <u>Determination of NEP Activity</u>

The activity of the previously aliquoted NEP is measured by its ability to cleave the NEP specific peptide substrate.

4.1 A 4% DMSO/NEP buffer solution is made (4mls DMSO in 96mls NEP buffer).

4.2 Substrate, total product, enzyme, and Phosphoramidon stocks are left on ice to thaw.

4.3 50µl of 4% DMSO/NEP buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:40 to make a 50µM solution. 100µl of 50µM substrate is added to each well (final concentration 25µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of NEP buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:80 to make a 25µM solution. 200µl of 25µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37oC in a shaking incubator for 60 minutes.

4.8 The 300µM Phosphoramidon stock is diluted 1:100 to 300nM. The reaction is stopped by the addition of 100µl 300nM Phosphoramidon and incubated at 37°C in a shaking incubator for 20 minutes before being read on the Fluostar (ex320/em420).

5 NEP Inhibition Assays

5.1 Substrate, total product, enzyme and Phoshoramidon stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in NEP buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO solution (4mls DMSO in 96mls NEP buffer).

5.3 50µl of compound in duplicate is added to the 96 well plate and 50µl of 4% DMSO/NEP buffer is added to control and blank wells (see appendix for plate layout). Alternatively see appendix for robotic dilutions.

5.4 The 2mM substrate stock is diluted 1:40 in NEP buffer to make a 50µM solution (275µl 2mM substrate to 10.73ml buffer is enough for 1 plate).

5.5 The enzyme stock diluted in NEP buffer (determined from activity checks).

5.6 The 2mM total product stock is diluted 1:80 in NEP buffer to make a 25µM solution. 200µl is added to the first four wells of a separate plate.

5.7 The 300µM Phosphoramidon stock is diluted 1:1000 to make a 300nM stock (11µl Phosphoramidon to 10.99ml NEP buffer.

5.8 To each well in the 96 well plate the following is added:

Table: Reagents to be added to 96 well plate.

	Compound/ DMSO	Tris Buffer	Substrate	NEP enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.9 The reaction is initiated by the addition of the NEP enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.10 The reaction is stopped with 100µl 300nM Phosphoramidon and incubated at 37°C for 20 minutes in a shaking incubator before being read on the Fluostar (ex320/em420).

6 Calculations

374

The activity of the NEP enzyme is determined in the presence and absence of compound and expressed as a percentage.

% Control activity (turnover of enzyme) =

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

% Activity with inhibitor =

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

Activity expressed as % of control =

$$\frac{\text{\% Activity with inhibitor}}{\text{\% Control activity}} \times 100$$

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC50 values calculated using LabStats fit-curve in Excel.

The specific examples herein all had an IC50 against NEP of less than 5000nM.

In addition (in a preferred embodiment) many of the examples tested also had a selectivity for NEP over ACE of at least 300 fold.

ACE Assay

The Preparation and Assay of Soluble Angiotensin Converting Enzyme (Ace), from Porcine and Human Kidney Cortex.

Soluble ACE activity is obtained from the kidney cortex and assayed by measuring the rate of cleavage of the ACE substrate Abz-Gly-p-nitro-Phe-Pro-OH to generate its fluorescent product, Abz-Gly.

1 Materials

All water is double de ionised.

1.1 Human Kidney: IIAM (Pennsylvania. U.S.A.) or UK Human

Tissue Bank (UK HTB)

1.2 Porcine kidney ACE Sigma (A2580)

1.3 Homogenisation buffer-1

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.4 Homogenisation buffer-2

100mM Mannitol, 20mM Tris @ pH7.1 and 10mM $MgCl_2.6H_2O$ (Fisher M0600/53)

To 500ml of the homogenisation buffer 1 (1.4) 1.017g of $MgCl_2$ is added.

1.5 Tris buffer (ACE buffer).

50mM Tris and 300mM NaCl @ pH 7.4

50ml of 50mM Tris pH 7.4 (Sigma T2663) and 17.52g NaCl (Fisher S/3160/60) are made up to 1000ml in water.

1.6 Substrate (Abz-D-Gly-p-nitro-Phe-Pro-OH) (Bachem M-1100)

ACE substrate is stored as a powder at –20°C. A 2mM stock is made by gently re-suspending the substrate in ACE buffer, this must not be vortexed or sonicated. 400µl aliquots of the 2mM stock are stored at –20°C for up to one month.

1.7 Total product

Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined (see calculations). The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.8 Stop solution.

0.5M EDTA (Promega CAS[6081/92/6]) is diluted 1:250 in ACE buffer to make a 2mM solution.

1.9 Dimethyl sulphoxide (DMSO).

1.10 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.11 Black 96 well flat bottom assay plates (Costar 3915 or Packard).

1.12 Topseal A (Packard 6005185).

1.13 Centrifuge tubes

2 <u>Specific Equipment</u>

375.

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 BMG Fluostar Galaxy.

2.5 Wesbart 1589 shaking incubator.

3 Methods

3.1 Tissue Preparation

3.2 Human ACE is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approximately 10 volumes of homogenisation buffer-1 (1.4) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.11) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer-2 (1.5) (5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,630rpm) for 12 minutes in a Beckman centrifuge before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer-2 (0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer. This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4 Determination Of ACE Activity

The activity of the previously aliquoted ACE is measured by its ability to cleave the ACE specific peptide substrate.

Porcine ACE (1.2) is defrosted and resuspended in ACE buffer (1.6) at

0.004U/µl, this is frozen down in 50µl aliquots.

4.1 A 4% DMSO/ACE buffer solution is made (4mls DMSO in 96mls ACE buffer).

4.2 Substrate (1.7), total product (1.8) and enzyme (1.1, 1.2, 1.3), are left on ice to thaw.

4.3 50µl of 4% DMSO/ACE buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:100 to make a 20µM solution. 100µl of 20µM substrate is added to each well (final concentration in the assay 10µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of ACE buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:200 to make 10µM solution. 200µl 10µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37°C in a shaking incubator for 60 minutes.

4.8 The enzyme reaction is stopped by the addition of 100µl 2mM EDTA in ACE buffer and incubated at 37°C in a shaking incubator for 20 minutes before being read on the BMG Fluostar Galaxy (ex320/em420).

5 <u>ACE Inhibition Assays</u>

5.1 Substrate, total product, and enzyme stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in ACE buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO/ACE buffer solution (4mls DMSO in 96mls ACE buffer).

5.3 50µl of compound, in duplicate, is added to the 96 well plate and 50µl of 4% DMSO/ACE buffer is added to control and blank wells (see appendix-1 for plate layout).

5.4 Steps 5.2 and 5.3 can be carried out either by hand or using the Packard multiprobe robots (see appendix-2 for details)

5.5 The 2mM substrate stock is diluted 1:100 in ACE buffer to make a 20µM solution (10µM final concentration in the assay) (110µl of 2mM substrate added to 10.89ml buffer is enough for 1 plate).

5.6 The enzyme stock is diluted in ACE buffer, as determined from activity checks (4.0).

5.7 The 2mM total product stock is diluted 1:200 in ACE buffer to make a 10µM solution. 200µl is added to the first four wells of a separate plate.

5.8 The 0.5mM EDTA stock is diluted 1:250 to make a 2mM stock (44µl EDTA to

376

10.96ml ACE buffer).

5.9 To each well of the 96 well plate the following reagents are added:

Table 1: Reagents added to 96 well plate.

	Compound/ DMSO	Tris Buffer	Substrate	ACE enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.10 50µl of the highest concentration of each compound used in the assay is added in duplicate to the same 96 well plate as the totals (5.7). 150µl of ACE buffer is added to determine any compound fluorescence.

5.11 The reaction is initiated by the addition of the ACE enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.12 The reaction is stopped by the addition of 100µl 2mM EDTA and incubated at 37°C for 20 minutes in a shaking incubator, before being read on the BMG Fluostar Galaxy (ex320/em420).

6 Calculations

The activity of the ACE enzyme is determined in the presence and absence of compound and expressed as a percentage. (FU = Fluorescence units)

(i) % Control activity (turnover of enzyme) =

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(ii) % Activity with inhibitor =

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(iii) Activity expressed as % of control =

$$\frac{\text{\% Activity with inhibitor}}{\text{\% Control activity}} \times 100$$

or

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of controls} - \text{Mean FU of blanks}} \times 100$$

(iv) % Inhibition = 100 - % control

(v) For fluorescent compounds the mean FU of blanks containing compound (5.10) is deducted from the mean FU of compound values used to calculate the % Activity.

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC_{50} values calculated using LabStats fit-curve in Excel.

Animal Model of arousal response

A particularly preferred compound of the invention (selected from the list of 10 compounds given herebefore) was administered according to the following protocol to show an increase in genital blood flow in the rabbit (it has previously been shown by Ottensen that an increase in vaginal blood flow increase vaginal lubrication-. Ottesen, B., Pedersen, B., Nielsen, J. *et al.* (1987). Vasoactive intestinal polypeptide (VIP) provokes vaginal lubrication in normal women. *Peptides*, 8, 797-800.

"The genital organs consist of an internal and external group. The internal organs are situated within the pelvis and consist of ovaries, the uterine tubes, uterus and the vagina. The external organsare superficial to the urogenital diaphragm and below the pelvic arch. They comprise the mons pubis, the labia majora and minora pudendi, the clitoris, the vestibule, the bulb of the vestibule, and the greater vestibular glands" (Gray's Anatomy, C.D. Clemente, 13th American Edition).

Methods

Anaesthetic Protocol

377

Female New Zealand rabbits (~2.5kg) were pre-medicated with a combination of Medetomidine (Domitor®) 0.5ml/kg *i.m.*, and Ketamine (Vetalar®) 0.25ml/kg *i.m.* whilst maintaining oxygen intake via a face mask. The rabbits were tracheotomised using a Portex™ uncuffed endotracheal tube 3 ID., connected to ventilator and maintained a ventilation rate of 30-40 breaths per minute, with an approximate tidal volume of 18-20 ml, and a maximum airway pressure of 10 cm H_2O. Anaesthesia was then switched to Isoflurane and ventilation continued with O_2 at 2l/min. The right marginal ear vein was cannulated using a 23G or 24G catheter, and Lactated Ringer solution perfused at 0.5ml/min. The rabbit was maintained at 3% Isoflurane during invasive surgery, dropping to 2% for maintenance anaesthesia.

Cannulation of Vessels

The left groin area of the rabbit was shaved and a vertical incision was made approximately 5cm in length along the thigh. The femoral vein and artery were exposed, isolated and then cannulated with a PVC catheter (17G) for the infusion of drugs and compounds. Cannulation was repeated for the femoral artery, inserting the catheter to a depth of 10cm to ensure that the catheter reached the abdominal aorta. This arterial catheter was linked to a Gould system to record blood pressure. Samples for blood gas analysis were also be taken via the arterial catheter. Systolic and diastolic pressures were measured, and the mean arterial pressure calculated using the formula (diastolic x2 + systolic) ÷3. Heart rate was measured via the pulse oxymeter and *Po-ne-mah* data acquisition software system (Ponemah Physiology Platform, Gould Instrument Systems Inc).

Stimulation of the Pelvic Nerve

A ventral midline incision was made into the abdominal cavity. The incision was about 5cm in length just above the pubis. The fat and muscle was *bluntly* dissected away to reveal the hypogastric nerve which runs down the body cavity. It was essential to keep close to the side curve of the pubis wall in order to avoid damaging the femoral vein and artery which lie above the pubis. The sciatic and pelvic nerves lie deeper and were located after further dissection on the dorsal side of the rabbit. Once the sciatic nerve is identified, the pelvic nerve was easily located. The term *pelvic nerve* is loosely applied; anatomy books on the subject fail to identify the nerves in sufficient detail.

However, stimulation of the nerve causes an increase in vaginal and clitoral blood flow, and innervation of the pelvic region. The pelvic nerve was freed away from surrounding tissue and a *Harvard* bipolar stimulating electrode was placed around the nerve. The nerve was slightly lifted to give some tension, then the electrode was secured in position. Approximately 1ml of light paraffin oil was placed around the nerve and electrode. This acts as a protective lubricant to the nerve and prevents blood contamination of the electrode. The electrode was connected to a *Grass* S88 Stimulator. The pelvic nerve was stimulated using the following parameters:- 5V, pulse width 0.5ms, duration of stimulus 10 seconds and a frequency range of 2 to 16Hz. Reproducible responses were obtained when the nerve was stimulated every 15-20 minutes.

A frequency response curve was determined at the start of each experiment in order to determine the optimum frequency to use as a sub-maximal response, normally 4Hz. The compound(s) to be tested were infused, via the femoral vein, using a *Harvard* 22 infusion pump allowing a continuous 15 minute stimulation cycle.

Positioning of the Laser Doppler Probes

A ventral midline incision was made, at the caudal end of the pubis, to expose the pubic area. Remove any connective tissue, and expose the tunica of the clitoris, ensuring that the wall is free from small blood vessels. The external vaginal wall was also exposed by removing any connective tissue. One laser Doppler flow probe was inserted 3cm into the vagina, so that half the probe shaft is still visible. A second probe was positioned so that it lies just above the external clitoral wall. The position of these probes was then adjusted until a signal was obtained. A second probe was placed just above the surface of a blood vessel on the external vaginal wall. Both probes were clamped in position.

Vaginal and clitoral blood flow was recorded either as numbers directly from the Flowmeter using *Po-ne-mah* data acquisition software (Ponemah Physiology Platform, Gould Instrument Systems Inc), or indirectly from Gould chart recorder trace. Calibration is set at the beginning of the experiment (0-125ml/min/100g tissue).

Infusion of Inhibitors

378

A particularly preferred NEP (Neutral Endopeptidase EC3.4.24.11) inhibitor chosen from the list of 10 given hereinbefore was made up in saline or 5% glucose solution (200µl 50% glucose in 1.8ml water for injection). The inhibitor and vehicle controls were infused using a Harvard 22 pump, infusing at 500µl/min via a 3-way tap into the femoral vein. After the infusion, the catheter was flushed with heparinised saline (Hepsaline) so that no NEP inhibitor was left in the catheter.

Results and Discussion

Animal model of sexual arousal

The major cause of FSAD is decreased genital blood flow and this manifests itself as reduced vaginal, labial and clitoral engorgement. Treatment of women with FSAD is achievable by restoration of the *normal* sexual arousal response. This can be achieved by enhancing genital blood flow.

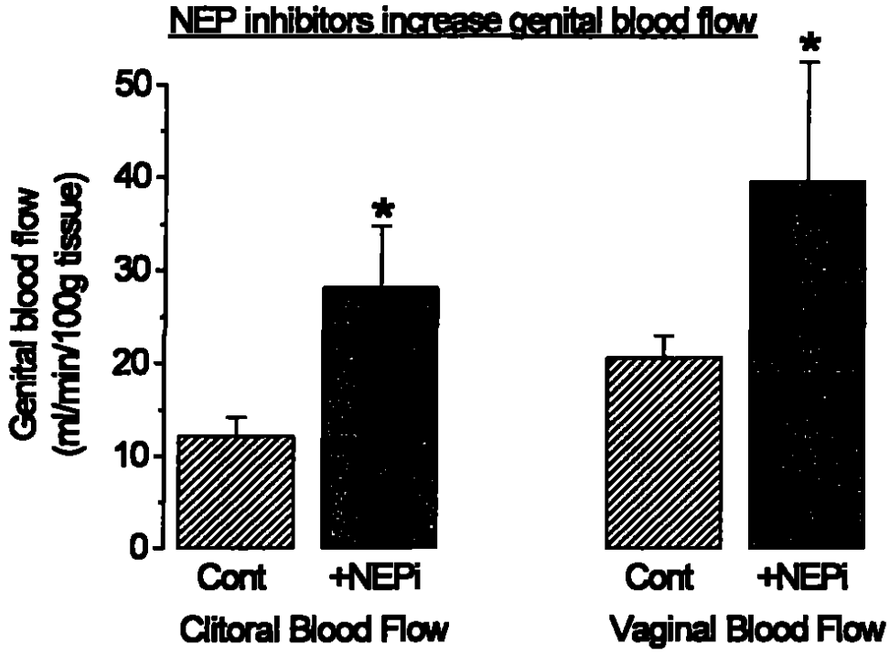
We have developed a robust reproducible model of the physiology of sexual arousal. Using this anaesthetised rabbit model, we are capable of measuring small changes in genital blood flow using Laser Doppler technology. Stimulation of the pelvic nerve is used to simulate the neuronal effects of sexual arousal.
FSAD is associated with and may result from reduced genital blood flow.

The selective NEP inhibitor tested, at clinically relevant doses, significantly enhanced pelvic nerve stimulated increases in genital blood flow (See Figure 1). The NEP inhibitor enhanced the peak increase in vaginal blood flow by up to 92% (n=3) and clitoral blood flow by 131% (n=3) compared to time matched control increases.

We have developed an animal model that mimics the physiological arousal response observed during female sexual arousal and directly reflects the clinical data obtained in human volunteers. The model uses Laser Doppler technologies to record small changes in vaginal and clitoral blood flow induced by pelvic nerve stimulation or vasoactive neurotransmitters. During sexual arousal, there is an increase in genital blood flow resulting from increased innervation from the pelvic nerve. The pelvic nerve-stimulated increase in vaginal and clitoral blood flow, observed in the animal model, represents the endogenous vascular effects observed during female sexual arousal. Therefore this model can be used to firstly, identify the mechanisms involved in the

regulation of vaginal and clitoral blood flow and secondly, use the model to validate novel approaches for the enhancement of genital blood flow.

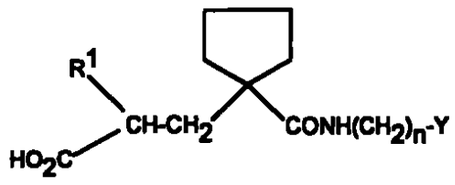
Figure 1:- The selective inhibitor of NEP EC 3.4.24.11 enhanced pelvic nerve stimulated (PNS) increases in genital blood flow in the anaesthetised rabbit model of sexual arousal. Repetitive PNS at 15 minute intervals induced reproducible increases in genital blood flow (Hatched Bars). Administration of a NEP inhibitor (Grey bar) enhanced the peak increase in clitoral and vaginal blood flow induced by submaximal stimulation frequencies (eg 4Hz) compared to increases observed during time matched control stimulations or vehicle controls (Hatched bar). The following simultaneous enhancements were observed following a 1.0mg/kg iv bolus – a 131% increase in clitoral and a 92% increase in vaginal blood flow (n=3). Data expressed as mean ± sem; all changes were monitored using laser Doppler technologies.



379

Claims

1 The use of a compound of formula I (or a pharmaceutically acceptable salt, solvate or prodrug thereof) in the preparation of a medicament for the treatment of sexual dysfunction:



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6}alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

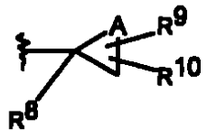
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or $-NR^2R^3$ wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

the $-(CH_2)_n-$ linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

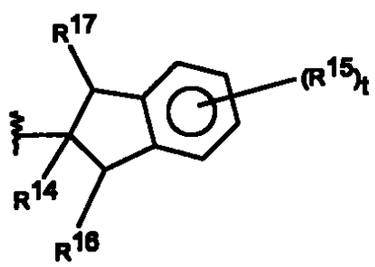
Y is the group



wherein A is $-(CH_2)_q-$ where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, $-CH_2OH$, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl, or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

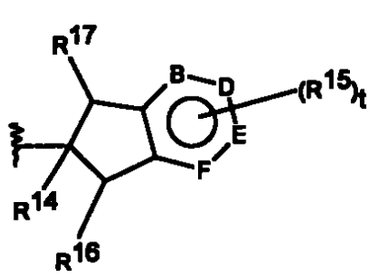
Y is the group, $-C(O)\ NR^{11}\ R^{12}$ wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and

R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

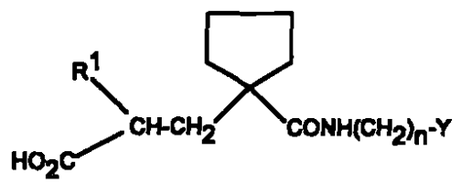
C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl; and

u is 0, 1, 2 or 3.

2 A compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6}alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, -

$S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

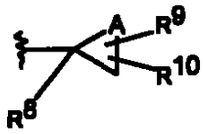
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or $-NR^2R^3$ wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

the $-(CH_2)_n-$ linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

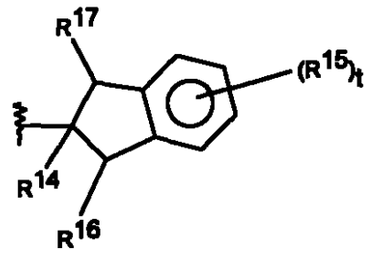
Y is the group



wherein A is $-(CH_2)_q-$ where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, $-CH_2OH$, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl, or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

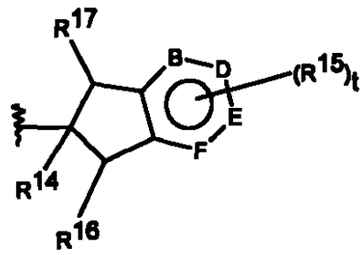
381

Y is the group,



wherein R^{14} is $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the

ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl; and

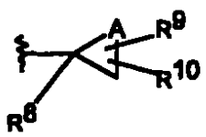
u is 0, 1, 2 or 3.

3 A compound as defined in claims 1 or 2 wherein R^1 is C_{1-6}alkyl, C_{1-6}alkoxy or C_{1-6}alkyl substituted with aryl.

4 A compound as defined in claim 3 wherein R^1 is C_{1-6}alkyl, C_{1-6}alkoxy or C_{1-6}alkoxy(C_{1-3})alkyl.

5 A compound as defined in claim 4 wherein R^1 is C_{1-4}alkyl.

6 A compound as defined in any one of the preceding claims wherein when Y is the group



and the carbocyclic ring is fully saturated, then one of R^9 or R^{10} is $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl.

7 A compound as defined in claim 6 wherein the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl.

8 A compound as defined in claim 7 wherein R^9 and R^{10} are attached to adjacent carbon atoms in the ring.

9 A compound as defined in any one of the preceding claims wherein R^8 is CH_2OH.

382

10 A compound as defined in any of claims 1 to 5 wherein when Y is the group - $NR^{18}S(O)_uR^{19}$, then R^{18} is H.

11 A compound as defined in any of claims 1 to 5 or 10 wherein R^{19} is benzyl or phenyl.

12 A compound as defined in any of claims 1 to 5 , 10 or 11 wherein u is 2.

13 A compound as defined in claim 1 wherein Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by one or more of C_{1-6} alkyl, phenyl, phenylC_{1-4}alkyl, C_{1-6} alkoxy, hydroxy, oxo, amino, mono or di-(C_{1-4}alkyl)amino or C_{1-4} alkanoylamino; with the proviso that when there is an oxo substitution, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring.

14 A compound as defined in claim 13 wherein the heterocyclic ring is a heteroaromatic ring.

15 A compound as defined in claim 14 wherein the heteroaromatic ring is selected from pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazole, thiazole, thiaiazole, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, pyridone, quinoxalinyl, and quinazolinyl each being optionally substituted as defined in claim 1.

16 A compound as defined in claim 15 wherein the heteroaromatic ring is selected from oxadiazole, pyridone and thiadiazole.

17 A compound as defined in claim 16 wherein the heteroaromatic ring is selected from 1,2,5 oxadiazole, 1,3,4 oxadiazole, 2-pyridone and 1,3,4 thiadiazole.

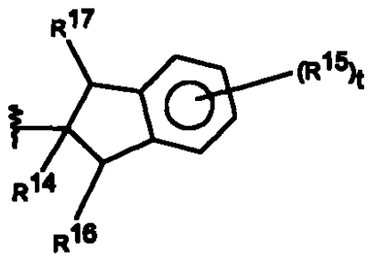
18 A compound as defined in any of claims 13 to 17 wherein the heterocyclic ring is substituted by one or more C_{1-6}alkyl, phenyl or phenylC_{1-4}alkyl.

19 A compound as defined in claim 18 wherein the substitution is C_{1-4}alkyl or benzyl.

20 A compound as defined in claims 16 to 19 wherein when Y is a pyridone, said pyridone is *N*-substituted (preferably with benzyl or C_{1-4}alkyl).

21 A compound as defined in claim 13 wherein Y is a lactam linked at the nitrogen.

22 A compound as defined in claim 1 and claims dependent thereon wherein Y is



wherein R^{14} is H, CH_2OH or $C(O)NR^{11}R^{12}$, R^{15} is one or more of H, OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; and R^{16} and R^{17} are independently H or C_{1-4}alkyl.

23 A compound as defined in claim 22 wherein R^{14} is CH_2OH or $C(O)NR^{11}R^{12}$.

24 A compound as defined in claim 23 wherein R^{14} is $C(O)NR^{11}R^{12}$.

25 A compound as defined in claims 2 and claims 22 to 24 wherein R^{16} and R^{17} are H.

26 A compound as defined in claims 2 and claims 21 to 24 wherein t is 0.

27 A compound according to any one of the preceding claims selected from the group consisting of:

2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-4-methoxybutanoic acid (Example 35),

2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid (Example 40),

(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (Example 44),
2-{(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]-4-phenylbutanoic acid (Example 43),
cis-3-(2-methoxyethoxy)-2-{(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}-cyclohexyl)amino]carbonyl}cyclopentyl)methyl]propanoic acid (Example 38),
(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 31),
(+)-2-[(1-{[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid (Example 30),
2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21),
2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]-pentanoic acid (Example 22), and
2-{[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 9).

28 A compound as defined in any one of the preceding claims wherein the chiral carbon attached to R^1 is preferably the R-enantiomer.

29 Use of a compound as defined in claim 2 and claims dependent thereon in the preparation of a medicament for the treatment or prophylaxis of a condition for which a beneficial therapeutic response can be obtained by the inhibition of neutral endopeptidase.

30 Use according to claim 29 for the treatment or prophylaxis of sexual dysfunction.

31 Use of a compound according to claims 1 or 30 wherein the sexual dysfunction treated is female sexual dysfunction.

32 Use according to claim 31 wherein the female sexual dysfunction(s) treated includes at least female sexual arousal dysfunction.

33 Use according to any one of claims 30 to 32 wherein the medicament is administered systemically.

34 Use according to claim 33 wherein the medicament is administered orally.

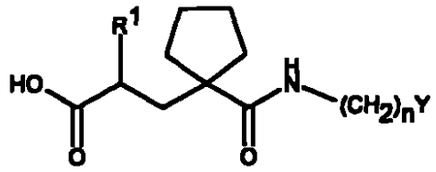
35 A compound as defined in claim 2 and claims dependent thereon for use in medicine.

36 A pharmaceutical formulation including a compound as defined in claim 2 and claims dependent thereon together with a pharmaceutically acceptable excipient.

37 A method for the treatment or prophylaxis of sexual dysfunction including administering to the patient a therapeutically effective amount of a compound as defined in any one of claims 1 to 28.

38 A sexual dysfunction pharmaceutical formulation including a therapeutically effective amount of a compound as defined in any one of claims 1 to 28 together with a pharmaceutically acceptable excipient.

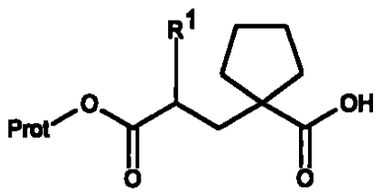
39 A process for preparing of a compound of formula I or salts thereof



(I)

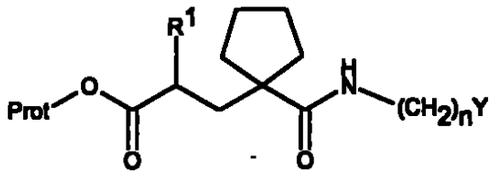
wherein R^1, n and Y are as defined in claim 2, comprising the steps of:

a) reacting a compound of formula II



(II)

wherein Prot is a suitable protecting group, with a compound of formula $Y(CH_2)_nNH_2$ (III), to give a compound of formula IV,



(IV)

then

b) reacting the compound of formula IV under suitable deprotecting conditions to give the compound of formula I; then

c) optionally forming a salt.

385

La Oficina de Patentes

La Oficina de Patentes

Concept House

Cardiff Road

Newport

South Wales

NP10 8QQ

Yo, el suscrito, siendo un oficial debidamente autorizado de acuerdo con la Sección 74(1) y (4) de la Ley de Deregulación y Contratación de 1994, para firmar y emitir certificados en nombre del Contralor-General, por la presente certifico que lo anexado es una copia fiel de los documentos como fueron originalmente radicados en conexión con la solicitud de Patente identificada allí dentro.

De acuerdo con las Reglas de Patentes (Re-inscripción de Compañías) de 1982, sí una compañía nombrada en este certificado y cualquier documento acompañante se ha re-inscrito bajo la Ley de Compañías de 1980 con el mismo nombre con el cual fue inscrito inmediatamente antes de la re-inscripción, salvo la substitución como, o la inclusión como, la última parte del nombre de las palabras "compañía

pública limitada" o sus equivalentes en Galés, referencias al nombre de la compañía en este certificado y cualquier documento acompañante serán tratados como referencias al nombre con el cual está re-inscrito.

De acuerdo con las reglas, las palabras "compañía pública limitada" pueden ser reemplazadas por p.l.c., plc, P.L.C. ó PLC.

Una re-inscripción bajo la Ley de Compañías no constituye una nueva entidad legal sino apenas sujeta a la compañía a ciertas reglas de ley corporativa adicionales.

Sello ilegible.

Firmado: ilegible

Fecha: 9 de mayo de 2001

Una Agencia Ejecutiva del Departamento de Comercio e Industria

La Oficina de Patentes

Sello de la oficina de Patentes con fecha 22 de Enero de 2001

Su Referencia PCS10910RCS-PROV2 **0101584.1**

Oficina de Patentes

Petición para el otorgamiento de una Patente

Formulario 1/77 **Patentes 1977**

---

1. Titulo de la Invención COMPOSICIÓN FARMACEUTICA

1 Por favor suministre el título de la invención

---

2. Detalles de la Solicitud

Primer o único solicitante

2a Si usted está aplicando como sociedad por favor dé:

Nombre corporativo

PFIZER LIMITED

País (y estado de la incorporación, si es apropiado)

REINO UNIDO

388

2b. Si usted está aplicando como individuo o uno de una sociedad por favor de por completo:

Apellidos

Nombres

2c. En todos los casos, por favor suministre los siguientes detalles:

Dirección

RAMSGATE ROAD

SÁNDWICH

KENT

Reino Unido Código PostalCT13 9NJ

(si aplica)

País Reino Unido

Número de ADP

(si lo conoce) 1271001

Segundo solicitante (si lo hay)

2d. Sí usted está aplicando como sociedad por favor dé:

Nombre corporativo

País (y estado de la incorporación, si es apropiado)

2e. Si usted está aplicando como individuo o uno de una sociedad por favor de por completo:

Apellidos

Nombres

2f. En todos los casos, por favor suministre los siguientes detalles:

Dirección

Reino Unido Código Postal

(si aplica)

País

Número ADP de patente

(si lo conoce)

---

3. Dirección para los detalles del servicio

3a. Tiene usted designado un agente para encargarse de su solicitud?

Si [X] No → ir a 3b

↓

Por favor suministre los detalles a continuación:

Nombre del Agente

DR. R. C. SEWELL

Dirección del Agente

PFIZER LIMITED

RAMSGATE ROAD

SANDWICH

KENT

Código postal CT13 9NJ

Número ADP del Agente 7964992001

3b. Si usted no ha designado un agente por favor suministre nombre y dirección en el Reino Unido en donde debe ser enviada toda la correspondencia:

Nombre

Dirección

Código Postal Teléfono diurno

Número ADP número (si esta disponible)

(si lo conoce)

391

4. Número de Referencia

4 Agente o solicitante
número de referencia PCS10910RCS-PROV2
(Si aplica)

---

5. Prioridad de una solicitud anterior

5 Está usted reclamando prioridad de una solicitud que haya sido presentada en una fecha anterior a esta solicitud?

Si [X] No [X] → ir a 6

↓

Por favor suministre detalles abajo
Número de solicitud anterior o número de patente
Fecha de presentación (día mes año)
Y la Sección del Acto de patentes 1977 bajo el cual usted esta reclamando:

15(4) Divisional [ ] 8(3)[ ] 12(6)[ ] 37(4) [ ]

---

6. Declaración de prioridad

6 Si está declarando prioridad de solicitud(es) previas, por favor suministre:

País de presentación	Número de solicitud de prioridad (si lo conoce)	Fecha de radicación (día, mes, año)

---

7. Inventoría

7 Es usted (el solicitante o solicitantes)el único inventor ó los inventores comunes?

Por favor marque caja correcta

Si ☐ No ☒ → Una Declaración de Inventoría formulario de Patentes 7/77 debe ser presentado (véase regla 15)

---

8. Lista de comprobación

8a Por favor indicar el número de hojas para cada uno de los siguientes tipos de documentos contenidos en esta solicitud.

Hojas de continuación de este Formulario de Patentes 1/77

393

Reivindicaciones	10	Descripción	132
Resumen	3	Dibujo(s)	1+1

8b. Cual de los siguientes documentos también acompañan la solicitud?

Documentos de prioridad (por favor indicar cuantos)

Traducción(es) de Documento(s) de Prioridad (por favor indicar cuantos)

Formulario de Patentes 7/77 Declaración de inventoría y Derecho de Otorgamiento (por favor indicar cuantos)

Formulario de Patentes 9/77 - Examen Preliminar/Búsqueda

Formulario de Patentes 10/77 - Petición para Examinación Substantiva

---

9. Petición

Yo/Nosotros pedimos el otorgamiento de una patente con base en esta solicitud.

Firma	Fecha
Ilegible	22/01/2001
	(dia, mes, año)

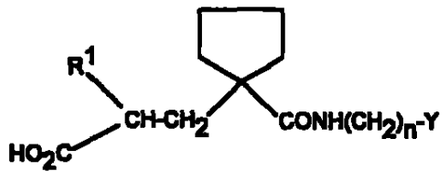
Por favor devolver el formulario completo, anexos y duplicados en donde han sido solicitados, junto con los honorarios descritos a:

The Comptroller
La Oficina de Patentes
Cardiff Road
NEWPORT
Gwent
NP9 1RH

The Comptroller
La Oficina de Patentes
25 Southampton Buildings
London
WC2A 1AY

REIVINDICACIONES:

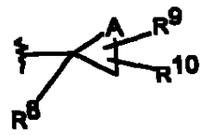
1. El uso de un compuesto de fórmula I, o una sal, solvato, polimorfo o profármacos farmacéuticamente aceptable, en la preparación de un medicamento para el tratamiento de la disfunción sexual:



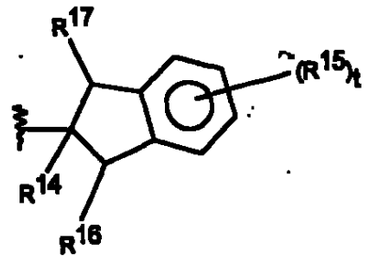
(I)

en donde R^1 es alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista: halo, hidroxi, alcoxi C_{1-6}, hidroxialcoxi C_{2-6}, alcoxi C_{1-6}(alcoxi C_{1-6}), cicloalquilo C_{3-7}, cicloalquenilo C_{3-7}, arilo, ariloxi (alcoxi C_{1-4})ariloxi, heterociclilo, heterocicliloxi, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ y $-CO_2$(alquilo C_{1-4}); o R^1 es cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar sustituido con uno o más sustituyentes de dicha lista, sustituyentes que pueden ser iguales o diferentes, cuya lista incluye además alquilo C_{1-6}; o R^1 es alcoxi C_{1-6}; $-NR^2R^3$ o $-NR^4SO_2R^5$; donde R^2 y R^3 son cada uno independientemente H, alquilo C_{1-4}, cicloalquilo C_{3-7}(opcionalmente sustituido con hidroxi o alcoxi C_{1-4}), arilo, (alquil C_{1-4})arilo, alcoxiarilo C_{1-6} o heterociclilo; R^2 y R^3 junto con el nitrógeno al que están unidos forman un grupo pirrolidinilo, piperidino, morfolino, piperazinilo o N-(alquil C_{1-4}) grupo piperazinilo; R^4 es H o alquilo C_{1-4};

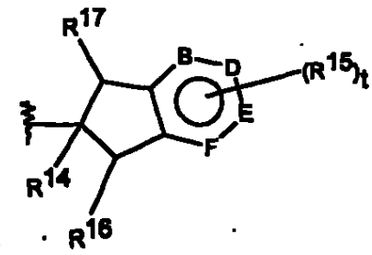
R^5 es alquilo C_{1-4}, CF_3, arilo, (alquil C_{1-4})arilo, (alcoxi C_{1-4})arilo; heterociclilo, alcoxi C_{1-4} o $-NR^2R^3$ donde R^2 y R^3 son como se han definido previamente; R^6 es alquilo C_{1-4}, arilo, heterociclilo o NR^2R^3 donde R^2 y R^3 son como se han definido previamente; y R^7 es alquilo C_{1-4}, cicloalquilo C_{3-7}, arilo o heterociclilo; n es 0, 1 ó 2; p es 0, 1, 2 ó 3; el enlace $-(CH_2)_n-$ está opcionalmente sustituido con alquilo C_{1-4}, alquilo C_{1-4}, sustituido con uno o más grupos fluoro o fenilo, alcoxi C_{1-4}, hidroxi, hidroxi(alquilo C_{1-3}), cicloalquilo C_{3-7}, arilo ó heterociclilo; Y es el grupo



donde A es $-(CH_2)_q-$, donde q es 1, 2, 3 ó 4 para completar un anillo carbocíclico de 3 a 7 miembros que pueden ser saturado o insaturado; R^8 es H, alquil C_{1-6}, $-CH_2OH$, fenilo, fenil(alquilo C_{1-4}) o $CONR^{11}R^{12}$; R^9 y R^{10} son cada uno independientemente H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$; alquilo C_{1-6}, fenilo (opcionalmente sustituido con alquilo C_{1-4}, halo o alcoxi C_{1-4}) o fenil(alquilo C_{1-4}), donde el grupo fenilo está opcionalmente sustituido con alquilo C_{1-4}, halo o alcoxi C_{1-4}, o R^9 y R^{10} juntos forman un dioxolano; R^{11} y R^{12}, que pueden ser iguales o diferentes, son H, alquilo C_{1-4}, R^{13} o $S(O)_rR^{13}$, donde r es 0, 1 ó 2 y R^{13} es fenilo opcionalmente sustituido con alquilo C_{1-4} o fenil-alquilo C_{1-4} donde el fenilo esta opcionalmente sustituido con alquilo C_{1-4}; o Y es el grupo $-C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se han definido previamente, excepto que R^{11} y R^{12} no son ambos H; o Y es el grupo,



donde R^{14} es H, CH_2OH o $C(O)NR^{11}R^{12}$ donde R^{11} y R^{12} son como se ha definido previamente; cuando está presente R^{15}, que puede ser igual o diferente a cualquier otro R^{15}, es OH, alquilo C_{1-4}, alcoxi C_{1-4}, halo o CF_3; t es 0, 1, 2, 3 ó 4; R^{16} Y R^{17} son independientemente H o alquilo C_{1-4}; o Y es el grupo



donde uno o dos de B, D o F es un nitrógeno, siendo los demás carbonos; y R^{14} a R^{17} y t son como se han definido previamente; o Y es un anillo heterociclico de 5 a 7 miembros opcionalmente sustituido, que puede ser saturado, insaturado o aromático y que contiene un nitrógeno, oxígeno o azufre y opcionalmente uno, dos o tres átomos de nitrógeno adicionales en el anillo y que puede estar opcionalmente condensado y opcionalmente sustituido con; alcoxi C_{1-6}; hidroxi, oxo, amino; mono o di-(alquilo C_{1-4})amino; alcanoilamino C_{1-4}; o alquilo C_{1-6} que puede estar sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionado de la lista: alcoxi C_{1-6}, haloalcoxi C_{1-6}; alquiltio C_{1-6}; halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo, o cicloalquilo C_{3-7}, arilo o heterociclilo, cada uno de los cuales puede estar

sustituido con uno o más sustituyentes, que pueden ser iguales o diferentes, seleccionados de la lista, alquilo C_{1-6}, alcoxi C_{1-6}, haloalcoxi C_{1-6}, alquiltio C_{1-6}, halógeno, cicloalquilo C_{3-7}, heterociclilo o fenilo; donde, cuando hay una sustitución oxo en el anillo heterociclico, el anillo contiene solamente uno o dos átomos de nitrógeno y la sustitución oxo es adyacente a un átomo de nitrógeno del anillo; o Y es $-NR^{18}S(O)_uR^{19}$, donde R^{18} es H o alquilo C_{1-4}; R^{19} es arilo, arilalquilo C_{1-4} o heterociclilo; (preferiblemente piridil); y u es 0, 1, 2 ó 3.

399
384

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed [redacted]

Dated 9 May 2001

400

For official use

THE PATENT OFFICE
K
22 JAN 2001
LONDON

23JAN01 E599922-1 D01298
P01/7700 0.00-0101584.1

Your reference
PCS10910RCS-PROV2

0101584.1

22 JAN 2001

Notes
Please type, or write in dark ink using CAPITAL letters. A prescribed fee is payable for a request for grant of a patent. For details, please contact the Patent Office (telephone 071-438 4700).

Rule 16 of the Patents Rules 1990 is the main rule governing the completion and filing of this form.

2 Do not give trading styles, for example, 'Trading as XYZ company', nationality or former names, for example, 'formerly (known as) ABC Ltd' as these are not required.

Warning
After an application for a Patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977 and will inform the applicant if such prohibition or restriction is necessary. Applicants resident in the United Kingdom are also reminded that under Section 23, applications may not be filed abroad without written permission unless an application has been filed not less than 6 weeks previously in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction revoked.

The Patent Office

Request for grant of a Patent

Form 1/77

Patents Act 1977

1 Title of invention

1 Please give the title of the invention

PHARMACEUTICAL COMPOSITION

2 Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate name
PFIZER LIMITED

Country *(and State of incorporation, if appropriate)*
UNITED KINGDOM

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c In all cases, please give the following details:

Address
RAMSGATE ROAD
SANDWICH
KENT

UK postcode CT13 9NJ
(if applicable)

Country UNITED KINGDOM

ADP number
(if known) 1271001

2d, 2e and 2f:
If there are further applicants please provide details on a separate sheet of paper.

☐ **Second applicant (if any)**

2d If you are applying as a corporate body please give:

Corporate name

Country *(and State of incorporation, if appropriate)*

2e If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2f **In all cases, please give the following details:**

Address

UK postcode
(if applicable)

Country

ADP number
(if known)

3
An address for service in the United Kingdom must be supplied.

Please mark correct box

3 Address for service details

3a Have you appointed an agent to deal with your application?

Yes [X] No [ ] ➡ *go to 3b*

⬇ *Please give details below*

Agent's name
DR. R.C. SEWELL
Agent's address
PFIZER LIMITED
RAMSGATE ROAD
SANDWICH
KENT
Postcode CT13 9NJ

Agent's ADP number 7960992001

3b:
If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.

3b If you have not appointed an agent please give a name and address in the United Kingdom to which all correspondence will be sent:

Name

Address

Postcode

Daytime telephone number *(if available)*

ADP number
(if known)

401

4 Reference number

4 Agent's or applicant's reference number *(if applicable)* PCS10910RCS-PROV2

5 Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box Yes ☐ No ☒ ➡ *go to 6*

⬇ *please give details below*

- ☐ number of earlier application or patent number
- ☐ filing date *(day month year)*
- ☐ and the Section of the Patents Act 1977 under which you are claiming:

Please mark correct box 15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

6

If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number.

Please give the date in all number format, for example, 31/05/90 for 31 May 1990.

6 Declaration of priority

6 If you are declaring priority from previous application(s), please give:

Country of filing	Priority application number *(if known)*	Filing date *(day,month,year)*

7

The answer must be 'No' if:
- any applicant is not an inventor
- there is an inventor who is not an applicant, or
- any applicant is a corporate body.

7 Inventorship

7 Are you (the applicant or applicants) the sole inventor or the joint inventors?

Please mark the correct box

Yes ☐ No ☒ ➡

A statement of inventorship on Patents Form 7/77 will need to be filed (see Rule 15).

8

Please supply duplicates of claim(s), abstract, description and drawing(s).

8 Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77			
Claim(s)	10	Description	132
Abstract	3	Drawing(s)	1 + 1

8b Which of the following documents also accompanies the application?

Please mark correct box(es)

Priority documents *(please state how many)*	
Translation(s) of Priority documents *(please state how many)*	
Patents Form 7/77 - Statement of Inventorship and Right to Grant *(please state how many)*	
Patents Form 9/77 - Preliminary Examination/Search	
Patents Form 10/77 - Request for Substantive Examination	

9

You or your appointed agent (see Rule 90 of the Patents Rules 1990) must sign this request.

9 Request

I/We request the grant of a patent on the basis of this application.

Please sign here ➡

Signed [signature] Date 22/01/2001

(day month year)

A completed fee sheet should preferably accompany the fee.

Please return the completed form, attachments and duplicates where requested, together with the prescribed fee to:

☐ ***The Comptroller***
The Patent Office
Cardiff Road
NEWPORT
Gwent
NP9 1RH

The Comptroller
The Patent Office
25 Southampton Buildings
London
WC2A 1AY

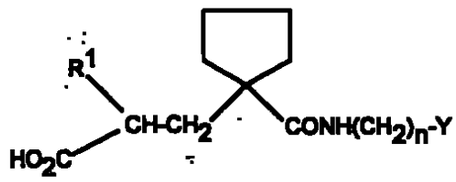
402

Pharmaceutical Composition

This invention relates to inhibitors of neutral endopeptidase enzyme (NEP) and derivatives thereof and to processes for the preparation of, intermediates used in the preparation of, compositions containing and the uses of said inhibitors. These inhibitors have utility in a variety of therapeutic areas including the treatment of sexual disorders in particular female sexual dysfunction, especially wherein the female sexual dysfunction treated includes female sexual arousal disorder.

NEP inhibitors are disclosed in WO 91/07386 and WO 91/10644.

According to a first aspect, the present invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, in the preparation of a medicament for the treatment of sexual dysfunction;



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6} alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl

(optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

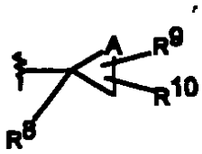
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or -NR^2R^3 wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

the -$(CH_2)_n$- linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

Y is the group



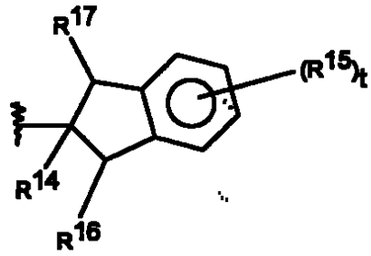
wherein A is -$(CH_2)_q$- where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, -CH_2OH, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, -CH_2OH, -$C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl (optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy) or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy, or R^9 and R^{10} together form a dioxolane;

$R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or

$S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

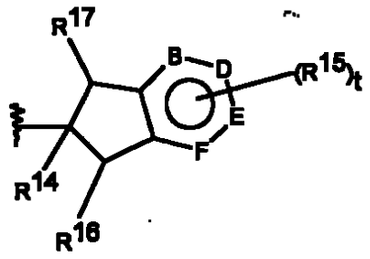
Y is the group, $-C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by: C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl (preferably pyridyl); and

u is 0, 1, 2 or 3.

Some of the compounds of formula I are disclosed in WO 91/10664 and WO 91/07386, but there is no teaching that they could be useful in the treatment of sexual dysfunction. The remaining compounds of formula I are novel.

Therefore according to a second aspect, the invention provides a (novel) compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, wherein R^1, n and Y are as defined in the first aspect with the proviso that Y is not the group $-C(O)NR^{11}R^{12}$ and when R^1 is propyl or phenylethyl, R^{14} is not $-CH_2OH$.

According to a third aspect, the invention provides a (novel) compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, wherein R^1, n and Y are as defined in the first aspect with the proviso that Y is not the group $-C(O)NR^{11}R^{12}$ and R^{14} is not H or $-CH_2OH$.

Unless otherwise specified, the compounds of the first, second and third aspects are hereinafter defined as compounds of the invention.

Where it is necessary to distinguish between the known and novel compounds of the invention, the novel compounds will be referred to as the compounds of the second and third aspects of the invention.

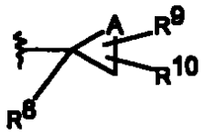
The following are preferred compounds of the invention, i.e. in accordance with the first, second and third aspects of the invention.

Preferred R^1 substituents are C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl, C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl or C_{1-6}alkyl substituted with aryl.

More preferred R^1 substituents are C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl (preferably methoxyethyl) or C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl (preferably methoxyethoxymethyl).

Even more preferred R^1 substituents are C_{1-4}alkyl (preferably propyl) or C_{1-6}alkoxy(C_{1-3})alkyl (preferably methoxyalkyl, more preferably methoxyethyl).

When Y is the group



and the carbocyclic ring is fully saturated, then preferably one of R^9 or R^{10} is $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl. More, preferably the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is

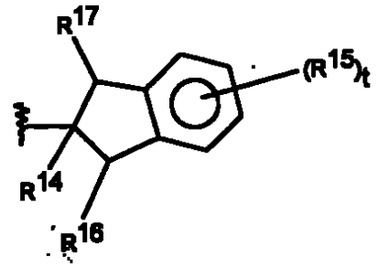
optionally substituted by C_{1-4}alkyl. More preferably, R^9 and R^{10} are attached to adjacent carbon atoms in the ring. More preferably, R^8 is CH_2OH.

When Y is the group $-NR^{18}S(O)_uR^{19}$, preferably R^{18} is H. More preferably, R^{19} is benzyl or phenyl. More preferably u is 2.

Preferably Y is an optionally substituted 5-7 membered heterocyclic ring. More preferably the ring is an optionally substituted aromatic ring, particularly pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazolyl, thiazolyl, thiadiazolyl, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, pyridonyl, quinoxalinyl or quinazolinyl [especially oxadiazole (preferably 1,2,5- or 1,3,4-oxadiazole), pyridone (preferably 2-pyridone) or thiadiazole (preferably 1,3,4-thiadiazole) each of which may be substituted as defined in the first aspect. Preferably the heterocyclic ring is substituted by one or more C_{1-6}alkyl, phenyl or phenylC_{1-4}alkyl, more preferably by C_{1-4}alkyl or benzyl. Preferably Y is an *N*-substituted pyridone, preferably by benzyl or C_{1-4}alkyl.

Preferably Y is a lactam linked at the nitrogen.

Preferably Y is



wherein R^{14} is preferably CH_2OH or $C(O)NR^{11}R^{12}$, especially $C(O)NR^{11}R^{12}$. Preferably R^{16} and R^{17} are hydrogen. Preferably t is 0.

405

The chiral carbon attached to R^1 is preferably the R-enantiomer.

Particularly preferred compounds of the invention (referred to hereinafter as the list of 21 preferred compounds) are:

2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]-4-methoxybutanoic acid (Example 35);

2-[[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid (Example 40);

(+)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid (Example 44);

2-[(1-[[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid (Example 43);

cis-3-(2-methoxyethoxy)-2-[(1-[[(4-[[(phenylsulfonyl)amino]carbonyl}cyclohexyl)-amino]carbonyl}cyclopentyl)methyl]propanoic acid (Example 38);

(+)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid (Example 31);

(+)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid (Example 30);

2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21);

2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid (Example 22);

2-[[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 9);

trans-3-[1-({[2-(4-chlorophenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxymethyl)propanoic acid (Example 46);

trans-3-[1-({[2-(4-methoxyphenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 47);

trans-3-[1-({[2-pentylcyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 48);

3-[1-({[5-benzyl-[1,3,4]-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 49);

3-[1-({[4-butylpyridin-2-yl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 50);

3-[1-({[4-phenylpyridin-2-yl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 51);

3-[1-({[1-hydroxymethyl-3-phenylcyclopentyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 52);

2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-methoxybutanoic acid (Example 53);

trans-3-[1-({[2-phenylcyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 54);

(*R*)- 2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-methoxybutanoic acid (Example 55); and

(*S*)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-methoxybutanoic acid (Example 56).

In the above definition, unless otherwise indicated, alkyl groups having three or more carbon atoms may be straight or branched-chain. The term aryl as used herein means an aromatic hydrocarbon group such as phenyl or naphthyl which may optionally be substituted with, for example, one or more of OH, CN, CF_3, C_1-C_4 alkyl, C_1-C_4 alkoxy, halo, carbamoyl, aminosulphonyl, amino, mono or di(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Halo means fluoro, chloro, bromo or iodo.

In the above definition, unless otherwise indicated the term heterocyclyl means a 5 or 6 membered nitrogen, oxygen or sulphur containing heterocyclic group which, unless otherwise stated, may be saturated, unsaturated or aromatic and which may optionally include a further oxygen or one to three nitrogen atoms in the ring and which may optionally be benzofused or substituted with for example, one or more halo, C_1-C_4 alkyl, hydroxy, carbamoyl, benzyl, oxo, amino or mono or di-(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Particular examples of heterocycles include pyridyl, pyridonyl, pyrazinyl, pyrimidinyl, pyridazinyl,

pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, furanyl, tetrahydrofuranyl, tetrahydropyranyl, dioxanyl, thienyl, oxazolyl, isoxazolyl, thiazolyl, oxadiazolyl, thiadiazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, quinoxalinyl, quinazolinyl and benzimidazolyl, each being optionally substituted as previously defined.

The compounds of the invention are inhibitors of the zinc-dependent, neutral endopeptidase EC.3.4.24.11., and it is proposed that the compounds of the invention will treat the disease states listed below. This enzyme is involved in the breakdown of several bioactive oligopeptides, cleaving peptide bonds on the amino side of hydrophobic amino acid residues. The peptides metabolised include atrial natriuretic peptides (ANP), bombesin, bradykinin, calcitonin gene-related peptide, endothelins, enkephalins, neurotensin, substance P and vasoactive intestinal peptide. Some of these peptides have potent vasodilatory and neurohormone functions, diuretic and natriuretic activity or mediate behaviour effects. Thus, the compounds of the invention, by inhibiting the neutral endopeptidase EC.3.4.24.11, can potentiate the biological effects of bioactive peptides. Thus, in particular the compounds have utility in the treatment of a number of disorders, including hypertension, heart failure, angina, renal insufficiency, cyclical oedema, Menières disease, hyperaldosteroneism (primary and secondary) and hypercalciuria. In addition, because of their ability to potentiate the effects of ANF the compounds have utility in the treatment of glaucoma. As a further result of their ability to inhibit the neutral endopeptidase E.C.3.4.24.11 the compounds of the invention may have activity in other therapeutic areas including for example the treatment of menstrual disorders, preterm labour, pre-eclampsia, endometriosis, and reproductive disorders (especially male and female infertility, polycystic ovarian syndrome, implantation failure). Also the compounds of the invention should treat asthma, inflammation, leukemia, pain, epilepsy, affective disorders, dementia and geriatric confusion, obesity and gastrointestinal disorders (especially diarrhoea and irritable bowel syndrome), wound healing (especially diabetic and venous ulcers and pressure sores), septic shock, the modulation of gastric acid secretion and the treatment of

hyperreninaemia. In a preferred embodiment the compounds of the invention are useful in the treatment of male and female sexual dysfunction.

We have found that the compounds of the invention inhibit the enzyme neutral endopeptidase. Therefore, according to a further aspect, the invention provides the use of a compound as defined in the second aspect in the preparation of a medicament for the treatment or prophylaxis of a condition for which a beneficial therapeutic response can be obtained by the inhibition of neutral endopeptidase.

The compounds of the invention are particularly beneficial for the treatment of sexual dysfunction in the male (e.g. male erectile dysfunction), more particularly in the female - female sexual dysfunction (FSD).

In accordance with the invention, FSD can be defined as the difficulty or inability of a woman to find satisfaction in sexual expression. FSD is a collective term for several diverse female sexual disorders (Leiblum, S.R. (1998). Definition and classification of female sexual disorders. *Int. J. Impotence Res.*, 10, S104-S106; , Berman, J.R., Berman, L. & Goldstein, I. (1999). Female sexual dysfunction: Incidence, pathophysiology, evaluations and treatment options. *Urology*, 54, 385-391). The woman may have lack of desire, difficulty with arousal or orgasm, pain with intercourse or a combination of these problems. Several types of disease, medications, injuries or psychological problems can cause FSD. Treatments in development are targeted to treat specific subtypes of FSD, predominantly desire and arousal disorders.

The categories of FSD are best defined by contrasting them to the phases of normal female sexual response: desire, arousal and orgasm (Leiblum, S.R. (1998). Definition and classification of female sexual disorders. *Int. J. Impotence Res.*, 10, S104-S106). Desire or libido is the drive for sexual expression. Its manifestations often include sexual thoughts either when in the company of an interested partner or when exposed to other erotic stimuli. Arousal is the vascular response to sexual stimulation, an important component of which is

genital engorgement and includes increased vaginal lubrication, elongation of the vagina and increased genital sensation/sensitivity. Orgasm is the release of sexual tension that has culminated during arousal.

Hence, FSD occurs when a woman has an inadequate or unsatisfactory response in any of these phases, usually desire, arousal or orgasm. FSD categories include hypoactive sexual desire disorder, sexual arousal disorder, orgasmic disorders and sexual pain disorders. Although the compounds of the invention will improve the genital response to sexual stimulation (as in female sexual arousal disorder), in doing so it may also improve the associated pain, distress and discomfort associated with intercourse and so treat other female sexual disorders.

Thus, in accordance with a preferred aspect of the invention, there is provided use of a compound of the invention in the preparation of a medicament for the treatment or prophylaxis of hypoactive sexual desire disorder, sexual arousal disorder, orgasmic disorder and sexual pain disorder, more preferably for the treatment or propylaxis of sexual arousal disorder, orgasmic disorder, and sexual pain disorder, and most preferably in the treatment or prophylaxis of sexual arousal disorder.

Hypoactive sexual desire disorder is present if a woman has no or little desire to be sexual, and has no or few sexual thoughts or fantasies. This type of FSD can be caused by low testosterone levels, due either to natural menopause or to surgical menopause. Other causes include illness, medications, fatigue, depression and anxiety.

Female sexual arousal disorder (FSAD) is characterised by inadequate genital response to sexual stimulation. The genitalia do not undergo the engorgement that characterises normal sexual arousal. The vaginal walls are poorly lubricated, so that intercourse is painful. Orgasms may be impeded. Arousal disorder can be caused by reduced oestrogen at menopause or after childbirth

and during lactation, as well as by illnesses, with vascular components such as diabetes and atherosclerosis. Other causes result from treatment with diuretics, antihistamines, antidepressants eg SSRIs or antihypertensive agents.

Sexual pain disorders (includes dyspareunia and vaginismus) is characterised by pain resulting from penetration and may be caused by medications which reduce lubrication, endometriosis, pelvic inflammatory disease, inflammatory bowel disease or urinary tract problems.

The prevalence of FSD is difficult to gauge because the term covers several types of problem, some of which are difficult to measure, and because the interest in treating FSD is relatively recent. Many women's sexual problems are associated either directly with the female ageing process or with chronic illnesses such as diabetes and hypertension.

Because FSD consists of several subtypes that express symptoms in separate phases of the sexual response cycle, there is not a single therapy. Current treatment of FSD focuses principally on psychological or relationship issues. Treatment of FSD is gradually evolving as more clinical and basic science studies are dedicated to the investigation of this medical problem. Female sexual complaints are not all psychological in pathophysiology, especially for those individuals who may have a component of vasculogenic dysfunction (eg FSAD) contributing to the overall female sexual complaint. There are at present no drugs licensed for the treatment of FSD. Empirical drug therapy includes oestrogen administration (topically or as hormone replacement therapy), androgens or mood-altering drugs such as buspirone or trazodone. These treatment options are often unsatisfactory due to low efficacy or unacceptable side effects.

Since interest is relatively recent in treating FSD pharmacologically, therapy consists of the following:- psychological counselling, over-the-counter sexual lubricants, and investigational candidates, including drugs approved for other

conditions. These medications consist of hormonal agents, either testosterone or combinations of oestrogen and testosterone and more recently vascular drugs, that have proved effective in male erectile dysfunction. None of these agents has been demonstrated to be very effective in treating FSD.

As discussed, the compounds of the invention are particularly useful for the treatment of female sexual arousal disorder (FSAD).

The Diagnostic and Statistical Manual (DSM) IV of the American Psychiatric Association defines Female Sexual Arousal Disorder (FSAD) as being:

> "a persistent or recurrent inability to attain or to maintain until completion of the sexual activity adequate lubrication-swelling response of sexual excitement. The disturbance must cause marked distress or interpersonal difficulty."

The arousal response consists of vasocongestion in the pelvis, vaginal lubrication and expansion and swelling of the external genitalia. The disturbance causes marked distress and/or interpersonal difficulty.

FSAD is a highly prevalent sexual disorder affecting pre-, peri- and post menopausal (±HRT) women. It is associated with concomitant disorders such as depression, cardiovascular diseases, diabetes and UG disorders.

The primary consequences of FSAD are lack of engorgement/swelling, lack of lubrication and lack of pleasurable genital sensation. The secondary consequences of FSAD are reduced sexual desire, pain during intercourse and difficulty in achieving an orgasm.

It has recently been hypothesised that there is a vascular basis for at least a proportion of patients with symptoms of FSAD (Goldstein *et al.*, Int. J. Impot. Res., 10, S84-S90,1998) with animal data supporting this view (Park *et al.*, Int. J. Impot. Res., 9, 27-37, 1997).

Drug candidates for treating FSAD, which are under investigation for efficacy, are primarily erectile dysfunction therapies that promote circulation to the male genitalia. They consist of two types of formulation, oral or sublingual medications (Apomorphine, Phentolamine, phosphodiesterase type 5 (PDE5) inhibitors e.g. Sildenafil), and prostaglandin (PGE_1) that are injected or administered transurethrally in men, and topically to the genitalia in women.

The present invention is advantageous as it provides a means for restoring a normal sexual arousal response - namely increased genital blood flow leading to vaginal, clitoral and labial engorgement. This will result in increased vaginal lubrication *via* plasma transudation, increased vaginal compliance and increased genital sensitivity. Hence, the present invention provides a means to restore, or potentiate, the normal sexual arousal response.

Without being bound by theory, we believe that neuropeptides such as vasoactive intestinal peptide (VIP) are major neurotransmitter candidates in the control of the female sexual arousal response, especially in the control of genital blood flow. VIP and other neuropeptides are degraded/ metabolised by NEP EC3.4.24.11. Thus, NEP inhibitors will potentiate the endogenous vasorelaxant effect of VIP released during arousal. This will lead to a treatment of FSAD, such as through enhanced genital blood flow and hence genital engorgement. We have shown that selective inhibitors of NEP EC 3.4.24.11 enhance pelvic nerve-stimulated and VIP-induced increases in vaginal and clitoral blood flow. In addition, selective NEP inhibitors enhance VIP and nerve-mediated relaxations of isolated vagina wall.

Thus the present invention is advantageous as it helps provide a means for restoring a normal sexual arousal response - namely increased genital blood flow leading to vaginal, clitoral and labial engorgement. This will result in increased vaginal lubrication *via* plasma transudation, increased vaginal compliance and

increased vaginal sensitivity. Hence, the present invention provides a means to restore, or potentiate the normal sexual arousal response.

Background teachings on NEP have been presented by Victor A. McKusick et al on http://www3.ncbi.nlm.nih.gov/Omim/searchomim.htm. The following information concerning NEP has been extracted from that source:

"Common acute lymphocytic leukemia antigen is an important cell surface marker in the diagnosis of human acute lymphocytic leukemia (ALL). It is present on leukemic cells of pre-B phenotype, which represent 85% of cases of ALL. CALLA is not restricted to leukemic cells, however, and is found on a variety of normal tissues. CALLA is a glycoprotein that is particularly abundant in kidney, where it is present on the brush border of proximal tubules and on glomerular epithelium. Letarte et al. (1988) cloned a cDNA coding for CALLA and showed that the amino acid sequence deduced from the cDNA sequence is identical to that of human membrane-associated neutral endopeptidase (NEP; EC 3.4.24.11), also known as enkephalinase. NEP cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin. By cDNA transfection analysis, Shipp et al. (1989) confirmed that CALLA is a functional neutral endopeptidase of the type that has previously been called enkephalinase. Barker et al. (1989) demonstrated that the CALLA gene, which encodes a 100-kD type II transmembrane glycoprotein, exists in a single copy of greater than 45 kb which is not rearranged in malignancies expressing cell surface CALLA. The gene was located to human chromosome 3 by study of somatic cell hybrids and in situ hybridization regionalized the location to 3q21-q27. Tran-Paterson et al. (1989) also assigned the gene to chromosome 3 by Southern blot analysis of DNA from human-rodent somatic cell hybrids. D'Adamio et al. (1989) demonstrated that the CALLA gene spans more than 80 kb and is composed of 24 exons."

1 Barker, P. E.; Shipp, M. A.; D'Adamio, L.; Masteller, E. L.; Reinherz, E. L. The common acute lymphoblastic leukemia antigen gene maps to chromosomal region 3(q21-q27). J. Immun. 142: 283-287, 1989.

2 D'Adamio, L.; Shipp, M. A.; Masteller, E. L.; Reinherz, E. L. : Organization of the gene encoding common acute lymphoblastic leukemia antigen (neutral endopeptidase 24.11): multiple miniexons and separate 5-prime untranslated regions. Proc. Nat. Acad. Sci. 86: 7103-7107, 1989.

3 Letarte, M.; Vera, S.; Tran, R.; Addis, J. B. L.; Onizuka, R. J.; Quackenbush, E. J.; Jongeneel, C. V.; McInnes, R. R. : Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. J. Exp. Med. 168: 1247-1253, 1988.

4 Shipp, M. A.; Vijayaraghavan, J.; Schmidt, E. V.; Masteller, E. L.; D'Adamio, L.; Hersh, L. B.; Reinherz, E. L. : Common acute lymphoblastic leukemia antigen (CALLA) is active neutral endopeptidase 24.11 ('enkephalinase'): direct evidence by cDNA transfection analysis. Proc. Nat. Acad. Sci. 86: 297-301, 1989.

5 Tran-Paterson, R.; Willard, H. F.; Letarte, M. : The common acute lymphoblastic leukemia antigen (neutral endopeptidase--3.4.24.11) gene is located on human chromosome 3. Cancer Genet. Cytogenet. 42: 129-134, 1989.

The compounds of formula (I) may contain several asymmetric centres and thus they can exist an enantiomers and diastereomers. The invention includes both the separated individual isomers as well as mixutres of isomers.

The pharmaceutically or veterinarily acceptable salts of the compounds of the invention which contain a basic centre are, for example, non-toxic acid addition salts formed with inorganic acids such as hydrochloric, hydrobromic, hydroiodic,

410

sulphuric and phosphoric acid, with carboxylic acids or with organo-sulphonic acids. Examples include the HCl, HBr, HI, sulphate or bisulphate, nitrate, phosphate or hydrogen phosphate, acetate, benzoate, succinate, saccarate, fumarate, maleate, lactate, citrate, tartrate, gluconate, camsylate, methanesulphonate, ethanesulphonate, benzenesulphonate, p-toluenesulphonate and pamoate salts. The pharmaceutically or veterinarily acceptable salts of the compounds of the invention which contain an acidic centre are those formed with bases which form non-toxic salts. Examples include the alkali metal salts such as the sodium, potassium or calcium salts or salts with amines such as diethylamine and dicyclohexylamine. For a review on suitable pharmaceutical salts see Berge et al, J. Pharm, Sci., 66, 1-19, 1977.

The pharmaceutically acceptable solvates of the compounds of the invention include the hydrates thereof. All polymorphs of the compounds of the invention are also included within the scope of the invention.

In cases where the compounds of the invention exist as the E and Z isomers, the invention includes individual isomers as well as mixtures thereof.

In cases where compounds of the invention exist as tautomeric isomers, the invention includes individual tautomers as well as mixtures thereof.

In cases where the compounds of the invention exist as optical isomers, the invention includes individual isomers as well as mixtures thereof.

In cases where the compounds of the invention exist as diastereoisomers, the invention includes individual diastereoisomers as well as mixtures thereof.

Separation of diastereoisomers or E and Z isomers may be achieved by conventional techniques, e.g. by fractional crystallisation, chromatography or H.P.L.C. An individual enantiomer of a compound of the invention may be prepared from a corresponding optically pure intermediate or by resolution, such

as by H.P.L.C. of the corresponding racemate using a suitable chiral support or by fractional crystallisation of the diastereoisomeric salts formed by reaction of the corresponding racemate with a suitable optically active acid or base, as appropriate.

The present invention also includes all suitable isotopic variations of compounds of the invention or pharmaceutically acceptable salt thereof. An isotopic variation of compounds of the invention or a pharmaceutically acceptable salt thereof is defined as one in which at least one atom is replaced by an atom having the same atomic number but an atomic mass different from the atomic mass usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention and pharmaceutically acceptable salts thereof include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulphur, fluorine and chlorine such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F and ^{36}Cl, respectively. Certain isotopic variations of the compounds of the invention and pharmaceutically acceptable salts thereof, for example, those in which a radioactive isotope such as ^{3}H or ^{14}C is incorporated, are useful in drug and/or substrate tissue distribution studies. Tritiated, i.e., ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with isotopes such as deuterium, i.e., ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements and hence may be preferred in some circumstances. Isotopic variations in compounds of the invention and pharmaceutically acceptable salts thereof can generally be prepared by conventional procedures such as by the illustrative methods or by the preparations described in the Examples and Preparations hereafter using appropriate isotopic variations of suitable reagents.

It will be appreciated by those skilled in the art that certain protected derivatives of the compounds of the invention, which may be made prior to a final deprotection stage, may not possess pharmacological activity as such, but may, in certain instances, be administered orally or parenterally and thereafter metabolised in the

411

body to form compounds of the invention which are pharmacologically active. Such derivatives may therefore be described as "prodrugs". Further, certain compounds of the invention may act as prodrugs of other compounds of the invention.

All protected derivatives, and prodrugs, of compounds of the invention are included within the scope of the invention. Examples of suitable pro-drugs for the compounds of the present invention are described in Drugs of Today, Volume 19, Number 9, 1983, pp 499 – 538 and in Topics in Chemistry, Chapter 31, pp 306 – 316 and in "Design of Prodrugs" by H. Bundgaard, Elsevier, 1985, Chapter 1 (the disclosures in which documents are incorporated herein by reference).

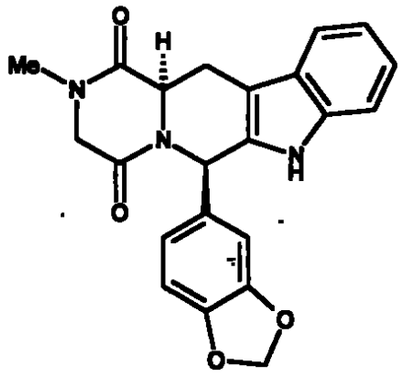
It will further be appreciated by those skilled in the art, that certain moieties, known to those skilled in the art as "pro-moieties", for example as described by H. Bundgaard in "Design of Prodrugs" (the disclosure in which document is incorporated herein by reference) may be placed on appropriate functionalities when such functionalities are present within compounds of the invention.

Preferred prodrugs for compounds of the invention include: esters, carbonate esters, hemi-esters, phosphate esters, nitro esters, sulfate esters, sulphoxides, amides, carbamates, azo-compounds, phosphamides, glycosides, ethers, acetals and ketals.

The compounds of the invention may also be combined with the following for the treatment of FSD (in particular FSAD):

Potentiators of cGMP (such as Sildenafil) and/or a centrally acting pharmaceutical (e.g. a dopamine agonist, such as apomorphine). Teachings on the use of apomorphine as a pharmaceutical may be found in US-A-5945117. In that particular document, apomorphine is delivered sub-lingually. In addition, or in the alternative, the agent may be used in combination with one or more of: one or more of a PDE inhibitor such as PDE2 (e.g. EHNA, and Example 100 of EP

0771799, incorporated herein by reference) and such as a PDE5 inhibitor (eg sildenafil, 1-[[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f]-as-trazin-2-yl)-4-ethoxyphenyl]sulfonyl]-4-ethylpiperazine i.e. vardenafil / Bayer BA 38-9456) and IC351 (see structure below, Icos Lilly), one or more of a dopamine receptor agonist (eg apomorphine), one or more of a melanocortin receptor agonist (eg Melanotan II), one or more of a potassium channel opener (eg a KATP channel opener and/or a calcium activated potassium channel opener (eg minoxdil, nicorandil)), one or more of a hormone replacement therapy (eg HRT) agent, one or more of a testosterone replacement agent (inc dehydroandrostendione), one or more of an estrogen agonists, one or more of a serotonin receptor agonist, one or more of a prostinoid receptor agonist (eg alprostadil), one or more of an NPY (e.g. NPYY1) antagonist, one or more of a VIP agonist .



IC351 (Icos Lilly)

If a combination of active agents are administered, then they may be administered simultaneously, separately or sequentially.

The compounds of the invention, their pharmaceutically acceptable salts, and pharmaceutically acceptable solvates of either entity can be administered alone but, in human therapy will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

The compositions of the present invention may be administered by direct injection. The composition may be formulated for parenteral, mucosal, intramuscular, intravenous, subcutaneous, ocular, intraocular or transdermal

412

administration. Depending upon the need, the agent may be administered at a dose of from 0.01 to 30 mg/kg body weight, such as from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight.

The term "administered" includes delivery by viral or non-viral techniques. Viral delivery mechanisms include but are not limited to adenoviral vectors, adeno-associated viral (AAV) vectos, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery mechanisms include lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by direct injection. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered topically. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by inhalation. In addition or in the alternative the compositions (or component parts thereof) of the present invention may also be administered by one or more of: a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution such as by an oral route, or by a parenteral route where delivery is by an injectable form, such as, for example, by a rectal, ophthalmic (including intravitreal or intracameral), nasal, topical (including buccal and sublingual), intrauterine, vaginal or parenteral (including subcutaneous, intraperitoneal, intramuscular, intravenous, intradermal, intracranial, intratracheal, and epidural) transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, or parenteral (e.g., intravenous, intraspinal, subcutaneous, transdermal or intramuscular) route.

By way of further example, the pharmaceutical composition of the present invention may be administered in accordance with a regimen of 1 to 10 times per day, such as once or twice per day. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy.

Hence, the term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular or subcutaneous route.

For example, the compounds of the invention or salts or solvates thereof can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed-, modified-, or controlled-release applications. The compounds of the invention may also be administered via intracavernosal injection.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethyl cellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

The compounds of the invention can also be administered parenterally, for example, intracavernosally, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. For such parenteral administration they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

For oral and parenteral administration to human patients, the daily dosage level of the compounds of the invention or salts or solvates thereof will usually be from 10 to 1000 mg (in single or divided doses).

Thus, for example, tablets or capsules of the compounds of the invention or salts or solvates thereof may contain from 5 to 1000mg, such as 5mg to 500 mg of active compound for administration singly or two or more at a time, as appropriate. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

The skilled person will also appreciate that, in the treatment of certain conditions (including FSD), compounds of the invention may be taken as a single dose on an "as required" basis (i.e. as needed or desired).

The compounds of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A [trade mark] or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA [trade mark]), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 1 to 50 mg of a compound of the invention for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 1 to 50 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, the compounds of the invention or salts or solvates thereof can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a gel, hydrogel, lotion, solution, cream, ointment or dusting powder. The compounds of the invention or salts or solvates thereof may also be dermally administered. The compounds of the invention or salts or solvates

thereof may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the ocular, pulmonary or rectal routes.

For ophthalmic use, the compounds can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds of the invention or salts or solvates thereof can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The compounds of the invention may also be used in combination with a cyclodextrin. Cyclodextrins are known to form inclusion and non-inclusion complexes with drug molecules. Formation of a drug-cyclodextrin complex may modify the solubility, dissolution rate, bioavailability and/or stability property of a drug molecule. Drug-cyclodextrin complexes are generally useful for most dosage forms and administration routes. As an alternative to direct complexation with the drug the cyclodextrin may be used as an auxiliary additive, e.g. as a carrier, diluent or solubiliser. Alpha-, beta- and gamma-cyclodextrins are most commonly used and suitable examples are described in WO-A-91/11172, WO-A-94/02518 and WO-A-98/55148.

In a preferred embodiment, the compounds of the invention are delivered systemically (such as orally, buccally, sublingually), more preferably orally.

Preferably such systemic (most preferably oral) administration is used to treat sexual dysfunction in a male or female, more preferably FSD and more preferably still FSAD.

Thus in a particularly preferred embodiment, there is provided the use of the compounds of the invention in the manufacture of a systemically delivered (preferably orally delivered) medicament for the treatment or prophylaxis of FSD, more preferably FSAD.

Since NEP is present throughout the body, it is very unexpected that the compounds of the invention can be administered systemically and achieve a therapeutic response in the genitalia (preferably the female genitalia) without provoking intolerable (adverse) side effects. Thus in the *in vivo* (rabbit) results hereafter, the compounds of the invention administered systemically increased genital blood flow, upon sexual arousal (mimiced by pelvic nerve stimulation) without adversely affecting cardiovascular parameters, such as causing a significant hypotensive or hypertensive.

Preferably the compounds of the invention are administered for the treatment of sexual dysfunction (more preferably FSD) in the sexually stimulated patient (by sexual stimulation we mean to include visual, auditory or tactile stimulation).The stimulation can be before, after or during said administration.

Thus the compounds of the invention enhance the pathways/mechanisms that underlie sexual aroual in the female gentialia restoring or improving the sexual arousal response to sexual stimulation.

Thus a preferred embodiment provides the use of a compound of the invention in the preparation of a medicament for the treatment or prophyaxis of sexual dysfunction (more preferably FSD) in the stimulated patient.

For veterinary use, a compound of the invention or a veterinarily acceptable salt thereof, or a veterinarily acceptable solvate or pro-drug thereof, is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

Compounds of the invention may be prepared, in known manner, in a variety of ways.

Throughout the specification, general formulae are designated by Roman numerals I, II, III, IV etc. Subsets of these general formulae are defined as Ia, Ib, Ic etc, ... IVa, IVb, IVc etc.

According to a further aspect of the invention, compounds of general formula I may be prepared according to reaction scheme 1, by reacting a compound of formula II (where Prot is a suitable protecting group) with a primary amine of formula III to give a compound of formula IV. Deprotection gives compounds of formula I.

Compounds of formula II and III are novel and form a further aspect of the invention.

Preferred reaction conditions for the acid/amine coupling step comprise reacting II with III (or its amine salt) in the presence of an activating agent, optionally a catalyst, and an excess of an acid acceptor, in a suitable solvent. Particularly preferred reaction conditions comprise reacting II (1-1.5 equivalents), III (or its salt 1-1.5 equivalents), in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCDI) or N,N'-dicyclohexylcarbodiimide (DCC) (1.1-1.3 equivalents), 1-hydroxybenzotrazole hydrate (HOBT) or dimethylaminopyridine (DMAP) (1.05-1.2 equivalents), *N*-methyl morpholine (NMM) or triethyamine (2.3-3 equivalents), in dimethylformamide or dichloromethane at between room temperature and 90°C for 16-18 hours.

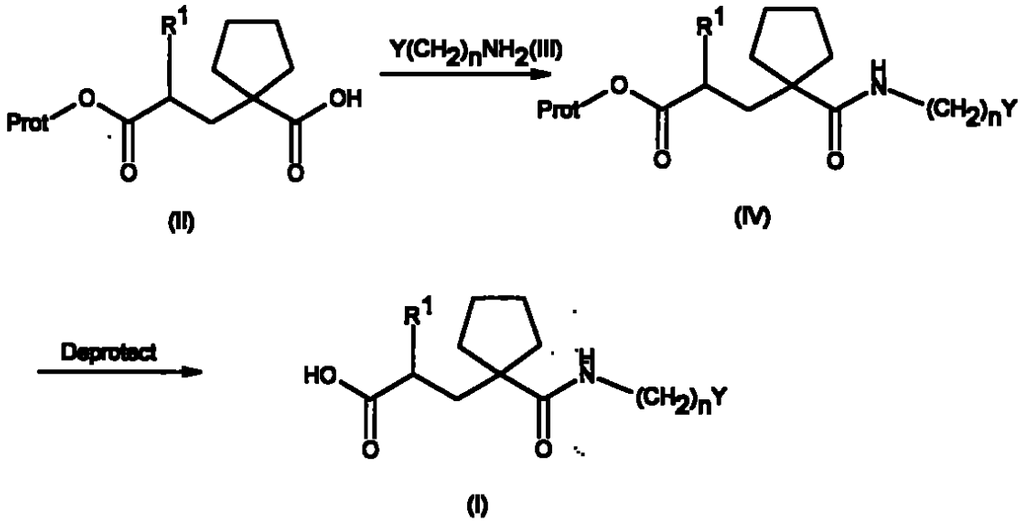
Alternatively, the acid/amine coupling step may be prepared via the acid chloride in the presence of an excess of acid acceptor, in a suitable solvent. The acid chloride may be isolated or it may be generated in situ. Preferred reaction conditions comprise reacting the acid chloride of II (1-1.1 equivalents), III (or its salt, 1 to 1.5 equivalents), triethyamine or *N*-methyl morpholine (1.4-10 equivalents), in dichloromethane at room temperature for 24 hours. Compounds of formula II can be converted to the acid chloride *in situ* by treatment with oxalyl chloride in dichloromethane in the presence of a catalytic amount of dimethylformamide for 2 hours at room temperature.

Methods for deprotection of an acid group depend on the protecting group. For examples of protection/deprotection methodology see "Protective groups in Organic synthesis", TW Greene and PGM Wutz.

For example, when Prot is a *tert*-butyl, deprotection conditions comprise reacting IV with trifluoroacetic acid/dichloromethane (1:1-1.5 by volume), at room temperature for 2-18 hours, optionally in the presence of a carbocation scavenger, e.g. anisole (10 equivalents). When Y contains a hydroxy group, base hydrolysis of the intermediate trifluoroacetic acid ester may be necessary. Alternative methodology for deprotection when Prot is *tert*-butyl comprises treating IV with hydrochloric acid in dichloromethane at room temperature for 3 hours. For the avoidance of doubt, Prot as *tert*-butyl is given by way of Example and is not intended to be limited to *tert*-Butyl.

When Prot is benzyl, deprotection conditions comprise reacting IV with palladium on charcoal (5-10%) in aqueous ethanol (40-95%) at 15-60 psi at room temperature for 2hrs to 3 days.

Scheme 1

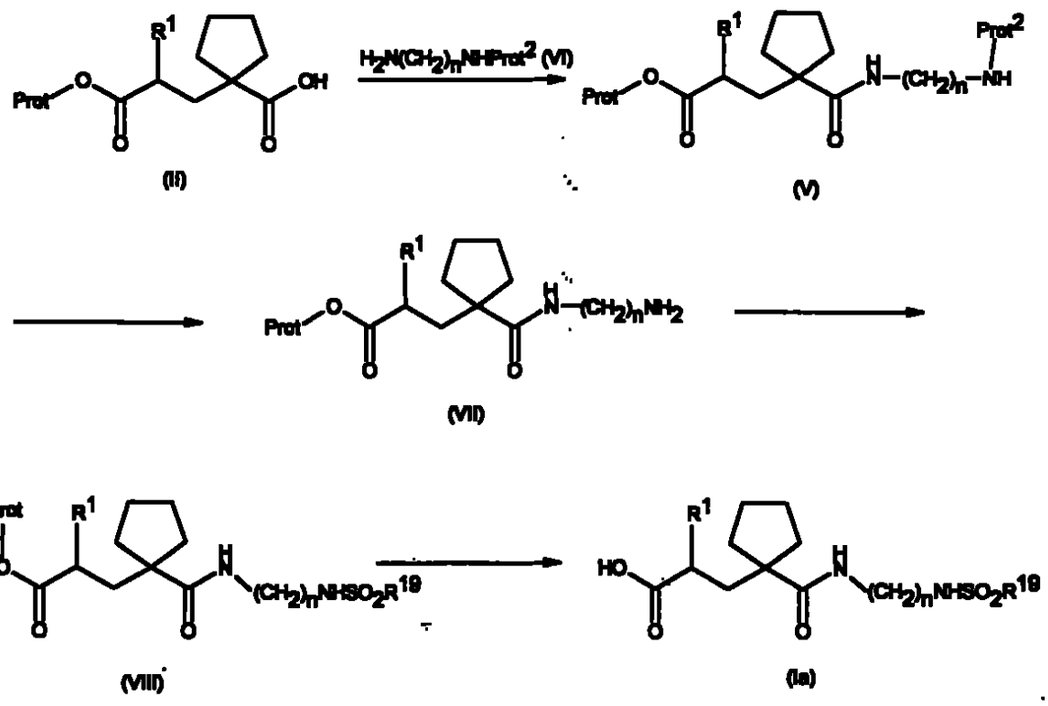


Compounds of formula Ia, i.e. compounds of general formula I where Y is $-NHSO_2R^{19}$, may be prepared according to reaction scheme 2. Compounds of formula V are first prepared by reacting compounds of formula II with compounds of formula VI where $Prot^2$ is a suitable amine protecting group. Preferred reaction conditions are analogous to those described the acid/amine coupling step for Scheme 1 above. Selective amine deprotection of compounds of formula V gives compounds of formula VII. Compounds of formula VII are reacted with $R^{19}SO_2Cl$ in the presence of an acid acceptor in a suitable solvent to form compounds of formula VIII. Deprotection of compounds of formula VIII under analogous conditions to those described for the deprotection step of Scheme 1 gives compounds of formula Ia.

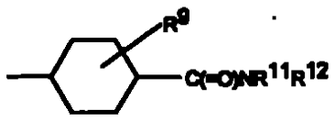
Methods for deprotection of an amine group depend on the protecting group. For examples of protection/deprotection methodology see "Protective groups in Organic Synthesis", TW Greene and PGM Wutz. For example, when $Prot^2$ is benzoyloxycarbonyl, deprotection conditions comprise reacting V with palladium on charcoal (10%) in ethanol at room temperature for 18 hours.

Preferred methods for preparation of the compounds of formula VIII comprise reaction of VII with $R^{19}SO_2Cl$ (1 equivalent) in the presence of triethyamine (1.5-2.5 equivalents) in dichloromethane at room temperature for 2 to 3 days.

Scheme 2



Compounds of formula Ib, i.e. compounds of formula I where n is 0 and Y is



, may be prepared according to reaction scheme 3.

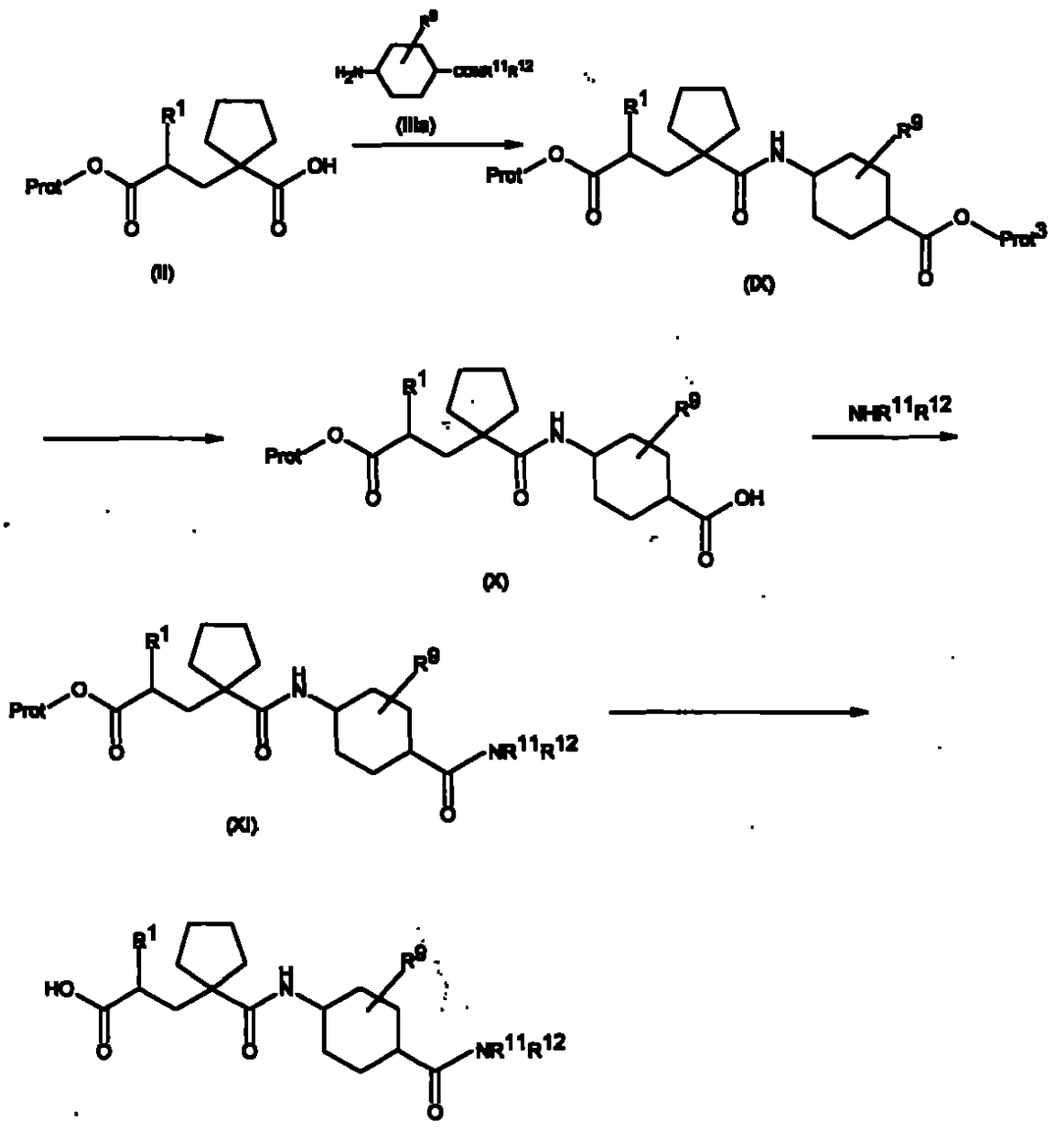
Compounds of formula II are reacted with compounds of formula IIIa under analogous conditions to acid/amine coupling conditions of Scheme 1 to give compounds of formula IX, where $Prot^3$ is a protecting group which can be selectively removed in the presence of protecting group Prot. A preferred protecting group $Prot^3$ is a base labile ester group. Consequently, treatment of compound of formula IX under basic conditions gives compounds of formula X. Compounds of formula X are reacted with compounds of formula $NHR^{11}R^{12}$ under analogous conditions to acid/amine coupling conditions of Scheme 1 to form compounds of formula XI. Deprotection of compounds of formula XI under

417

analogous conditions to the deprotection step in Scheme 1 gives compounds of formula Ib.

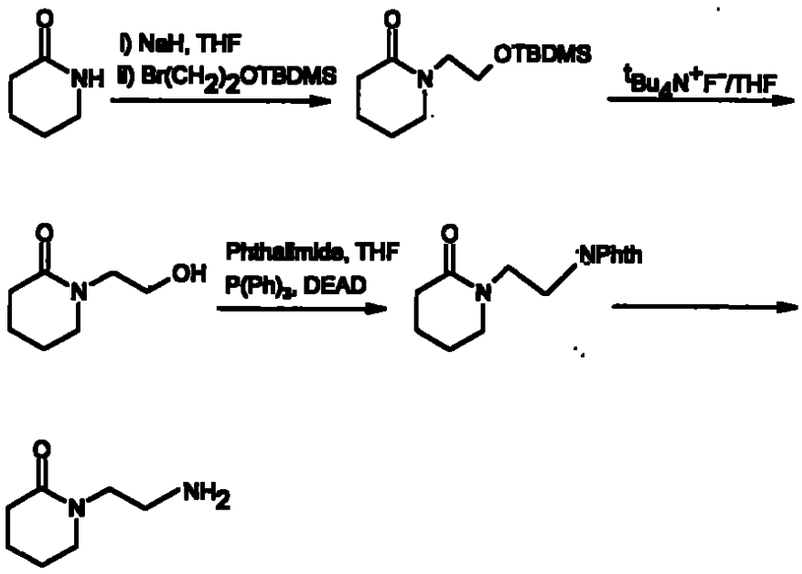
Preferred conditions for removal of protecting group $Prot^3$ from IX comprise treatment of IX with sodium hydroxide (1N) in methanol at room temperature for 22 hours.

Scheme 3



Compounds of formula IIIb, i.e. compounds of general formula III where n is 2 and Y is 2-oxopiperidino, may be prepared according to reaction scheme 4.

Scheme 4



(IIIb)

Compounds of formula IIIc where n is 1 or 2, may be prepared according to reaction scheme 5. Compounds of formula XII are protected at the amine moiety with a suitable protecting group $Prot^4$ to form compounds of formula XIII. A preferred protecting group is *tert*-butyloxycarbonyl. Compounds of formula XIII are reacted under typical acid/amine coupling conditions with $NHR^{11}R^{12}$ to form compounds of formula XIV, which on deprotection form compounds of formula IIIc.

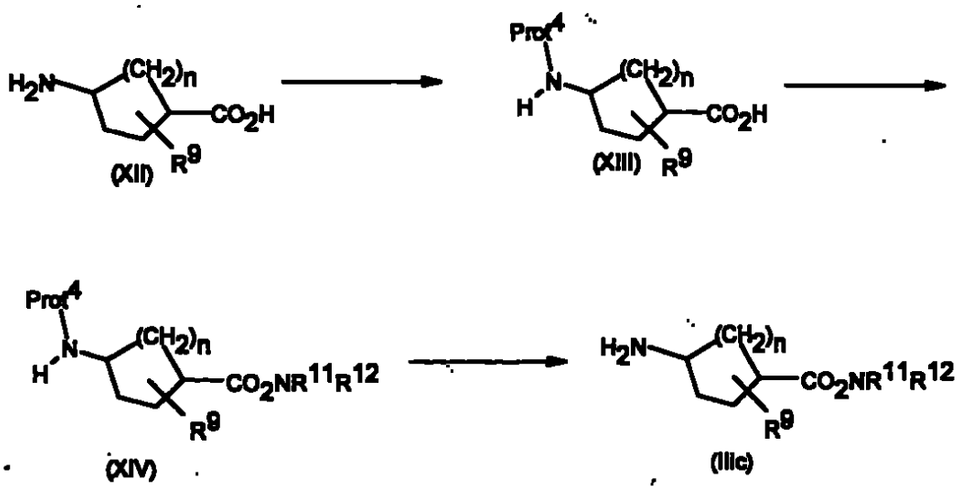
Typical reaction conditions for introducing the *tert*-butyloxycarbonyl protecting group comprise treating XII with (*tert*-butyloxycarbonyl)$_2$O in dioxan and 2N sodium hydroxide at room temperature for 18 hrs.

Typical acid/amine coupling conditions comprise treating XIII and $NHR^{11}R^{12}$ with benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PYBOP), 1-hydroxybenzotrazole hydrate (HOBT), Hünigs base, an amine (eg

triethylamine), in dimethylformamide at room temperature for 2hrs. Alternatively, XIII and $NHR^{11}R^{12}$ may be treated with1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOBT, N-methyl morpholine (NMM), in dimethylformamide at room temperature for 18 hrs.

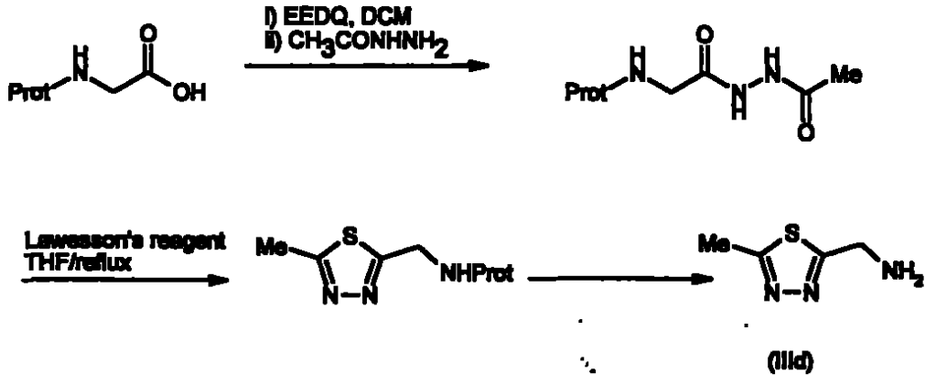
Typical reaction conditions for deprotection when $Prot^4$ is *tert*-butyloxycarbonyl comprise reacting XIV with hydrochloric acid or trifluoroacetic acid in dichloromethane at room temperature for 2 to 4 hrs

Scheme 5



Compounds of formula IIId can be prepared according to reaction scheme 6. The protecting group is preferably *tert*-butyloxycarbonyl, which is removed under standard conditions, as previously described.

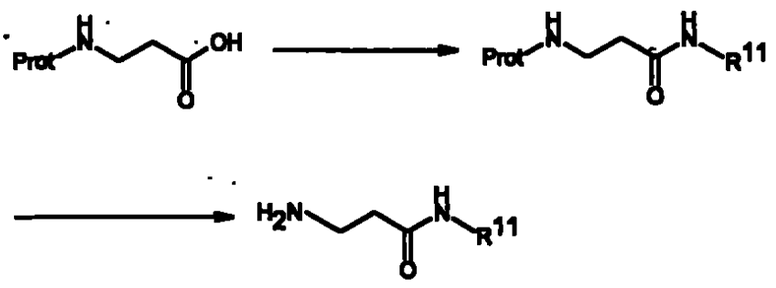
Scheme 6



(IIId)

Compounds of formula IIIe are prepared according to reaction scheme 7 using standard acid/amine coupling reactions, as previously described. The protecting group is preferably benzyloxycarbonyl which may be removed under standard conditions, typically palladium on charcoal (5-10%) in ethanol at room temperature and 50 psi for 4 hrs.

Scheme 7

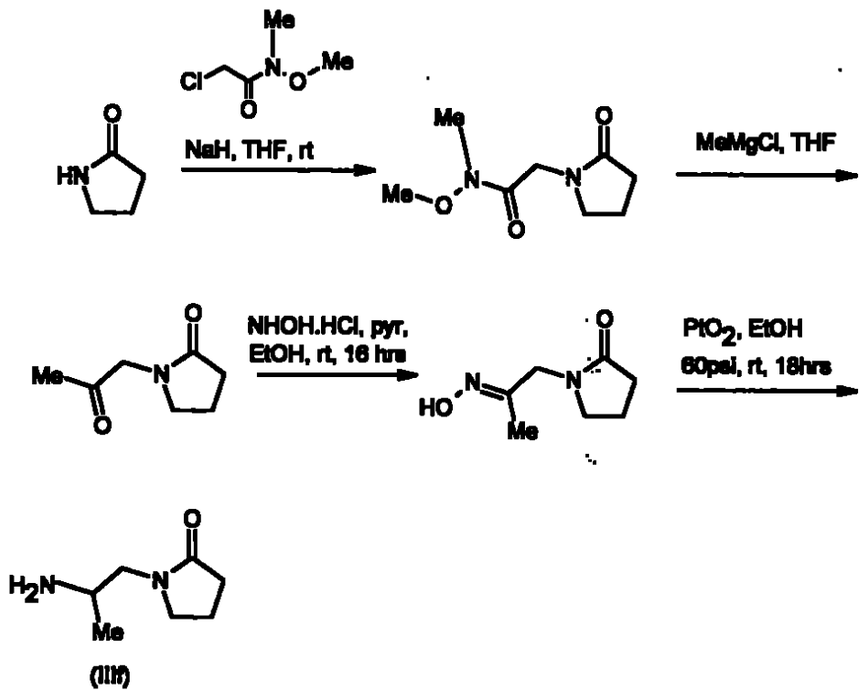


(IIIe)

Compounds of formula IIIf may be prepared according to reaction scheme 8.

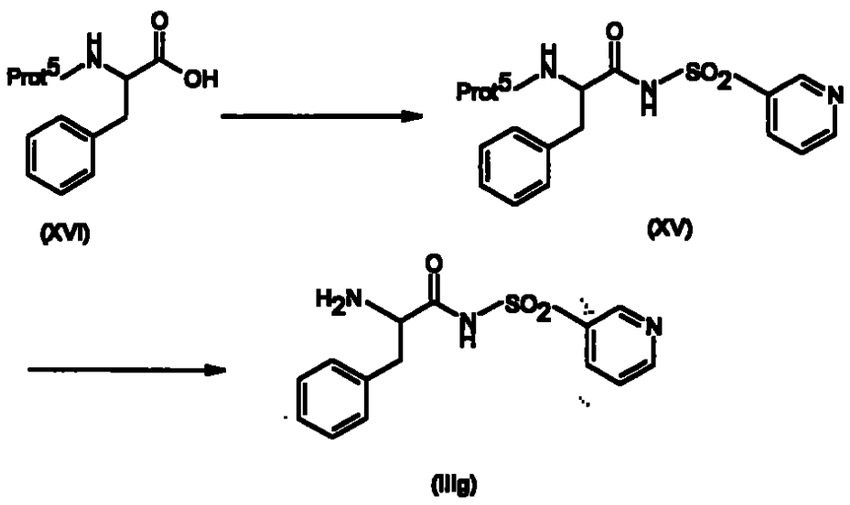
419

Scheme 8



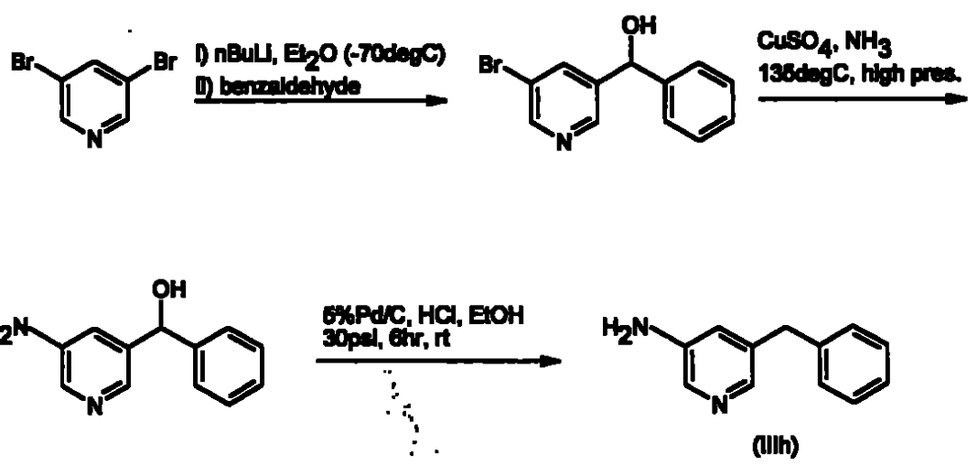
Compounds of formula IIIg may be prepared in two steps according to reaction scheme 9. As a first step, compounds of formula XV are prepared from compounds of formula XVI using standard acid/amine coupling methodology analogous to the acid/amine coupling conditions described for reaction scheme 1. Prot^5 represents a suitable leaving group, preferably *tert*-butyloxycarbonyl. The second step comprises removal of Prot^5. When Prot^5 is *tert*-butyloxycarbonyl then preferred reaction conditions comprise treatment with hydrochloric acid in diethyl ether/ethyl acetate at room temperature for 18 hrs.

Scheme 9



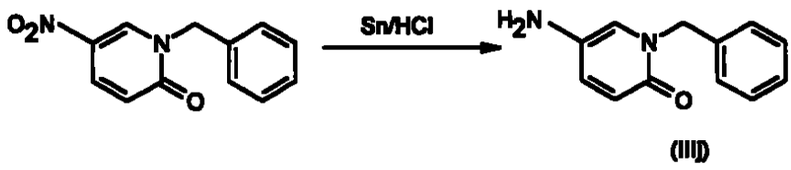
Compounds of formula IIIh may be prepared in three steps according to reaction scheme 10.

Scheme 10



Compounds of formula IIIj may be prepared by reduction of a nitro group according to reaction scheme 11.

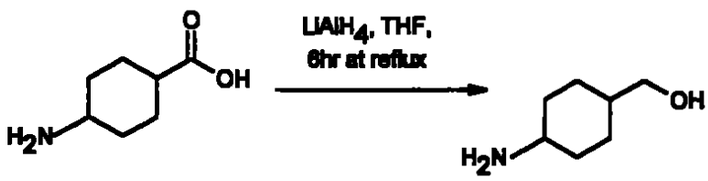
Scheme 11

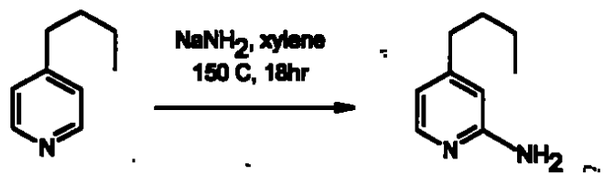


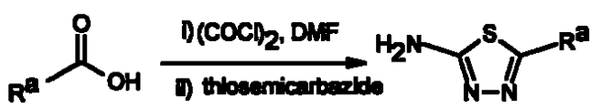
(III)

Further methods for preparing compounds of formula III are give in Scheme 12 below, where R^a is C_{1-6}alkyl or alkoxy.

Scheme 12







All of the above reactions and the preparations of novel starting materials used in the preceding methods are conventional. Appropriate reagents and reaction conditions for their performance or preparation as well as procedures for isolating the desired products will be well-known to those skilled in the art with reference to literature precedents and the Examples and Preparations hereinbelow.

A pharmaceutically acceptable salt of a compound of the formula (I) may be readily prepared by mixing together solutions of a compound of the formula (I) and the desired acid or base, as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent.

It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention . "Active ingredient" means a compound according to formula I or a pharmaceutically acceptable salt thereof.

Formulation 1: A tablet is prepared using the following ingredients:

	weight/mg
Active ingredient	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	5
Total	665

the components are blended and compressed to form tablets each weighing 665mg.

Formulation 2: An intravenous formulation may be prepared as follows:

Active ingredient	100mg
Isotonic saline	1,000ml

The invention additionally includes:

(i) a pharmaceutical composition including a compound of the second aspect of the invention, together with a pharmaceutically acceptable excipient, diluent or carrier;

(ii) a compound of the second aspect of the invention for use as a medicament;

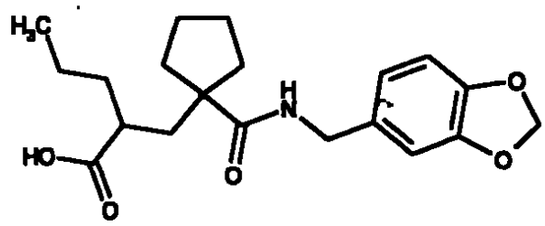
(iii) a method of treating sexual dysfunction (preferably FSD) in a mammal including treating said mammal with an effective amount of a compound of the invention; and

(iv) a sexual dysfunction treating pharmaceutical composition comprising a compound of the invention together with a pharmaceutically acceptable excipient, diluent or carrier.

The following Examples illustrate the preparation of the compounds of general formula I.

Example 1

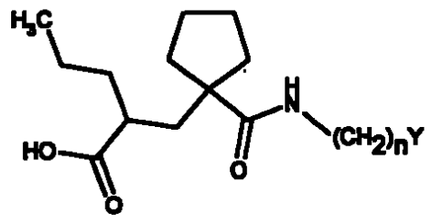
2-({1-[(1,3-Benzodioxol-5-ylamino)carbonyl]cyclopentyl}methyl)pentanoic acid



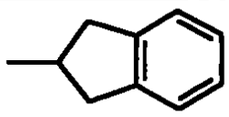
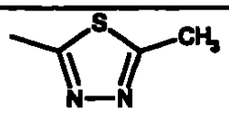
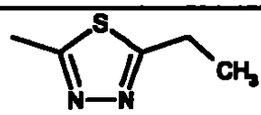
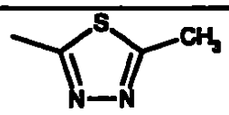
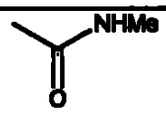
Trifluoroacetic acid (5ml) was added to a solution of the *tert*-butyl ester from preparation 34 (130mg, 0.31mmol) in dichloromethane (5ml), and the solution stirred at room temperature for 4 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with toluene and dichloromethane to afford the title compound as a clear oil, 112 mg, ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.83 (t, 3H), 1.22-1.40 (m, 3H), 1.50-1.72 (m, 8H), 1.95 (m, 1H), 2.10 (m, 2H), 2.19 (m, 1H), 4.30 (m, 2H), 5.93 (s, 2H), 5.99 (bs, 1H), 6.74 (m, 3H); LRMS: m/z 380 (MH^-).

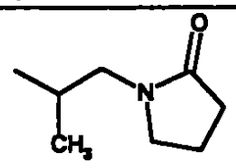
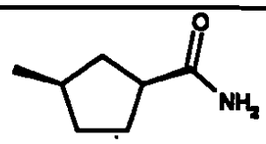
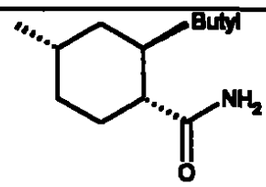
Examples 2 to 9

Compounds of formula Ic, i.e. compounds of general formula I where R^1 is propyl, were prepared from the corresponding *tert*-butyl ester, following a similar procedure to that described in Example 1.



(Ic)

Ex	n	R	Yield	Data
2[1]	0		78	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (s, 3H), 1.17-2.04 (m, 14H), 2.27-2.38 (m, 1H), 2.64-2.80 (m, 2H), 3.20-3.31 (m, 2H), 4.60-4.72 (m, 1H), 5.97 (d, 1H), 7.03-7.18 (m, 4H). LRMS : m/z 343.8 (M^+).
3[2,3]	0		81	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.30-1.42 (m, 4H), 1.59-1.81 (m, 7H), 2.18 (m, 1H), 2.30 (m, 1H), 2.42 (m, 1H), 2.55 (m, 1H), 2.61 (s, 3H). LRMS : m/z 324 (MH^+). Mp 184-186°C Anal. Found: C, 55.50; H, 7.22; N, 12.61. $C_{16}H_{23}N_3O_3S$ requires C, 55.36; H, 7.14; N, 12.91%.
4[3]	0		86	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.92 (t, 3H), 1.35 (t, 3H), 1.25-1.80 (m, 11H), 2.20-2.50 (m, 4H), 2.95 (q, 2H), 12.10 (bs, 1H). LRMS : m/z 339.8 (MH^+) Anal. Found: C, 56.46; H, 7.46; N, 12.36. $C_{16}H_{25}N_3O_3S$ requires C, 56.62; H, 7.44; N, 12.37%.
5[2]	1		81	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 1.20-1.70 (m, 11H), 1.90-2.20 (m, 3H), 2.25 (m, 1H), 2.70 (s, 3H), 4.75 (m, 2H), 7.10 (bs, 1H). LRMS : m/z 340.6 (MH^+)
6[2]	2		45	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.25-1.40 (m, 3H), 1.41-1.70 (m, 8H), 1.92 (m, 1H), 2.00-2.18 (m, 2H), 2.38 (m, 1H), 2.42 (t, 2H), 2.80 (d, 3H), 3.40-3.60 (m, 2H), 6.50 (bs, 1H), 6.74 (bs, 1H). LRMS : m/z 313.2 (MH^+)

Ex	n	R	Yield	Data
7	0		93	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.19 (d, 3H), 1.21-1.69 (m, 11H), 1.89-2.10 (m, 5H), 2.30 (m, 1H), 2.41 (m, 2H), 2.95 (m, 1H), 3.35 (m, 1H), 3.63 (m, 2H), 4.20 (m, 1H), 6.58-6.70 (m, 1H). LRMS : m/z 353.1 (MH^+)
8	0		99	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.39 (m, 3H), 1.41-2.10 (m, 1H), 2.80 (m, 1H), 4.35 (m, 17H), 5.81 (d, 1H), 6.30 (bs, 0.5H), 6.43 (bs, 0.5H), 7.40 (bd, 0.5H), 7.61 (bd, 0.5H). LRMS : m/z 339.8 (MH^+)
9	0			^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (m, 6H), 1.08-2.08 (m, 29H), 4.29 (m, 1H), 5.95 (d, 1H), 6.43 (s, 1H), 7.80 (d, 1H). LRMS : m/z 409.5 (MH^+)

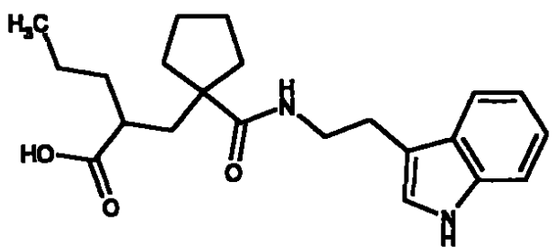
1 = additionally purified by column chromatography on silica gel using ethyl acetate:pentane as eluant.

2 = additionally purified by column chromatography on silica gel using dichloromethane:methanol as eluant.

3 = recrystallised from ether

Example 10

2-{[1-({[2-(1*H*-Indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid

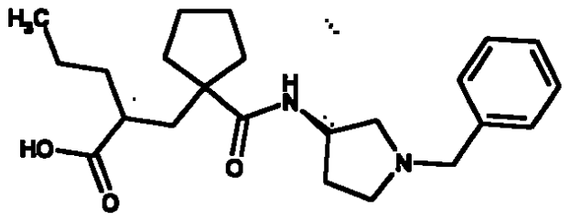


Trifluoroacetic acid (2.61ml, 33.9mmol) was added to a solution of the *tert*-butyl ester from preparation 44 (482mg, 1.13mmol) and anisole (1.23ml, 11.3mmol) in dichloromethane (4ml), and the reaction stirred at room temperature for 4 hours. The mixture was washed with water, then brine, dried ($MgSO_4$), concentrated under reduced pressure and the residue azeotroped with toluene. The residual brown oil was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant, and re-columned using an elution gradient of ethyl acetate:pentane (30:70 to 50:50) to afford the title compound as

a clear foam, 136mg, 32%; ^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.82 (s, 3H), 1.16-1.77 (m, 12H), 1.78-2.03 (m, 2H), 2.36 (m, 1H), 2.97 (m, 2H), 3.61 (m, 2H), 5.83 (m, 1H), 7.04 (s, 1H), 7.09-7.23 (m, 2H), 7.39 (d, 1H), 7.61 (d, 1H), 8.15 (m, 1H); LRMS : m/z 371.8 (MH^+.

Example 11

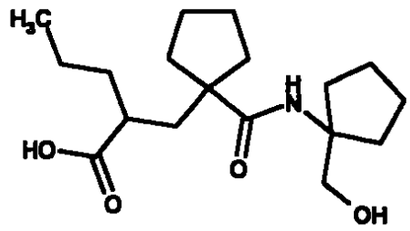
2-[[1-({[(3*S*)-1-Benzylpyrrolidinyl]amino}carbonyl)cyclopentyl]methyl]pentanoic acid



A solution of the *tert*-butyl ester from preparation 45 (70mg, 0.16mmol) in trifluoroacetic acid (1ml) and dichloromethane (1ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with dichloromethane. The residue was partitioned between water (1ml) and ethyl acetate (5ml), and the pH of the aqueous layer adjusted to 6 using sodium bicarbonate solution. The layers were separated, the organic phase dried (Na_2SO_4), evaporated under reduced pressure and the residue azeotroped with dichloromethane, to give the title compound as a beige foam, 45mg, 73%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-2.95 (m, 19H), 3.52 (m, 1H), 3.75 (m, 1H), 3.95 (m, 1H), 4.25 (m, 1H), 4.45 (m, 1H), 6.96 (bs, 1H), 7.39 (m, 5H); LRMS : m/z 387 (MH^+); Anal. Found: C, 61.11; H, 7.69; N, 6.00. $C_{23}H_{34}N_2O_3$:CH_2Cl_2 requires C, 61.14; H, 7.70; N, 5.94%.

Example 12

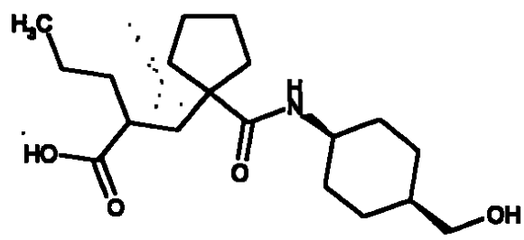
2-[[1-({[1-(Hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid



A solution of the *tert*-butyl ester from preparation 33 (38mg, 0.1mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with toluene and then dichloromethane to give a colourless gum. This was suspended in a solution of potassium carbonate (50mg, 0.3mmol) in methanol, and the mixture stirred for 2 hours at room temperature. The methanol was removed under reduced pressure, the residual aqueous mixture diluted with water (20ml), and acidifed to pH 2 using 2N hydrochloric acid. This solution was extracted with ethyl acetate (2x20ml), and the combined organic solutions dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil, 32mg, 97%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.20-1.40 (m, 3H), 1.41-1.90 (m, 17H), 2.01-2.20 (m, 2H), 2.40 (m, 1H), 3.71 (dd, 2H), 5.80 (bs, 1H); LRMS : m/z 326.1 (MH^+)

Example 13

Cis-2-[[1-({[4-(Hydroxymethyl)cyclohexyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid

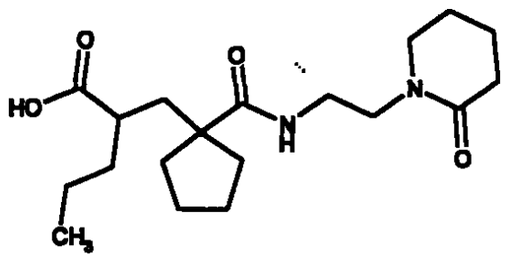


The title compound was obtained as a colourless gum in 68%, from the *tert*-butyl ester from preparation 43, following the procedure described in example 12,

except the product was additionally purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.21-1.40 (m, 6H), 1.52-1.70 (m, 15H), 1.92-2.11 (m, 3H), 2.39 (m, 1H), 3.55 (d, 2H), 4.01 (m, 1H), 5.90 (m, 1H); LRMS : m/z 340.3 (MH^+).

Example 14

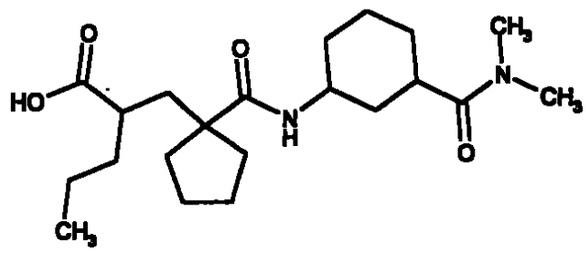
2-{[1-({[2-(2-Oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



Hydrogen chloride gas was bubbled through an ice-cold solution of the *tert*-butyl ester from preparation 47 (43mg, 0.105mmol) in dichloromethane (10ml), for 20 minutes. The solution was then stirred at room temperature for 3 hours. The mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give a glass-like solid. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound, 6mg; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.36 (m, 4H), 1.41-1.69 (m, 7H), 1.79 (m, 4H), 1.90-2.10 (m, 3H), 2.30 (m, 1H), 2.38 (t, 2H), 3.30-3.60 (m, 6H), 7.00 (bs, 1H); LRMS : m/z 351 $(M-H)^-$.

Example 15

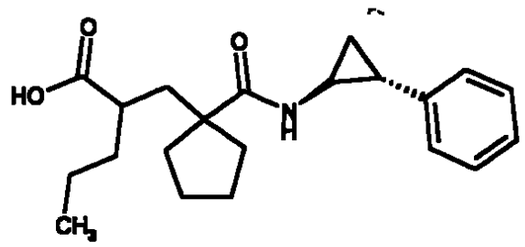
2-({1-[({3-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl) pentanoic acid



The title compound was obtained as a solid in 85% yield, from the *tert*-butyl ester from preparation 42, following a similar method to that described in example 14, except that dichloromethane:methanol:acetic acid (95:3:2) was used as the chromatographic eluant; ^{1}H NMR ($CDCl_3$, 400MHz) d: 0.89 (t, 3H), 1.09-1.76 (m, 12H), 1.80-2.17 (m, 10H), 2.37 (m, 1H), 2.68 (m, 1H), 2.95 (s, 3H), 3.04 (s, 3H), 3.83 (m, 1H), 6.06 (m, 1H); LRMS : m/z 381 (MH^+); Anal. Found: C, 63.31; H, 9.17; N, 6.53. $C_{21}H_{36}N_2O_4$;H_2O requires C, 63.29; H, 9.61; N, 7.03%.

Example 16

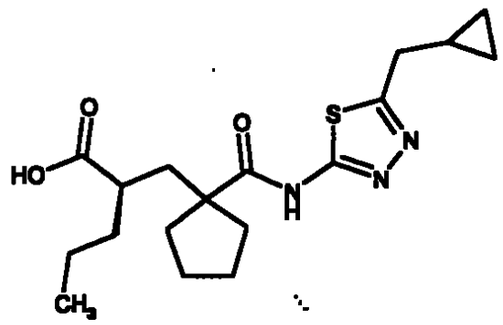
2-[[1-({[(1*R*,2*R*)-2-Phenylcyclopropyl]amino}carbonyl)cyclopentyl]-methyl]pentanoic acid



The title compound was obtained quantitatively as an orange gum from the *tert*-butyl ester from preparation 46, following a similar procedure to that described in example 14; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.12-2.14 (m, 17H), 2.38 (m, 1H), 2.87 (m, 1H), 6.10 (s, 1H), 7.13 (m, 3H), 7.25 (m, 2H); LRMS : m/z 344.3 (MH^+).

Example 17

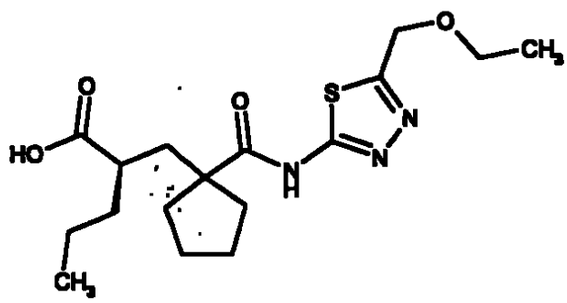
(2*R*)-2-[[1-({[5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)-cyclopentyl]methyl]pentanoic acid



A solution of the *tert*-butyl ester from preparation 50 (63mg, 0.15mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml), was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant , to give the title compound as a white foam, 46mg, 83%; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.38 (m, 2H), 0.62 (m, 2H), 0.82 (t, 3H), 1.12 (m, 1H), 1.26 (m, 2H), 1.38 (m, 1H), 1.52 (m, 1H), 1.78-1.78 (m, 6H), 1.90 (m, 1H), 2.23 (m, 4H), 2.92 (d, 2H); LRMS : m/z 366.0 (MH^+); [α]$_D$ = -7.75° (c = 0.08, methanol).

Example 18

(2*R*)-2-[[1-({[5-(Ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]-methyl]pentanoic acid

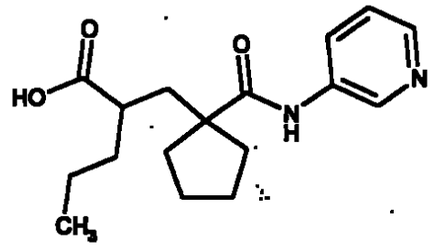


The title compound was obtained as a white foam in 62% yield, from the *tert*-butyl ester from preparation 51, following a similar procedure to that described in example 17; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.82 (t, 3H), 1.21-1.40 (m, 7H), 1.50

(m, 1H), 1.60-1.77 (m, 7H), 1.88 (m, 1H), 2.23 (m, 4H), 3.62 (q, 2H); $[\alpha]_D$ = -6.08° (c = 0.25, methanol).

Example 19

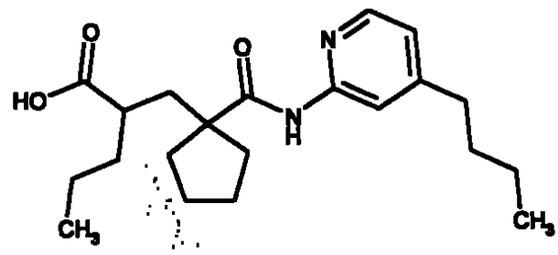
2-({1-[(3-Pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 52 (130mg, 0.33mmol) and 10% palladium on charcoal (20mg) in 95% aqueous ethanol (3ml) was hydrogenated at 15psi and room temperature for 2 hours. The reaction was filtered through Arbocel®, washing through with ethanol, and the filtrate evaporated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 103mg, 83%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 2H), 1.44 (m, 1H), 1.58-1.82 (m, 8H), 2.19 (m, 1H), 2.39 (m, 2H), 2.52 (m, 1H), 6.88 (m, 1H), 7.67 (m, 1H), 7.82 (d, 1H), 8.38 (d, 1H), 9.78 (s, 1H); LRMS : m/z 305 (MH^+).

Example 20

2-[(1-{[(4-Butyl-2-pyridinyl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid

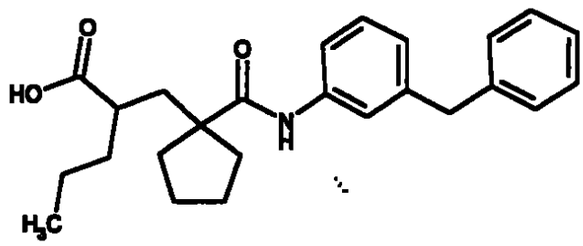


The title compound was obtained in 92% yield from the benzyl ester from preparation 55, following a similar procedure to that described in example 19; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (m, 6H), 1.28-1.50 (m, 5H), 1.58-1.81 (m, 10H),

2.20 (m, 1H), 2.40 (m, 2H), 2.58 (m, 3H), 6.70 (d, 1H), 7.68 (d, 1H), 8.22 (s, 1H), 9.90 (bs, 1H).

Example 21

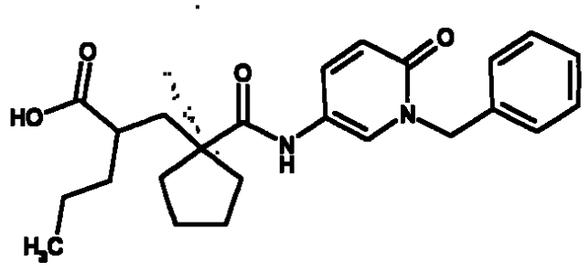
2-({1-[(3-Benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 53 (1.3mg, 2.47mmol) and 5% palladium on charcoal (130mg) in water (10ml) and ethanol (40ml) was hydrogenated at 30 psi and room temperature for 2 hours. The reaction mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure, and the residue triturated with dichloromethane. The residual gum was triturated with ether, then hexane, and dried at 50°C, to give the title compound as a solid, 0.79g, 81%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.95 (t, 3H), 1.24-1.51 (m, 3H), 1.58-1.80 (m, 7H), 1.88 (dd, 1H), 2.15 (m, 2H), 2.24 (m, 1H), 2.48 (m, 1H), 4.00 (s, 2H), 6.98 (d, 1H), 7.24 (m, 6H), 7.40 (m, 3H); Anal. Found: C, 75.48; H, 7.76; N, 3.59. $C_{25}H_{31}NO_3$;0.25H_2O requires C, 75.44; H, 7.98; N, 3.51%.

Example 22

2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)-methyl]pentanoic acid.

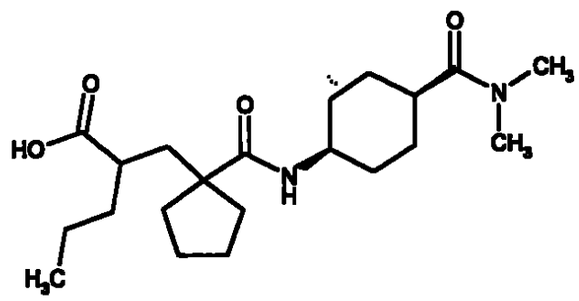


The title compound was obtained as a white foam in 51% yield from the benzyl ester from preparation 56, following a similar procedure to that described in

example 21, except, the product was purified by column chromatography on silica gel, using ethyl acetate as eluant; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.28-1.80 (m, 12H), 2.01 (m, 1H), 2.30-2.52 (m, 2H), 5.02 (dd, 2H), 6.60 (d, 1H), 7.27 (m, 5H), 7.70 (s, 1H), 8.34 (s, 1H); Anal. Found: C, 69.52; H, 7.41; N, 6.51. $C_{24}H_{30}N_2O_4$;0.25H_2O requires C, 69.45; H, 7.41; N, 6.75.

Example 23

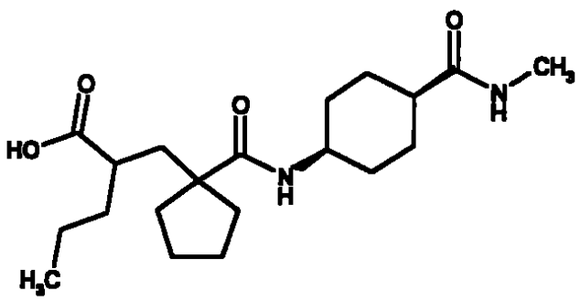
Cis-2-({1-[({4-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 58 (150mg, 0.33mmol) and 10% palladium on charcoal (20mg) in water (0.3ml) and ethanol (3.5ml) was hydrogenated at 15 psi and room temperature for 3 days. The reaction mixture was filtered through Arbocel®, and the filtrate concentrated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 85mg, 65%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.29-1.96 (m, 18H), 2.01-2.23 (m, 4H), 2.37 (m, 1H), 2.62 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.96 (m, 1H), 5.98 (m, 1H); LRMS : m/z 381.8 (MH^+); Anal. Found: C, 63.81; H, 9.58; N, 6.99. $C_{21}H_{36}N_2O_4$;0.2CH_2Cl_2 requires C, 64.06; H, 9.23; N, 7.05%.

Example 24

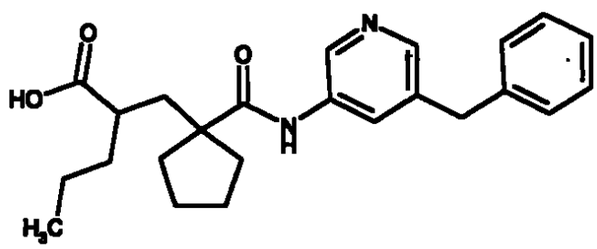
Cis-2-{[1-[({4-[(Methylamino)carbonyl]cyclohexyl}amino)carbonyl]cyclopentyl]-methyl}pentanoic acid



The title compound was obtained as a white solid in 34% yield from the benzyl ester from preparation 59, following the procedure described in example 23; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.90 (t, 3H), 1.26-2.02 (m, 20H), 2.19 (m, 3H), 2.39 (m, 1H), 2.82 (d, 3H), 4.00 (m, 1H), 5.69 (m, 1H), 6.00 (d, 1H); LRMS : m/z 365 ($M-H^-$).

Example 25

2-[(1-{[(5-Benzyl-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid.

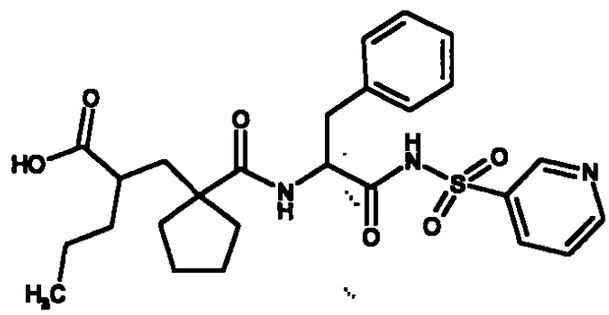


A mixture of the benzyl ester from preparation 54 (850mg, 1.76mmol) and 5% palladium on charcoal (100mg) in 20% aqueous ethanol (30ml) was hydrogenated at 30 psi and room temperature for 2 hours. The mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure, and the residue azeotroped with dichloromethane to give the title compound as a foam, 0.63g; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.92 (t, 3H), 1.30-1.83 (m, 11H), 2.07 (m, 1H), 2.42 (m, 3H), 3.82 (s, 2H), 7.15-7.38 (5H), 7.80 (s, 1H), 8.48 (s, 1H), 8.59 (s, 1H), 8.62 (s, 1H); Anal. Found: C, 72.29; H, 7.70; N, 6.90. $C_{24}H_{30}N_2O_3$:0.25H_2O requires C, 72.24; H, 7.70; N, 7.02%.

427

Example 26

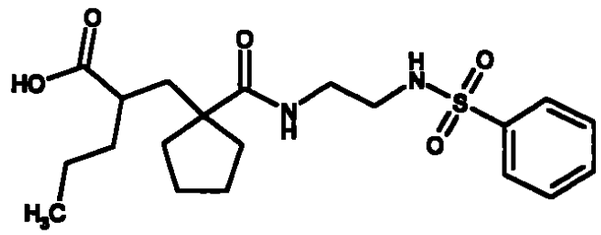
2-({1-[({1-Benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoic acid.



A mixture of the benzyl ester from preparation 57 (918mg, 1.52mmol) and 10% palladium on charcoal (90mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 4 ½ hours. Tlc analysis showed starting material remaining, so additional catalyst (70mg) was added, and the mixture hydrogenated for a further 18 hours. Tlc analysis, again showed starting material remaining, so further catalyst (70mg) was added, and hydrogenation continued for an additional 6 hours. The reaction mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:acetic acid:ethanol (99:1:0 to 79.1:0.9:20) to afford the title compound as a white foam, 271mg, 35%; ^{1}H NMR (DMSOd_6, 300MHz) δ: 0.75 (m, 3H), 0.96-1.42 (m, 11H), 1.61-1.99 (m, 4H), 2.75-3.02 (m, 2H), 4.45 (m, 1H), 7.20 (m, 6H), 7.62 (m, 1H), 8.24 (m, 1H), 8.83 (s, 1H), 9.01 (s, 1H), 11.98 (bs, 1H), 12.70 (bs, 1H); IR (KBr disc) 1185, 1195 (m), 1455, 1515, 1640, 1704, 2870, 2930, 2960 (s).

Example 27

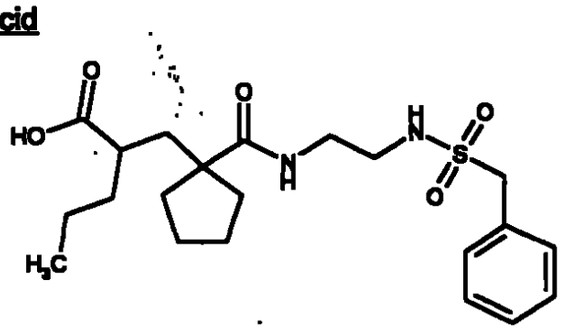
2-({1-[({2-[(Phenylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}-methyl)pentanoic acid



A mixture of the amine from preparation 61 (235mg, 0.72mmol), benzenesulphonyl chloride (127mg, 0.72mmol) and triethylamine (150µl, 1.08mmol) in dichloromethane (6ml) was stirred at room temperature for 2 days. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to give a clear oil. This was then dissolved in trifluoroacetic acid (3ml) and dichloromethane (3ml) and the solution stirred at room temperature for 6 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) to afford the title compound as a clear oil, 204mg, 69%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.22-1.43 (m, 4H), 1.43-2.18 (m, 10H), 2.36 (m, 1H), 3.11 (m, 2H), 3.20-3.31 (m, 1H), 3.42-3.53 (m, 1H), 6.13-6.24 (m, 1H), 7.42-7.59 (m, 3H), 7.84 (m, 2H); LRMS : m/z 411.8 (MH^+); Anal. Found: C, 57.26; H, 7.40; N, 6.61. $C_{20}H_{30}N_2O_5S$ requires C, 57.18; H, 7.22; N, 6.62%.

Example 28

2-({1-[({2-[(Benzylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}-methyl)pentanoic acid



The title compound was obtained as a clear oil in 97% yield, from the amine from preparation 61, following the procedure described in example 27, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.87 (t, 3H), 1.19-1.72 (m, 11H), 1.80-1.96 (m, 1H), 2.00-2.16 (m, 2H), 2.27-2.38 (m, 1H), 2.92-3.21 (m, 3H), 3.23-3.39 (m, 1H), 4.25 (s, 2H), 5.80-6.06 (m, 1H), 6.38 (m, 1H), 7.29-7.43 (m, 5H); LRMS : m/z 425.8 (MH^+).

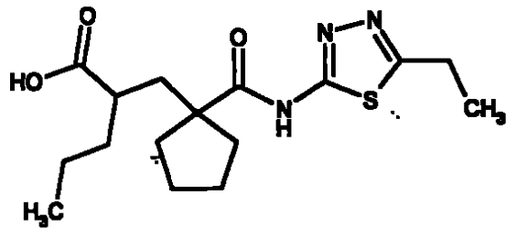
Example 29

(-)-2-[(1-[[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)methyl]pentanoic acid

and

Example 30

(+)-2-[(1-[[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)methyl]pentanoic acid



The acid from Example 4 (824mg) was further purified by HPLC using an AD column and using hexane:*iso*-propanol:trifluoroacetic acid (85:15:0.2) as eluant to give the title compound of example 29 as a white foam, 400mg, 99.5% ee, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.36 (m, 6H), 1.50-1.80 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.30 (bs, 1H), LRMS : m/z 338 (MH^+), $[\alpha]_D$ = -9.0° (c = 0.1, methanol), and the title compound of example 30 as a white foam, 386mg, 99% ee, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 6H), 1.50-1.79 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.27 (bs, 1H); LRMS: m/z 338 (MH^+); and $[\alpha]_D$ = +3.8° (c = 0.1, methanol)

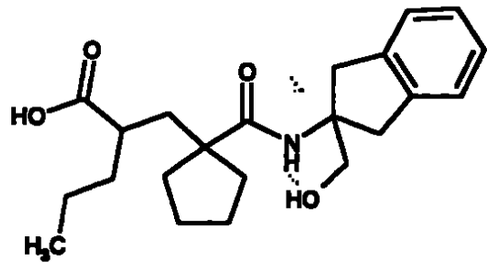
Example 31

(+)-2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]pentanoic acid

and

Example 32

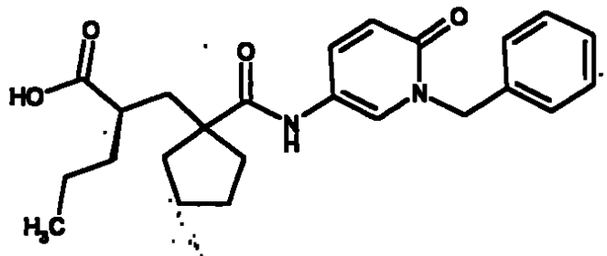
(-)-2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1H-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl]pentanoic acid



2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl]pentanoic acid (WO 9110644) was further purified by HPLC using an AD column and hexane:isopropanol:trifluoroacetic acid (90:10:0.1) as eluant, to give the title compound of example 31, 99% ee, $[\alpha]_D$ = +10.4° (c = 0.067, ethanol) and the title compound of example 32, 99% ee, $[\alpha]_D$ = -10.9° (c = 0.046, ethanol).

Example 33

(2*R*)-2-[(1-[[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]-cyclopentyl)methyl]-pentanoic acid

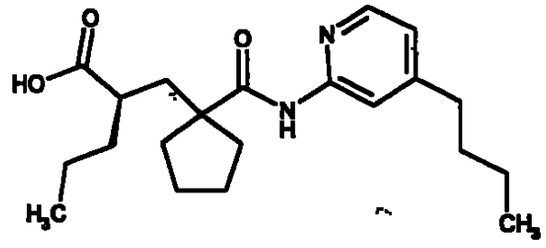


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (191mg, 1.0mmol), 1-hydroxybenzotriazole hydrate (135mg, 01.0mmol), N-methylmorpholine (165µl, 1.5mmol) and finally the amine from preparation 28 (150mg, 0.69mmol) were added to a solution of the acid from preparation 2 (284mg, 1.0mmol) in N,N-dimethylformamide (8ml), and the reaction stirred at 90°C for 18 hours. The

cooled solution was diluted with ethyl acetate (90ml), washed with water (4x50ml), and brine (50ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using ethyl acetate:pentane (30:70) to give a yellow oil, 191mg. This intermediate was dissolved in dichloromethane (3ml) and trifluoroacetic acid (3ml) and the solution stirred at room temperature for 5 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to give the title compound as a foam, 77mg, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (t, 3H), 1.20-1.76 (m, 12H), 1.93-2.02 (m, 1H), 2.20-2.46 (m, 3H), 4.95 (d, 1H), 5.04 (d, 1H), 6.61 (d, 1H), 7.21 (m, 1H), 7.50 (s, 1H), 8.23 (s, 1H); LRMS : m/z 411.6 $(MH)^+$; $[\alpha]_D$ = -3.8° (c = 0.052, ethanol).

Example 34

(2*R*)-2-[(1-[[(4-Butyl-2-pyridinyl)amino]carbonyl]cyclopentyl)methyl]pentanoic acid

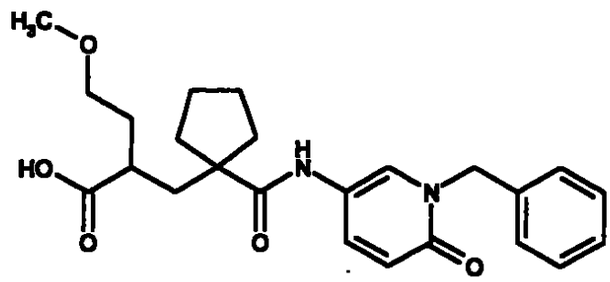


The title compound was obtained in 43% yield from the acid from preparation 2 and the amine from preparation 30, following a similar procedure to that described in example 33, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80-1.00 (m, 6H), 1.22-1.84 (m, 18H), 2.03-2.56 (m, 3H), 2.77 (m, 1H), 7.14 (d, 1H), 8.08 (d, 1H), 8.23 (s, 1H), 11.71 (brs, 1H).

LRMS : m/z 361.7 $(MH)^+$, $[\alpha]_D$ = -1.4° (c = 0.14, ethanol).

Example 35

2-[(1-[[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]cyclopentyl)methyl]-4-methoxybutanoic acid

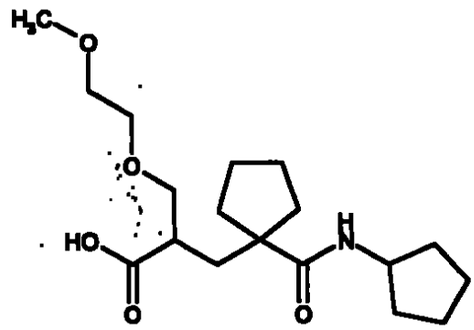


A mixture of the benzyl ester from preparation 62 (850mg, 1.64mmol), and 5% palladium on charcoal (250mg) in 40% aqueous ethanol (21ml), was hydrogenated at 30 psi and room temperature for 30 minutes. The reaction mixture was filtered through Hyflo®, and the filtrate evaporated under reduced pressure. The residual foam was purified by column chromatography on silica gel using dichloromethane:methanol (97:3) as eluant to give the title compound as a white foam, 550mg, 79%; ^{1}H NMR (DMSO-d_6, 300MHz) δ: 1.24-2.17 (m, 12H), 2.18-2.31 (m, 1H), 3.07 (s, 3H), 3.21 (t, 2H), 5.08 (s, 2H), 6.63 (d, 1H), 7.23-7.41 (m, 5H), 7.72 (d, 1H), 8.24 (s, 1H).

Anal. Found: C, 67.46; H, 7.18; N, 6.24. $C_{24}H_{30}N_2O_5$ requires C, 67.58; H, 7.09; N, 6.57%.

Example 36

3-[1-[(Cyclopentylamino)carbonyl]cyclopentyl]-2-[(2-methoxyethoxy)methyl]propanoic acid

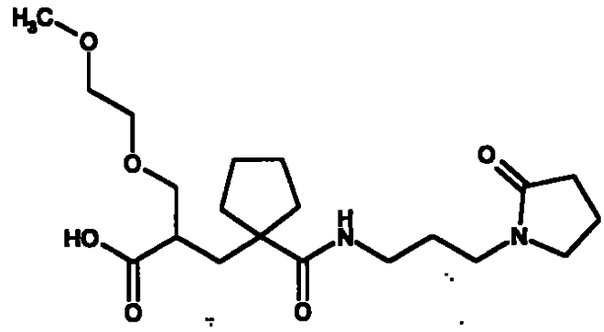


A solution of the *tert*-butyl ester from preparation 64 (320mg, 0.80mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room

temperature for 8 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) to give the title compound as a clear oil, 171mg, 62%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.29-1.40 (m, 2H), 1.42-1.69 (m, 10H), 1.75 (dd, 1H), 1.87-2.03 (m, 5H), 2.64 (m, 1H), 3.34 (s, 3H), 3.43-3.52 (m, 3H), 3.57 (m, 2H), 3.61 (m, 1H), 4.08-4.20 (m, 1H), 5.89 (d, 1H); LRMS : m/z 340 (MH^+).

Example 37

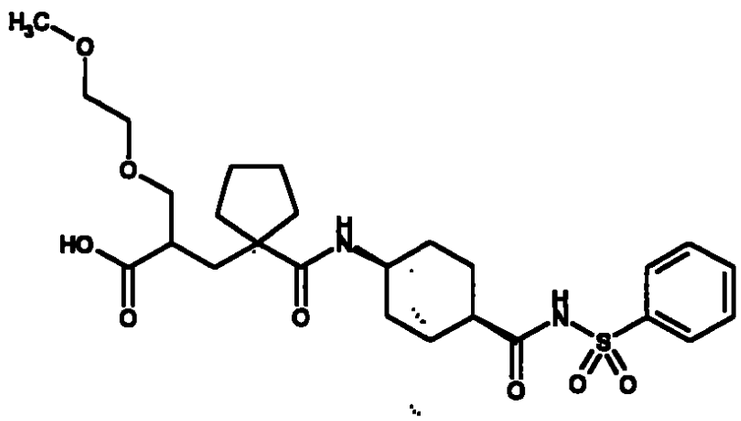
3-(2-Methoxyethoxy)-2-[[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)-cyclopentyl]methyl]propanoic acid



The title compound was obtained as a clear oil in 57% yield from the *tert*-butyl ester of preparation 65, following the procedure described in example 36, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.56-1.78 (m, 8H), 1.94-2.17 (m, 6H), 2.44 (m, 2H), 2.68-2.76 (m, 1H), 3.10-3.21 (m, 1H), 3.22-3.31 (m, 1H), 3.37 (s, 3H), 3.40 (m, 2H), 3.44-3.56 (m, 5H), 3.60 (m, 2H), 3.68 (m, 1H), 6.91-7.01 (m, 1H); LRMS : m/z 398.7 (M^+)

Example 38

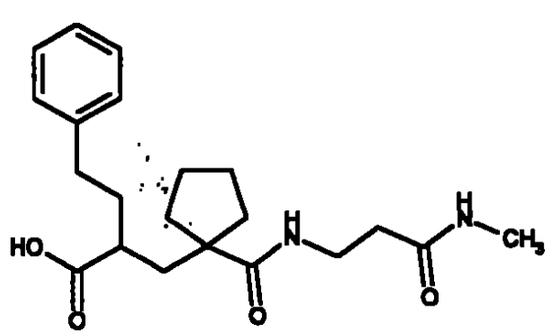
cis-3-(2-Methoxyethoxy)-2-[(1-[[(4-[[(phenylsulfonyl)amino]carbonyl]cyclohexyl)-amino]carbonyl]cyclopentyl)methyl]propanoic acid



A solution of the *tert*-butyl ester from preparation 66 (446mg, 0.75mmol) in dichloromethane (5ml) and trifluoroacetic acid (5ml) was stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and the residue azeotroped with dichloromethane, then toluene, and finally ether, to afford the title compound as a white foam, 385mg, 95%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.48-2.17 (m, 18H), 2.40 (s, 1H), 2.66 (s, 1H), 3.37 (s, 3H), 3.50-3.70 (m, 6H), 3.94 (s, 1H), 6.10 (d, 1H), 6.59 (s, 1H), 7.55 (t, 2H), 7.61 (m, 1H), 8.02 (d, 2H), 9.11 (s, 1H); Anal. Found: C, 54.88; H, 6.90; N, 5.04. $C_{28}H_{38}N_2O_8S;1.7H_2O$ requires C, 57.97; H, 7.11; N, 5.20%.

Example 39

2-[[1-([[3-(Methylamino)-3-oxopropyl]amino]carbonyl)cyclopentyl]methyl]-4-phenylbutanoic acid

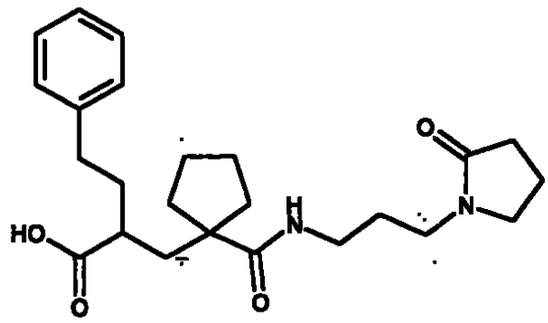


A mixture of the benzyl ester from preparation 68 (160mg, 0.34mmol) and 10% palladium on charcoal (100mg) in ethanol (30ml) was hydrogenated at room

temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel and the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:acetic acid (95:5:0 to 95:5:0.5) to afford the title compound as a white foam, 100mg, 79%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.40-1.70 (m, 8H), 1.95 (m, 3H), 2.10 (m, 1H), 2.35 (d, 3H), 2.59 (m, 2H), 2.75 (t, 3H), 3.42 (m, 2H), 6.25 (bs, 1H), 6.70 (bs, 1H), 7.13-7.25 (m, 5H); and LRMS: m/z 375.0 (MH^+).

Example 40

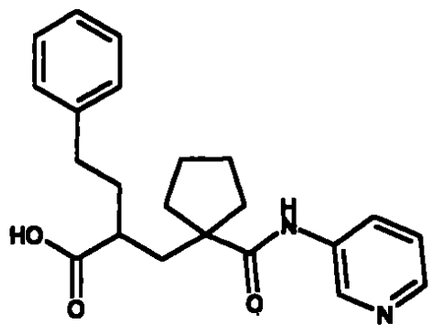
2-[[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]-methyl]-4-phenylbutanoic acid.



A mixture of the benzyl ester from preparation 67 (780mg, 1.55mmol) and 10% palladium on charcoal (100mg) in ethanol:water (90:10 by volume), (30ml) was hydrogenated at room temperature under 60psi H_2 pressure for 1.5 hours. The catalyst was filtered off, and the filtrate evaporated under reduced pressure to provide the title compound as a white foam, 473mg, 74%; ^{1}H NMR ($CDCl_3$, 300MHz) d : 1.26-1.77 (m, 10H), 1.78-2.46 (m, 11H), 2.49-2.70 (m, 2H), 2.95-3.36 (m, 4H), 6.92-7.38 (m, 5H); Anal. Found: C, 64.05; H, 7.73; N, 6.22. $C_{24}H_{34}N_2O_4$;0.75H_2O requires C, 65.88; H, 7.83; N, 6.40%.

Example 41

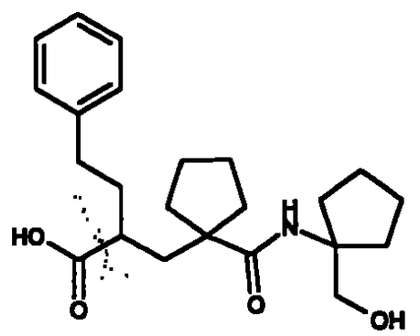
4-Phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoic acid



A mixture of the benzyl ester from preparation 71 (700mg, 1.53mmol) and 5% palladium on charcoal (70mg) in ethanol:water (90:10 by volume, 50ml) was hydrogenated at room temperature under 30 psi H_2 pressure for 5 hours. The catalyst was filtered through Arbocel®, washing well with ethanol, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant to provide the title compound as a white foam, 510mg, 91%; mp 80-85°C (collapses to a gum); ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.40-2.78 (m, 15H), 6.93-7.39 (m, 5H), 7.93 (m, 1H), 8.59 (d, 1H), 9.17 (d, 1H), 9.41 (s, 1H); Anal. Found: C, 70.83; H, 7.10; N, 7.64. $C_{22}H_{28}N_2O_3$;0.3H_2O requires C, 70.94; H, 7.22; N, 7.52%.

Example 42

2-{[1-({[1-(Hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid

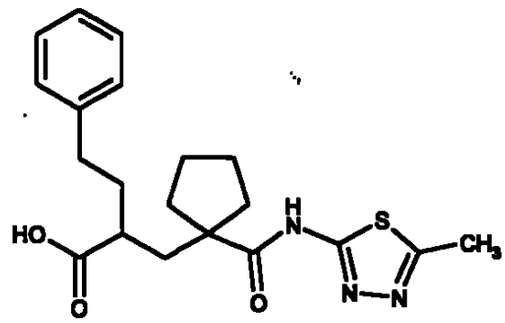


A mixture of the benzyl ester from preparation 69 (118mg, 0.25mmol) and 10% palladium on charcoal (100mg) in ethanol (20ml) was hydrogenated at room temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel ,

the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane to give the title compound as a colourless gum, 95mg, 98%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.41-1.80 (m, 17H), 1.90 (m, 1H), 1.92-2.20 (m, 3H), 2.40 (m, 1H), 2.60 (m, 2H), 3.60 (d, 1H), 3.71 (d, 1H), 5.80 (bs, 1H), 7.15-7.30 (m, 5H); LRMS : m/z 388.1 (MH^+)

Example 43

2-[(1-[[(5-Methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl]cyclopentyl)methyl]-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 70 (187mg, 0.39mmol) and 10% palladium on charcoal (80mg) in ethanol (20ml) was hydrogenated at 60 psi for 18 hours. Tlc analysis showed starting material remaining, so additional 10% palladium on charcoal (100mg) was added, and the reaction continued for a further 5 hours. Tlc analysis again showed starting material remaining, so additional catalyst (100mg) was added, and hydrogenation continued for 18 hours. The mixture was filtered through Arbocel®, and the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane. The crude product was purified by chromatography on silica gel using a Biotage® column, and dichloromethane:methanol (95:5) as eluant to afford the title compound as a clear oil, 80mg, 53%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.51-1.89 (m, 9H), 2.03 (m, 1H), 2.20 (m, 1H), 2.40 (m, 2H), 2.60 (m, 5H), 7.15-7.30 (m, 5H); LRMS : m/z 387.8 (MH^+).

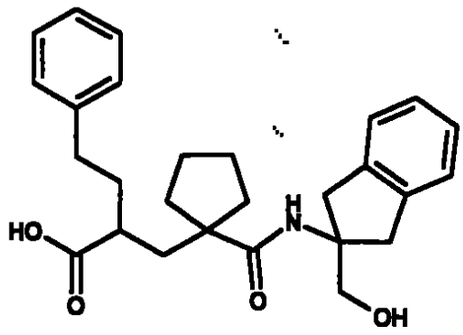
Example 44

(+)-2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-phenylbutanoic acid

and

Example 45

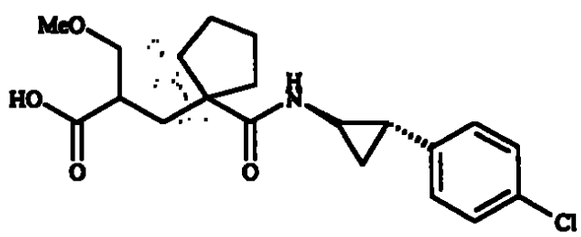
(-)-2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-phenylbutanoic acid



2-[[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]-methyl]-4-phenylbutanoic acid (WO 91/10644) may be purified by standard HPLC procedures using an AD column and hexane:isopropanol:trifluoroacetic acid (70:30:0.2) as eluant, to give the title compound of example 44, 99.5% ee; $[\alpha]_D$ = +9.1° (c = 1.76 in ethanol); and the title compound of example 45, 99.5% ee; $[\alpha]_D$ = -10.5° (c = 2.2 in ethanol).

Example 46

trans-3-[1-({[2-(4-Chlorophenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxymethyl)propanoic acid

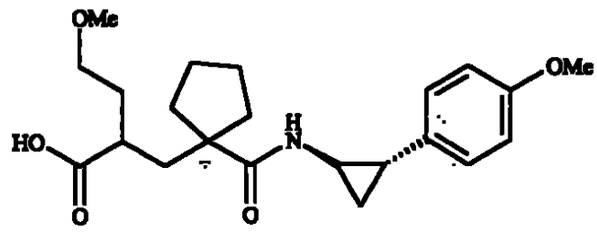


The product from preparation 72 (160mg, 0.39mmol) was taken up in 50ml DCM

and cooled to 0°C. Hydrogen chloride gas was then bubbled through the solution for 15mins and then allowed to stir at room temperature for 16h. The reaction mixture was concentrated *in vacuo* and then purified by column chromatography using 5:95 MeOH:DCM as eluant to provide the acid as a colourless film (18mg, 12%); R_f 5:95 (DCM:MeOH) 0.2; ^{1}HNMR (400MHz, $CDCl_3$) 1.04-1.18 (m, 2H), 1.20-1.36 (m, 2H), 1.36-1.79 (m, 7H), 1.83-2.08 (m, 4H), 2.57-2.66 (m, 1H), 2.73-2.83 (m, 1H), 3.27 (s, 3H, OMe), 3.32-3.41 (m, 1H), 3.48 (app. dd, 1H, CHOMe), 6.21 (s, NH), 7.03 (d, 2H, Ar), 7.18 (d, 2H, Ar); LRMS : m/z, M-H 378; HRMS Found MH+ 380.1622. Calculated MH+ 380.1623.

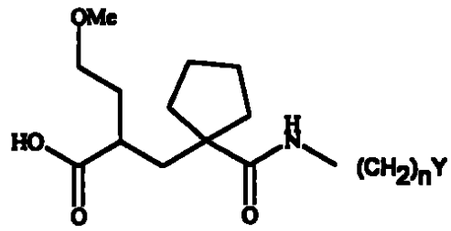
Example 47

trans-3-[1-({[2-(4-Methoxyphenyl)cyclopropyl]amino}carbonyl)cyclopentyl]-2-(methoxyethyl)propanoic acid

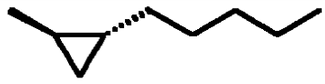
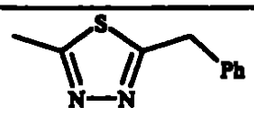
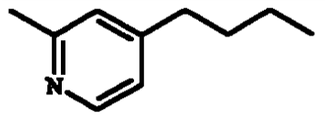
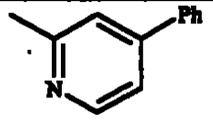
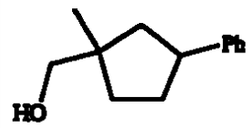
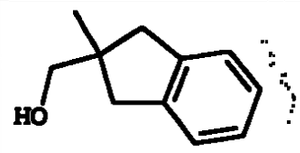


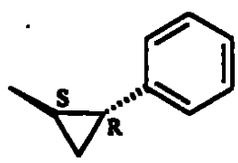
The product from preparation 81 (113mg, 0.25mmol) was taken up in a 4M solution of hydrogen chloride in dioxane (10mls) and stirred for 3h. Concentrated *in vacuo* and purified by column chromatography using 5:95 (MeOH:DCM) as eluant to provide the acid as a colourless film (45mg, 44%);
R_f 95:5 (DCM:MeOH) 0.2; LRMS : m/z, M-H, 388; ^{1}HNMR (400MHz, $CDCl_3$) 1.01-1.22 (m,.2H), 1.40-2.22 (m, 15H), 2.42-2.57 (m, 1H), 2.73-2.82 (m, 1H), 3.23 (s, 3H, OMe), 3.27-3.44 (m, 2H), 3.72 (s, 3H, OMe), 6.12 (s, 1H, NH), 6.78 (d, 2H, Ar), 7.06 (d, 2H, Ar).

Compounds of formula Id, *i.e.* compounds of formula I where R^1 is methoxyethyl, were prepared from the corresponding *tert*-butyl ester, following a similar procedure to that described in Example 47.



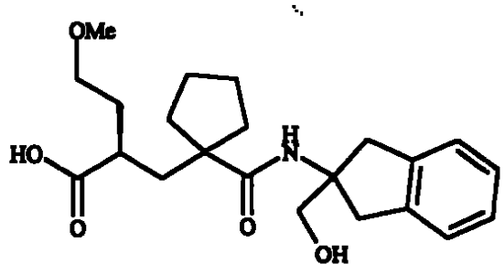
(Id)

Ex	n	Y	Data
48	0		^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.50-0.63 (m, 3H), 0.82 (t, 3H, Me), 0.77-0.84 (m, 1H), 1.01-1.18 (m, 1H), 1.20-1.78 (m,), 1.82-2.08 (m, 2H), 3.27 (s, 3H, OMe), 3.33-3.41 (m, 2H), 5.92 (s, 1H, NH). LRMS : m/z 352 (M-H)
49	0		^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.62-2.57 (m, 13H), 3.07 (s, 3H, OMe), 2.96-3.44 (m, 2H), 4.09 (s, 2H), 7.20 (brs, 5H). HRMS : Found m/z 418.1796. $C_{21}H_{27}N_3O_4S$ requires m/z 418.1795.
50	0		^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.92 (t, 3H, Me), 1.24-2.60 (m, 19H), 3.25 (s, 3H, OMe), 3.39 (t, 2H, CH_2OMe), 6.68-6.71 (m, 1H, Ar), 7.63-7.70 (m, 1H, Ar), 8.21 (s, 1H, Ar), 9.77 (brs, NH). LRMS : m/z 376 (M^+).
51	0		^{1}HNMR ($CDCl_3$, 400MHz) δ: 1.24-2.34 (m, 10H), 2.37-2.54 (m, 1H), 2.54-2.73 (m, 2H), 3.33 (s, 3H, OMe), 3.38-4.49 (m, 2H), 7.00 (d, 1H. Ar), 7.38-7.56 (m, 3H), 7.59-7.69 (m, 2H), 7.80 (t, 1H, Ar), 8.66 (s, 1H, Ar), 9.77-9.93 (m, NH). LRMS : m/z 395 (M-H).
52	0		^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.84 (br.t, 3H), 1.20-2.20 (m, 19H), 2.24-2.58 (m, 2H), 3.07-3.33 (m, 1H), 3.60-3.96 (m, 2H), 5.82-5.98 (m, 1H), 7.14-7.36 (m, 5H). LRMS m/z M-H 400
53	0		^{1}HNMR ($CDCl_3$, 400MHz) δ: 1.43-1.76 (m, 7H), 1.80-2.24 (m, 4H), 2.57-2.68 (m, 2H), 3.06 (d, 1H), 3.12 (d, 1H), 3.27 (d, 1H), 3.32 (s, 3H), 3.36-3.48 (m, 2H), 3.80 (d, 1H), 3.87 (d, 1H), 6.04 (s, 1H), 7.16-7.22 (m, 4H).

Ex	n	Y	Data
54	0		^{1}HNMR ($CDCl_3$, 400MHz) d : 1.02-1.26 (m, 2H), 1.37-1.84 (m, 7H), 1.85-2.16 (m, 4H), 2.62 (br.s, 1H), 2.80-2.93 (m, 1H), 3.29 (s, 3H, Me), 3.22-3.58 (m, 2H), 6.21 (br.s, 1H), 7.03-7.34 (m, 5H). LRMS m/z M+H 346 HRMS m/z M+H Found 346.2011. $C_{20}H_{27}NO_4$ requires 346.2013.

Example 55

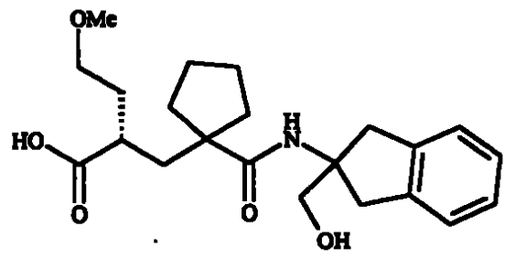
(*R*)- 2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}-4-methoxybutanoic acid



and

Example 56

(*S*)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}-4-methoxybutanoic acid



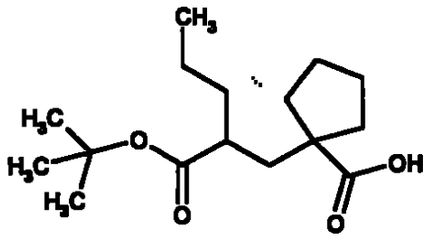
Racemic 2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}-carbonyl)-cyclopentyl]methyl}-4-methoxybutanoic acid from Example 53 was purified by HPLC using a Chiralcel OD column (250*20mm) at ambient temperature using a mixture of 70% hexane containing 0.3% TFA and 0.2% DEA and 30% IPA containing 0.3% TFA and 0.2% DEA at a flow rate of 10ml/min. Example 55 is

the R enantiomer which eluted first after 6mins (α_D 11.00 c1mg/ml in EtOH). Example 56 is the S enantiomer which eluted second after 7mins (α_D –8.62 c1.07mg/ml in EtOH).

The following Preparations describe the preparation of certain intermediates used in the preceding Examples.

Preparation 1

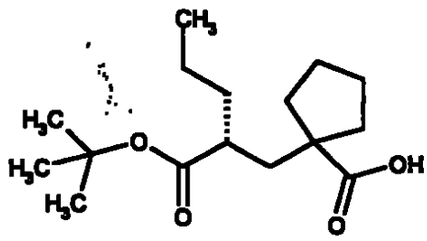
1-[2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid



A mixture of 1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (EP 274234) (23g, 81.5mmol) and 10% palladium on charcoal (2g) in dry ethanol (200ml) was hydrogenated at 30psi and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel, using ethyl acetate:pentane (40:60) as the eluant, to provide the desired product as a clear oil, 21g, 91%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); LRMS : m/z 283 $(M-H)^-$

Preparation 2

1-[(2*R*)-2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid

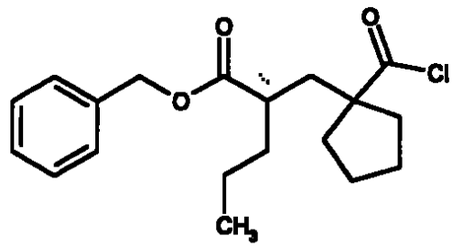


A mixture of (R)-1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic

acid (WO 9113054) (10g, 35.4mmol) and 10% palladium on charcoal (600mg) in dry ethanol (25ml) was hydrogenated at 1 atm. and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give the title compound as a yellow oil, 9.6g, 95%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); $[\alpha]_D$ = -3.3° (c = 0.09, ethanol).

Preparation 3

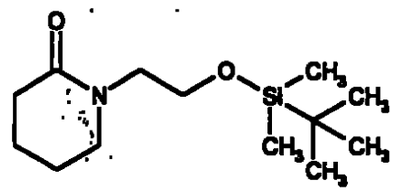
Benzyl 2-{[1-(chlorocarbonyl)cyclopentyl]methyl}pentanoate



Oxalyl chloride (1.15ml, 13.2mmol) was added to an ice-cooled solution of 1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentanecarboxylic acid (EP 274234) (2.0g, 6.3mmol) in dry dichloromethane (20ml), and the solution stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give the title compound as a golden oil, 2.1g; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.28 (m, 2H), 1.43 (m, 2H), 1.63 (m, 6H), 2.00 (m, 1H), 2.08-2.35 (m, 3H), 2.44 (m, 1H), 5.15 (s, 2H), 7.28 (m, 5H).

Preparation 4

1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-2-piperidinone

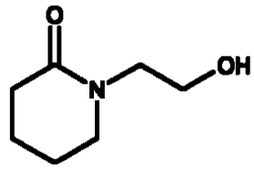


Sodium hydride (807mg, 60% dispersion in mineral oil, 20.18mmol) was added portionwise to a solution of d-valerolactam (2.0g, 20.2mmol) in tetrahydrofuran (100ml) under nitrogen. (2-Bromoethoxy)(*tert*-butyl)dimethylsilane (4.33ml,

20.2mmol) was added portionwise, and the reaction heated at 70°C for 18 hours. Water (50ml) was added to the cooled reaction, the mixture concentrated *in vacuo*, to remove the tetrahydrofuran, and extracted with ethyl acetate (200ml). The organic solution was dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 97:3) to give the title compound, 3.25g; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.00 (s, 6H), 0.83 (s, 9H), 1.75 (m, 4H), 2.35 (m, 2H), 3.39 (m, 4H), 3.75 (t, 2H); LRMS : m/z 257.9 (M^+)

Preparation 5

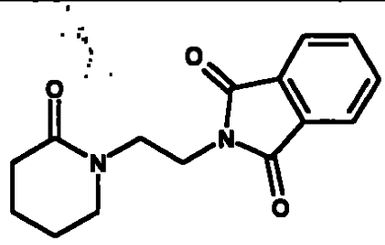
1-(2-Hydroxyethyl)-2-piperidinone



Tetra-n-butylammonium fluoride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added to a solution of the lactam from preparation 4 (3.3g, 12.8mmol) in tetrahydrofuran (50ml), and the reaction stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure, the residue azeotroped with dichloromethane, and purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 95:5) to give the title compound as an oil; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.80 (m, 4H), 2.40 (t, 2H), 3.38 (t, 2H), 3.42 (t, 1H), 3.56 (t, 2H), 3.80 (t, 2H).

Preparation 6

2-[2-(2-Oxo-1-piperidinyl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione

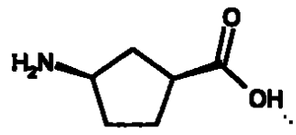


Pthalimide (952mg, 6.47mmol) was added to a solution of the product from

preparation 5 (842mg, 5.88mmol) in tetrahydrofuran (30ml), and the mixture sonicated until a solution was obtained. Polymer supported triphenyl phosphine (2.5g, 7.5mmol) and diethyl azodicarboxylate (1.15ml, 7.31mmol) were added, and the reaction stirred at room temperature for 18 hours. The mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 100:0), to give the title compound as a white foam, 1.6g (containing some impurities); ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.60-1.80 (m, 4H), 2.17 (m, 2H), 3.30 (m, 2H), 3.60 (m, 2H), 3.83 (m, 2H), 7.62 (m, 2H), 7.79 (m, 2H); LRMS : m/z 273.2 (MH^+).

Preparation 7

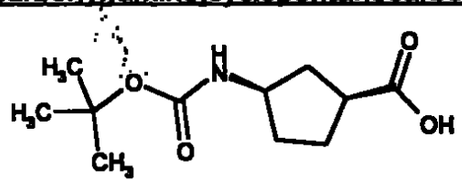
(1*S*,3*R*)-3-Aminocyclopentanecarboxylic acid



Platinum oxide (1g) was added to a solution of (1*R*,4*S*)-4-amino-cyclopent-2-ene carboxylic acid (5.3g, 41.7mmol) in water (70ml), and the mixture was hydrogenated at 45 psi and room temperature for 18 hours. The mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure, and the residue azeotroped with toluene, to afford the title compound as an off-white solid; ^{1}H NMR (D_2O, 400MHz) δ: 1.70-1.92 (m, 3H), 2.00 (m, 2H), 2.18 (m, 1H), 2.77 (m, 1H), 3.68 (m, 1H); LRMS : m/z 129.8 (MH^+).

Preparation 8

(1*S*,3*R*)-3-[(*tert*-Butoxycarbonyl)amino]cyclopentanecarboxylic acid

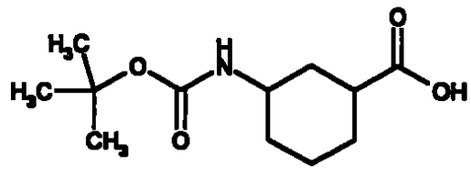


Di-*tert*-butyl dicarbonate (10g, 45.8mmol) was added to an ice-cooled solution of the amino acid from preparation 7 (5.4g, 41.8mmol) in dioxan (42.5ml) and

sodium hydroxide solution (42.5ml, 1N, 42.5mmol), and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure to remove the dioxan, then acidifed to pH 2 using 2N hydrochloric acid. The aqueous solution was extracted with ethyl acetate (5x100ml), the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure to give a white solid. This was triturated with hexane, to give the desired compound as a crystalline solid, 8.0g, 83%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.58-2.06 (m, 5H), 2.21 (m, 1H), 2.84 (m, 1H), 4.01 (m, 1H), 4.84 (m, 1H); LRMS: m/z 228 $(M-H)^-$.

Preparation 9

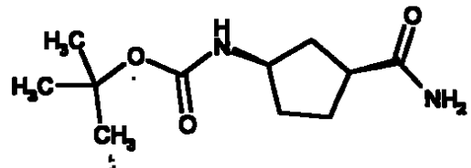
3-[(*tert*-Butoxycarbonyl)amino]cyclohexanecarboxylic acid



The title compound was obtained as a white solid in 81% yield, from 3-aminocyclohexanecarboxylic acid, following the procedure described in preparation 8; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.04 (m, 1H), 1.19-1.50 (m, 13H), 1.83 (m, 1H), 1.97 (m, 2H), 2.24 (m, 1H), 2.40 (m, 1H), 3.44 (bs, 1H), 4.42 (bs, 1H).

Preparation 10

tert-Butyl (1*R*,3*S*)-3-(aminocarbonyl)cyclopentylcarbamate

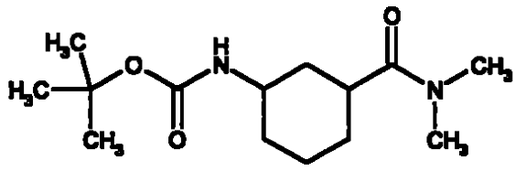


Benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (3.4g, 6.54mmol), 1-hydroxybenzotriazole hydrate (883mg, 6.54mmol), ammonium chloride (467mg, 8.72mmol) and N-ethyldiisopropylamine (3.04ml, 17.5mmol) were added sequentially to a solution of the acid from preparation 8 (1.0g, 4.37mmol) in N,N-dimethylformamide (16ml), and the reaction stirred at room

temperature for 2 hours. The mixture was diluted with ethyl acetate (100ml), washed with water (3x), and brine, then dried ($MgSO_4$) and evaporated under reduced pressure. The residual gum was purified by chromatography on silica gel using a Biotage® column, and an elution gradient of dichloromethane:methanol (98:2 to 95:5). The product was triturated with ether to afford the title compound as a white solid, 438mg, 44%; ^1H NMR ($DMSOd_6$, 400MHz) δ: 1.34 (s, 9H), 1.40 (m, 2H), 1.64 (m, 3H), 1.90 (m, 1H), 2.55 (m, 1H), 3.70 (m, 1H), 6.70 (bs, 1H), 6.80 (d, 1H), 7.22 (bs, 1H).

Preparation 11

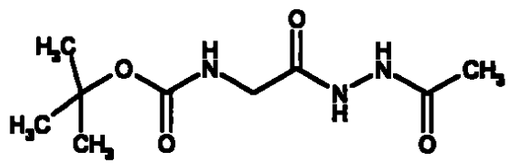
tert-Butyl 3-[(dimethylamino)carbonyl]cyclohexylcarbamate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.19g, 6.19mmol), 1-hydroxybenzotriazole hydrate (840mg, 6.19mmol), N-methylmorpholine (1.1ml, 10.1mmol) and finally 33% ethanolic dimethylamine (1.5ml) were added to a solution of the acid from preparation 9 (1.37g, 5.6mmol) in N,N-dimethylformamide (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure, the residue diluted with ethyl acetate and washed with water (2x). The mixture was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of methanol:dichloromethane (5:95 to 10:90), to give the title compound, 998mg, 66%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.12 (m, 1H), 1.40 (m, 11H), 1.70 (m, 2H), 1.85 (m, 1H), 2.00 (m, 2H), 2.62 (m, 1H), 2.96 (s, 3H), 3.05 (s, 3H), 3.50 (m, 1H), 4.50 (m, 1H).

Preparation 12

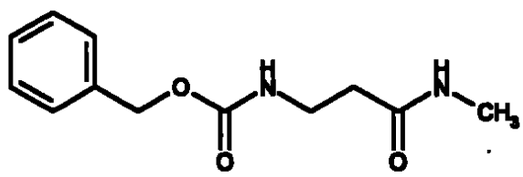
tert-Butyl 2-(2-acetylhydrazino)-2-oxoethylcarbamate



2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (7.06g, 28.5mmol) was added to a solution of N-*tert*-butoxycarbonylglycine (5.0g, 28.6mmol) in dichloromethane (75ml), and the solution stirred for 15 minutes. Acetic hydrazide (2.6g, 35.1mmol) was added, and the reaction stirred at room temperature for 18 hours. The resulting precipitate was filtered off, and dried *in vacuo*, to afford a white crystalline solid, 2.42g. The filtrate was concentrated under reduced pressure, diluted with ether, and the resulting precipitate filtered and dried *in vacuo*, to afford additional product as a white solid, 4.4g, 67% in total; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 2.02 (s, 3H), 3.87 (d, 2H), 5.22 (bs, 1H), 8.27 (bs, 1H), 8.84 (bs, 1H); LRMS : m/z 249.2 (MNH_4^+); Anal. Found: C, 46.41; H, 7.36; N, 17.98, $C_9H_{17}N_3O_4$ requires C, 46.66; H, 7.41; N, 18.13%.

Preparation 13

Benzyl 3-(methylamino)-3-oxopropylcarbamate

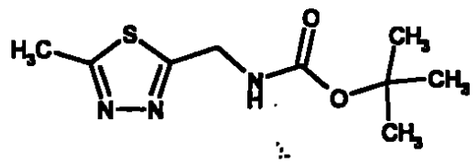


A mixture of N-[(benzyloxy)carbonyl]-β-alanine (10g, 44.8mmol), methylamine hydrochloride (3.33g, 49.28mmol), 1-hydroxybenzotriazole hydrate (6.05g, 44.8mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.3g, 53.76mmol) and N-methylmorpholine (11.33ml, 103mmol) in dichloromethane (200ml) was stirred at room temperature for 18 hours. The resulting precipitate was filtered off to give the desired product as a colourless foam, and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:hexane (90:10 to 100:0) to give additional product, 7.96g, 75% in total; ^{1}H NMR ($CDCl_3$,

300MHz) δ: 2.42 (t, 2H), 2.80 (s, 3H), 3.50 (m, 2H), 5.21 (s, 2H), 5.49 (bs, 1H), 5.63 (bs, 1H), 7.36 (m, 5H); Anal. Found: C, 60.68; H, 7.00; N, 11.95. $C_{12}H_{16}N_2O_3$ requires C, 61.00; H, 6.83; N, 11.86%.

Preparation 14

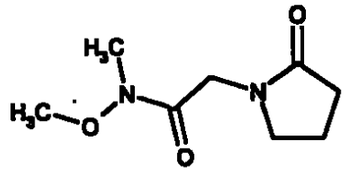
tert-Butyl (5-methyl-1,3,4-thiadiazol-2-yl)methylcarbamate



Lawesson's reagent (960mg, 2.38mmol) was added to a solution of the hydrazide from preparation 12 (500mg, 2.16mmol) in tetrahydrofuran (40ml), and the reaction heated under reflux for 3 hours, then stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 80:20) to give an oil. This was dissolved in ethyl acetate (100ml), charcoal (2g) added, the mixture stirred for 10 minutes then filtered. The filtrate was concentrated under reduced pressure, and the residue azeotroped with dichloromethane to afford the title compound as a crystalline solid, 441mg, 89%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.45 (s, 9H), 2.77 (s, 3H), 4.66 (d, 2H), 5.22 (bs, 1H); LRMS : m/z 230.1 (MH^+).

Preparation 15

N-Methoxy-*N*-methyl-2-(2-oxo-1-pyrrolidinyl)acetamide

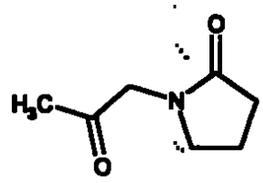


2-Chloro-N-methoxy-N-methylacetamide (3.2g, 23.3mmol) was added to a suspension of 2-pyrrolidinone (2.0g, 23.5mmol) and sodium hydride (940mg, 60% dispersion in mineral oil, 23.5mmol) in tetrahydrofuran (60ml), and the reaction stirred at room temperature for 48 hours. The mixture was quenched with water (150ml), and extracted with ethyl acetate (200ml) and

dichloromethane (200ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was triturated with hexane, then ether to afford the title compound as white crystals, 1.8g, 41%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.02 (m, 2H), 2.40 (t, 2H), 3.17 (s, 3H), 3.48 (t, 2H), 3.72 (s, 3H), 4.19 (s, 2H); LRMS : m/z 186.9 (MH^+).

Preparation 16

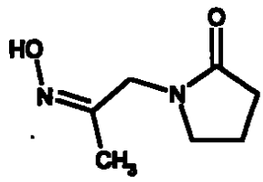
1-(2-Oxopropyl)-2-pyrrolidinone



Methylmagnesium chloride (2.7ml, 3M in tetrahydrofuran, 8.1mmol) was added to a cooled (-20°C) solution of the amide from preparation 15 (1.5g, 8.1mmol) in tetrahydrofuran (50ml), and the reaction allowed to warm to room temperature, then stirred for an hour. The mixture was quenched by the addition of aqueous ammonium chloride solution, then extracted with ethyl acetate (3x50ml). The combined organic solutions were dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as an oil, 645mg, 56%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.07 (m, 2H), 2.17 (s, 3H), 2.42 (t, 2H), 3.42 (t, 2H), 4.10 (s, 2H).

Preparation 17

1-[2-(Hydroxyimino)propyl]-2-pyrrolidinone

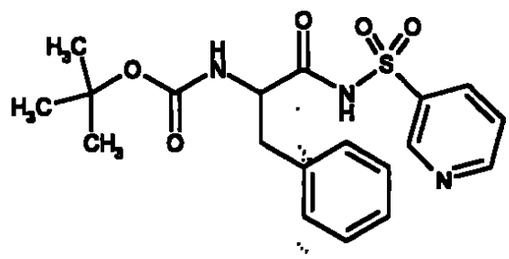


Hydroxylamine hydrochloride (316mg, 4.55mmol) and then pyridine (370µl, 4.58mmol) were added to a solution of the amide from preparation 16 (643mg, 4.55mmol) in ethanol (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 90:10). The product was triturated with ether

to give the title compound as a white solid, 375mg, 53%; ^{1}H NMR (DMSOd_6, 400MHz) δ: 1.60 (s, 3H), 1.87 (m, 2H), 2.20 (t, 2H), 3.19 (t, 2H), 3.78 (s, 2H), 10.77 (s, 1H); LRMS : m/z 157.4 (MH^+).

Preparation 18

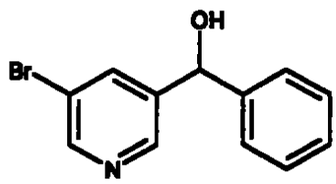
tert-Butyl 1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethylcarbamate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (939mg, 4.9mmol), 1-hydroxybenzotriazole hydrate (562mg, 4.15mmol), and N-methylmorpholine (952mg, 9.42mmol) were added to an ice-cold solution of N-*tert*-butoxycarbonyl-L-phenylalanine (1.0g, 3.77mmol) in dichloromethane (20ml), and the mixture stirred for 15 minutes. 3-Pyridinesulphonamide (Mon. für Chemie; 72; 77; 1938) (596mg, 3.77mmol) was added, and the reaction stirred at room temperature for 24 hours. The mixture was evaporated under reduced pressure and the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The aqueous layer was extracted well with ethyl acetate, then dichloromethane, the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified twice by column chromatography on silica gel, using an elution gradient of ethyl acetate:ethanol (100:0 to 90:10) to give the desired product as a white foam, 1.01g, 66; ^{1}H NMR (DMSOd_6, 300MHz) δ: 1.30 (s, 9H), 2.77 (m, 1H), 2.97 (m, 1H), 3.84 (m, 1H), 5.95 (bs, 1H), 6.96 (m, 2H), 7.08 (m, 3H), 7.42 (m, 1H), 8.05 (d, 1H), 8.60 (d, 1H), 8.84 (m, 1H); $[\alpha]_D$ = -10° (0.1% solution in methanol).

Preparation 19

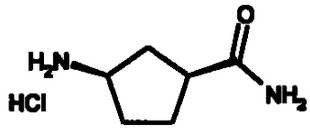
(5-Bromo-3-pyridinyl)(phenyl)methanol



n-Butyl lithium (17ml, 2.5M in hexanes, 42.5mmol) was added dropwise to cooled (-78°C) solution of 3,5-dibromopyridine (10g, 42.2mmol) in ether (200ml), so as to maintain an internal temperature <-70°C. The mixture was then stirred for 15 minutes, and a solution of benzaldehyde (4.5g, 42.5mmol) in ether (20ml) was added dropwise, again maintaining the temperature <-70°C. The mixture was stirred for 15 minutes, then allowed to warm to room temperature over an hour. The reaction was quenched by the addition of 0.9M ammonium chloride solution (200ml), the layers separated, and the aqueous phase extracted with ether. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:ether (95:5 to 80:20) to give the title compound as a yellow oil, 7.6g, 68%; ^1H NMR (D_2O, 300MHz) δ: 5.80 (s, 1H), 7.37 (m, 5H), 7.90 (s, 1H), 8.40 (s, 1H), 8.44 (s, 1H).

Preparation 20

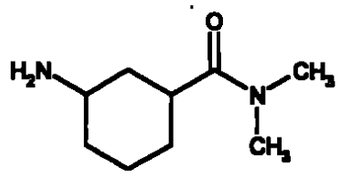
(1*S*,3*R*)-3-Aminocyclopentanecarboxamide hydrochloride



Hydrogen chloride gas was bubbled through an ice-cooled solution of the amide from preparation 10 (438mg, 1.92mmol) in dichloromethane (50ml) for 10 minutes, and the resulting suspension stirred at room temperature for 2 hours. The mixture was purged with nitrogen, then evaporated under reduced pressure. The residue was triturated with ether, to afford the title compound as a solid; ^1H NMR (D_2O, 400MHz) δ: 1.63-1.82 (m, 3H), 1.92-2.07 (m, 2H), 2.19 (m, 1H), 2.82 (m, 1H), 3.62 (m, 1H).

Preparation 21

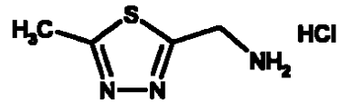
3-Amino-*N,N*-dimethylcyclohexanecarboxamide



A solution of the amide from preparation 11 (997mg, 3.69mmol) in trifluoroacetic acid (8ml) and dichloromethane (8ml) was stirred at room temperature for 4 hours. The mixture was concentrated under reduced pressure and the residue partitioned between dichloromethane (25ml) and sodium bicarbonate solution (25ml). The pH was adjusted to 9 using sodium hydroxide solution, the layers separated, and the aqueous phase evaporated under reduced pressure. The resulting solid was triturated with hot ethyl acetate, the suspension filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (84:14:2) to afford the title compound as a colourless oil, 346mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.08 (m, 1H), 1.25-1.54 (m, 6H), 1.72 (m, 1H), 1.86 (m, 2H), 2.53-2.75 (m, 2H), 2.96 (s, 3H), 3.03 (s, 3H);

Preparation 22

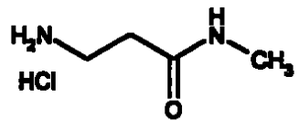
(5-Methyl-1,3,4-thiadiazol-2-yl)methylamine hydrochloride



Hydrogen chloride gas was bubbled through an ice-cooled solution of the thiadiazole from preparation 14 (425mg, 1.85mmol) in dichloromethane (50ml) for 15 minutes, and the reaction stirred at room temperature for 1 hour. The mixture was purged with nitrogen, then evaporated under reduced pressure to afford the title compound as a white solid; ^{1}H NMR ($DMSOd_6$, 400MHz) δ: 2.75 (s, 3H), 4.48 (m, 2H), 8.80 (bs, 3H).

Preparation 23

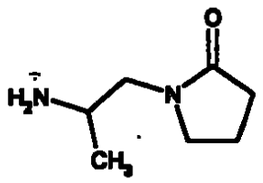
3-Amino-N-methylpropanamide hydrochloride



A mixture of the benzyl carbamate from preparation 13 (7.92g, 33.5mmol) and 5% palladium on charcoal (800mg) in ethanol (300ml) was hydrogenated at 50 psi and room temperature for 4 hours. The reaction mixture was filtered through Arbocel®, washing through with ethanol, and 1N hydrochloric acid (36.9ml, 36.9mmol) was added to the combined filtrate. This solution was evaporated under reduced pressure and the residue azeotroped with dichloromethane to afford the title compound as a colourless foam, 4.66g, ^{1}H NMR (DMSOd_6, 300MHz) δ: 2.46 (t, 2H), 2.60 (s, 3H), 2.95 (m, 2H), 7.98-8.16 (m, 2H).

Preparation 24

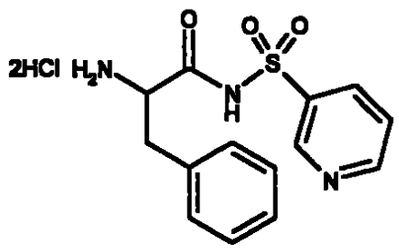
1-(2-Aminopropyl)-2-pyrrolidinone



A mixture of the oxime from preparation 17 (375mg, 2.40mmol) and platinum oxide (300mg) in ethanol (20ml) was hydrogenated at 60psi and room temperature for 18 hours. Tlc analysis showed starting material remaining, so additional platinum oxide (100mg) was added and the reaction continued for a further 4 hours. The mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:0.88 ammonia (95:5:0.5 to 90:10:1) to give the title compound as a clear oil, 170mg, 50%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.02 (d, 3H), 1.36 (bs, 2H), 2.00 (m, 2H), 2.38 (t, 2H), 3.00-3.16 (m, 2H), 3.21 (m, 1H), 3.35-3.45 (m, 2H); LRMS : m/z 143 (MH^+).

Preparation 25

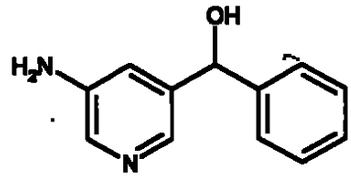
N-(2-Amino-3-phenylpropanoyl)-3-pyridinesulphonamide dihydrochloride



Saturated ethereal hydrochloric acid (40ml) was added to an ice-cold solution of the sulphonamide from preparation 18 (959mg, 2.37mmol) in ethyl acetate (30ml) and ether (10ml), and the solution stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x) to afford the title compound as a white solid, 959mg; ^{1}H NMR (DMSOd_6, 300MHz) δ: 3.23-3.50 (m, 1H), 3.70-3.98 (m, 1H), 4.13 (m, 1H), 7.05 (m, 2H), 7.20 (m, 3H), 7.78 (m, 1H), 8.36 (d, 1H), 8.44 (bs, 2H), 8.95 (d, 1H), 9.02 (s, 1H);$[\alpha]_D$ = +138° (0.5% solution in methanol).

Preparation 26

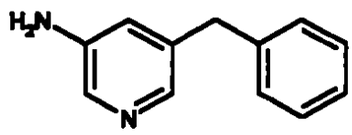
(5-Amino-3-pyridinyl)(phenyl)methanol



A mixture of the bromide from preparation 19 (2.0g, 7.60mmol) and copper (II) sulphate pentahydrate (350mg, 1.40mmol) in 0.88 ammonia (18ml) was heated at 135°C in a sealed vessel for 24 hours. Sodium hydroxide solution (1N, 10ml) was added to the cooled solution, and the mixture was then extracted with ether (6x). The combined organic extracts were dried ($MgSO_4$), and concentrated under reduced pressure to a low volume. The resulting precipitate was filtered, washed with ether and dried to give the title compound as a solid, 1.25g, 83%; mp 92-94°C; ^{1}H NMR (DMSOd_6, 300MHz) δ: 5.22 (s, 2H), 5.59 (d, 1H), 5.86 (d, 1H), 6.83 (s, 1H), 7.20 (m, 1H), 7.34 (m, 4H), 7.78 (m, 2H).

Preparation 27

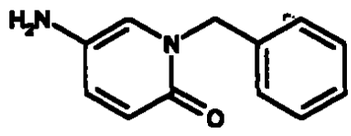
5-Benzyl-3-pyridinylamine



A mixture of the alcohol from preparation 26 (700mg, 3.5mmol) and 5% palladium on charcoal (70mg) in hydrochloric acid (5ml, 1N) and ethanol (20ml) was hydrogenated at 30 psi and room temperature for 6 hours. The mixture was filtered through Arbocel®, and the filtrate concentrated under reduced pressure. The residue was basified using aqueous sodium bicarbonate solution, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (92:8:0.4) as eluant, to give the title compound as a solid, 500mg, 78%; mp 107-109°C; ^1H NMR ($CDCl_3$, 300MHz) δ: 3.61 (bs, 2H), 3.94 (s, 2H), 6.78 (s, 1H), 7.24 (m, 5H), 7.98 (s, 2H).

Preparation 28

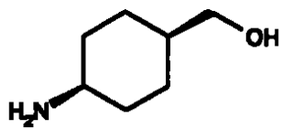
5-Amino-1-benzyl-2(1*H*)-pyridinone



A mixture of 1-benzyl-5-nitro-1H-pyridin-2-one (Justus Liebigs Ann. Chem. 484; 1930; 52) (1.0g, 4.35mmol), and granulated tin (3.5g, 29.5mmol) in concentrated hydrochloric acid (14ml) was heated at 90°C for 1.5 hours. The cooled solution was diluted with water, neutralised using sodium carbonate solution, and extracted with ethyl acetate (250ml in total). The combined organic extracts were filtered, dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as a pale green solid, (turned blue with time), 440mg, 51%; ^1H NMR ($CDCl_3$, 250MHz) δ: 4.12-4.47 (bs, 2H), 5.00 (s, 2H), 6.31 (d, 1H), 6.86 (s, 1H), 7.07 (m, 1H), 7.14-7.42 (m, 5H).

Preparation 29

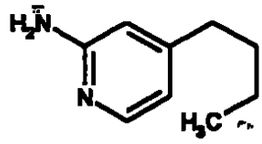
Cis-(4-Aminocyclohexyl)methanol



Lithium aluminium hydride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added dropwise to an ice-cooled solution of cis-4-aminocyclohexanecarboxylic acid (1.33g, 9.29mmol) in tetrahydrofuran (50ml), and once addition was complete, the reaction was heated under reflux for 6 hours. The resulting suspension was cooled to 5°C, and water (0.6ml), aqueous sodium hydroxide solution (1.1ml, 2M), then water (0.6ml) were added sequentially. The resulting suspension was filtered, and the filtrate evaporated under reduced pressure to give an oil, which was used without further purification; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.40-1.80 (m, 12H), 3.00 (m, 1H), 3.55 (d, 2H); LRMS : m/z 130.2 (MH^+).

Preparation 30

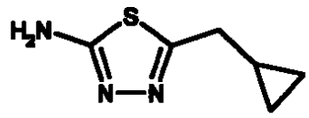
2-Amino-4-butylpyridine



A mixture of 4-butylpyridine (5.0g, 37.0mmol) and 95% sodium amide (1.7g, 40.7mmol) in xylene (10ml) was heated at 150°C for 18 hours. The cooled mixture was diluted with ether (100ml) and extracted with 2N hydrochloric acid (twice). The aqueous extracts were basified using sodium hydroxide solution, and re-extracted with ether. These combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (97:3:0.15) as eluant, to afford the title compound as a crystalline solid, 2.1g, 38%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.38 (m, 2H), 1.60 (m, 2H), 2.52 (t, 2H), 4.38 (bs, 2H), 6.38 (s, 1H), 6.55 (d, 1H), 7.98 (d, 1H); Anal. Found: C, 72.01; H, 9.47; N, 18.53. $C_9H_{14}N_2$ requires C, 71.96; H, 9.39; N, 18.65%.

Preparation 31

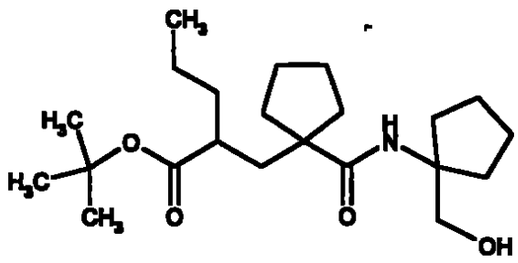
5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-amine



Oxalyl chloride (3.13ml, 35.9mmol) and N,N-dimethylformamide (1 drop) were added to a solution of cyclopropylacetic acid (3g, 29.9mmol) in dichloromethane (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure and azeotroped with dichloromethane to give a brown oil. A mixture of this intermediate acid chloride (887mg, 7.48mmol) and thiosemicarbazide (455mg, 4.99mmol) were heated at 70°C for 18 hours, then cooled. Water was added, the mixture basified to pH 9 using 50% aqueous sodium hydroxide solution, and the resulting precipitate filtered and dried, to give a cream solid, 410mg, 53%; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.28 (m, 2H), 0.60 (m, 2H), 1.02 (m, 1H), 2.77 (d, 2H); LRMS : m/z 155.2 (MH^+).

Preparation 33

tert-Butyl 2-{[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate

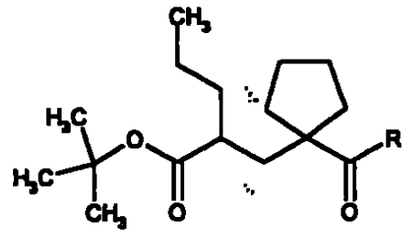


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (41mg, 0.21mmol), 1-hydroxybenzotriazole hydrate (27mg, 0.2mmol), N-methylmorpholine (35µl, 0.31mmol) and finally 1-amino-1-cyclopentanemethanol (25mg, 0.22mmol) were added to a solution of the acid from preparation 1 (150mg, 0.53mmol) in N,N-dimethylformamide (3ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (3x25ml), and brine (25ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using

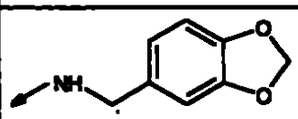
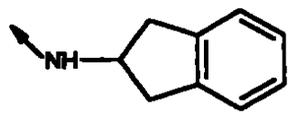
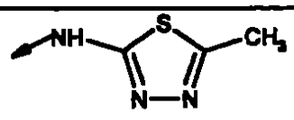
ethyl acetate:pentane (30:70) as the eluant to afford the title compound, 38mg, 57%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.29 (m, 3H), 1.41-1.78 (m, 26H), 1.78-1.98 (m, 4H), 2.04 (m, 1H), 2.26 (m, 1H), 3.59 (dd, 1H), 3.70 (dd, 1H), 4.80 (t, 1H), 5.81 (s, 1H); LRMS : m/z 380 (MH^+).

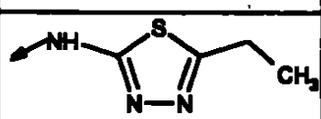
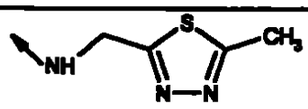
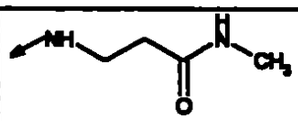
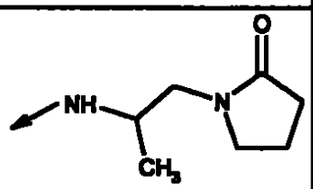
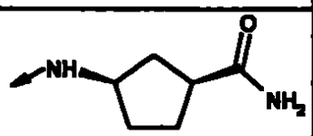
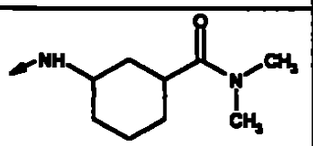
Preparations 34 to 43

The following compounds of general structure:

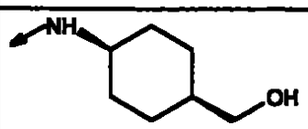


were prepared from the acid from Preparation 1 and the appropriate amine compound, following a similar procedure to that described in preparation 33.

Prep	R	Starting amine	Yield (%)	Data
34		Piperonylamine	88	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.26 (m, 4H), 1.42 (s, 9H), 1.46 (m, 2H), 1.59-1.75 (m, 5H), 1.95 (m, 2H), 2.06 (m, 1H), 2.22 (m, 1H), 4.26 (dd, 1H), 4.39 (dd, 1H), 5.95 (m, 3H), 6.78 (m, 3H). LRMS : m/z 418.3 (MH^+)
35[1]		2-Aminoindan hydrochloride	40	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.25 (m, 3H), 4H), 1.42 (m, 12H), 1.56-1.70 (m, 4H), 1.90 (m, 2H), 2.02 (m, 1H), 2.22 (m, 1H), 2.80 (m, 2H), 3.35 (m, 2H), 4.76 (m, 1H), 5.86 (d, 1H), 7.19 (m, 4H). LRMS : m/z 400.3 (MH^+)
36[2]		2-Amino-5-methyl-1,3,4-thiadiazole	76	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.20-1.85 (m, 20H), 2.18 (m, 4H), 2.67 (s, 3H), 9.80 (bs, 1H). LRMS : m/z 382.3 (MH^+)

Prep	R	Starting amine	Yield (%)	Data
37[2]		2-Amino-5-ethyl-1,3,4-thiadiazole	92	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.20-1.80 (m, 22H), 1.84 (m, 1H), 2.20 (m, 4H), 3.04 (q, 2H), 9.10 (bs, 1H). LRMS : m/z 396.2 (MH^+)
38		Preparation 22	77	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 4H), 1.42 (s, 9H), 1.44-1.76 (m, 7H), 1.95-2.12 (m, 3H), 2.20 (m, 1H), 2.76 (s, 3H), 4.74 (dd, 1H), 4.82 (dd, 1H), 6.54 (bs, 1H). LRMS : m/z 396.2 (MH^+)
39[1,2]		Preparation 23	60	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.21-1.38 (m, 3H), 1.40-1.70 (m, 17H), 1.88-2.04 (m, 3H), 2.20 (m, 1H), 2.39 (t, 2H), 2.80 (d, 3H), 3.53 (m, 2H), 6.13 (bs, 1H), 6.40 (m, 1H). LRMS : m/z 389.5 (MH^+)
40[2]		Preparation 24	70	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (m, 3H), 1.16 (2xd, 3H), 1.20-1.72 (m, 21H), 1.83 (m, 1H), 1.98 (m, 3H), 2.17 (m, 1H), 2.38 (m, 2H), 1.96 (m, 1H), 3.34 (m, 1H), 3.54-3.62 (m, 2H), 4.15-4.20 (m, 1H), 6.21-6.35 (2xbd, 1H). LRMS : m/z 409.3 (MH^+).
41[2]		Preparation 20	94	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.19-1.38 (m, 4H), 1.42 (m, 12H), 1.60 (m, 3H), 1.74-2.02 (m, 10H), 2.18 (m, 1H), 2.78 (m, 1H), 4.38 (m, 1H), 5.32 (bs, 1H), 5.57 (bs, 1H), 7.28 (bs, 1H). LRMS : m/z 395 (MH^+)
42[2]		Preparation 21	91	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (t, 3H), 1.18-1.78 (m, 25H), 1.84-2.03 (m, 6H), 2.22 (m, 1H), 2.68 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.84 (m, 1H), 5.78 (m, 1H). LRMS : m/z 437.7 (MH^+)

444

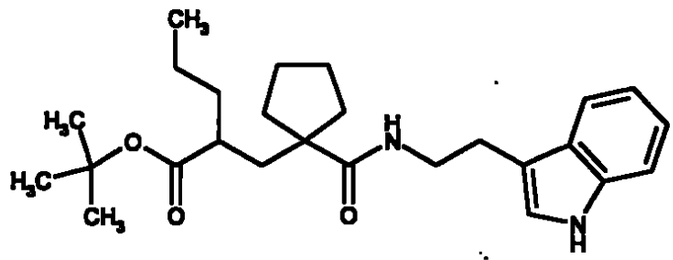
Prep	R	Starting amine	Yield (%)	Data
43[2]		Preparation 29	99	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.20-1.79 (m, 30H), 1.90 (m, 2H), 2.05 (m, 1H), 2.24 (m, 1H), 3.56 (m, 2H), 4.04 (m, 1H), 5.82 (bd, 1H). LRMS : m/z 396.4 (MH^+)

1 = reaction conducted at room temperature

2 = Methanol:dichloromethane was used as the column eluant

Preparation 44

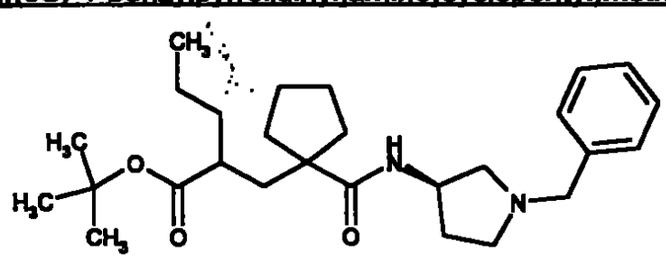
tert-Butyl 2-[[1-({[2-(1*H*-indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]methyl]pentanoate



The title compound was obtained as a pale yellow oil in 80% yield from the acid from preparation 1 and tryptamine, following a similar procedure to that described in preparation 33, except the reaction was performed in dichloromethane at room temperature; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.86 (t, 3H), 1.26 (m, 3H), 1.42 (m, 11H), 1.50-1.69 (m, 6H), 1.83 (m, 1H), 1.90-2.05 (m, 2H), 2.22 (m, 1H), 2.99 (t, 3H), 3.60 (m, 2H), 5.78 (m, 1H), 7.06 (s, 1H), 7.14 (m, 1H), 7.20 (m, 1H), 7.38 (d, 1H), 7.63 (d, 1H), 8.02 (bs, 1H); LRMS : m/z 427.5 (MH^+).

Preparation 45

tert-Butyl 2-[(1-{[(3*S*)-1-benzylpyrrolidinyl]amino}cyclopentyl)methyl]pentanoate

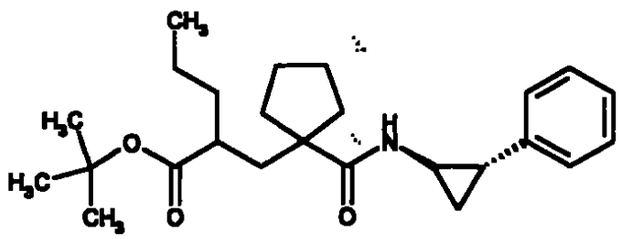


The title compound was obtained quantitatively from the acid from preparation 1

and (3S)-1-benzyl-3-aminopyrrolidine, following a similar procedure to that described in preparation 44; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.10-1.76 (m, 21H), 1.90-2.05 (m, 3H), 2.20-2.38 (m, 3H), 2.58 (m, 2H), 2.84 (m, 1H), 3.62 (s, 2H), 4.45 (m, 1H), 6.02 (m, 1H), 7.33 (m, 5H).

Preparation 46

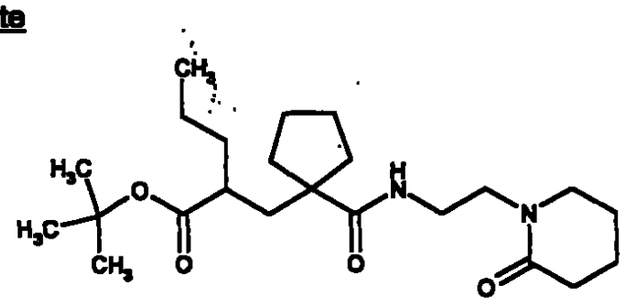
tert-Butyl 2-[1-({[cis-2-phenylcyclopropyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (81mg, 0.42mmol), N-methylmorpholine (0.15ml, 1.06mmol) and tranylcypromine hydrochloride (80mg, 0.35mmol) were added to a solution of the acid from preparation 1 (100mg, 0.35mmol) in dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The reaction mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 95:5) to afford the title compound as a yellow oil, 85mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.16 (m, 1H), 1.20-1.58 (m, 16H), 1.63 (m, 5H), 1.90-2.14 (m, 4H), 2.23 (m, 1H), 2.90 (m, 1H), 6.00 (m, 1H), 7.19 (m, 3H), 7.24 (m, 2H); LRMS : m/z 400 (MH^+).

Preparation 47

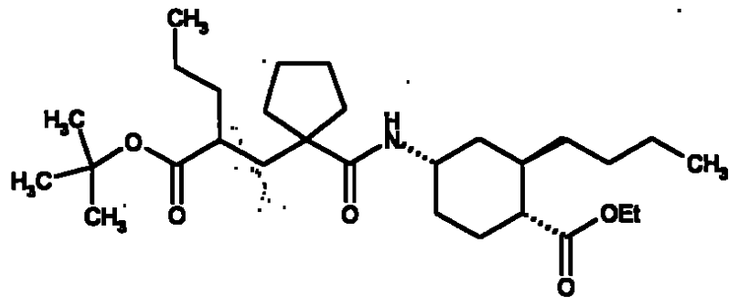
tert-Butyl 2-[1-({[2-(2-oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]-methyl}pentanoate



Hydrazine monohydrate (34µl, 0.70mmol) was added to a solution of the compound from preparation 6 (171mg, 0.63mmol) in ethanol (10ml), and the reaction heated under reflux for 5 hours. The cooled mixture was filtered, the filtrate concentrated under reduced pressure, the residue suspended in dichloromethane, and the suspension re-filtered. The resulting filtrate was concentrated under reduced pressure, and the residue purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (90:10:1) as eluant to give the amine, 16mg. The acid from preparation 1 (32mg, 0.11mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (25mg, 0.13mmol), 1-hydroxybenzotriazole hydrate (17mg, 0.13mmol), and N-methylmorpholine (25µl, 0.23mmol) were added to a solution of this amine in N,N-dimethylformamide (2ml), and the reaction stirred at room temperature for 18 hours. The mixture was partitioned between ethyl acetate and water, and the layers separated. The organc phase was washed with water (2x), dried ($MgSO_4$), and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98.5:1.5 to 95:5) to afford the title compound as an oil, 43mg, 17%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.22 (m, 3H), 1.38-1.65 (m, 17H), 1.58 (m, 4H), 1.95 (m, 3H), 2.17 (m, 1H), 2.37 (m, 2H), 3.30 (m, 2H), 3.38 (m, 2H), 3.50 (m, 2H), 6.76 (m, 1H); LRMS : m/z 409.2 (MH^+)

Preparation 48

Ethyl (1*R*,2*R*,4*S*)-4-[({1-[2-(*tert*-butoxycarbonyl)pentyl]cyclopentyl}-carbonyl)amino]-2-butylcyclohexanecarboxylate

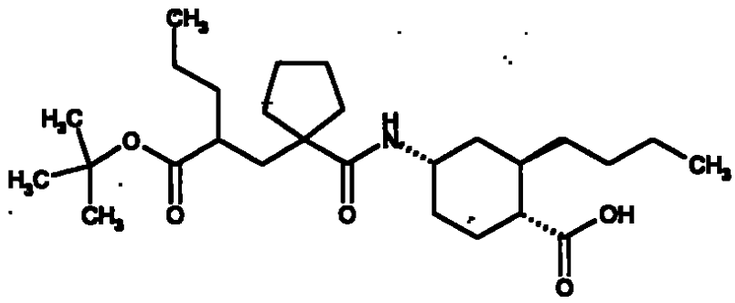


A mixture of the acid from preparation 1 (109mg, 0.38mmol), (1*R*,2*R*,4*S*)-4-amino-2-butyl-cyclohexanecarboxylic acid ethyl ester hydrochoride (WO,

9009374), (101mg, 0.38mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (95mg, 0.50mmol), 1-hydroxybenzotriazole hydrate (60mg, 0.40mmol) and triethylamine (0.12ml, 0.87mmol) in dichloromethane (3ml), was stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue treated with sodium bicarbonate solution and extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as eluant, and azeotroped with dichloromethane to afford the title compound, 190mg; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.88 (m, 6H), 1.20-1.40 (m, 13H), 1.40-2.10 (m, 25H), 2.16-2.30 (m, 2H), 4.18 (m, 3H), 5.83 (d, 1H).

Preparation 49

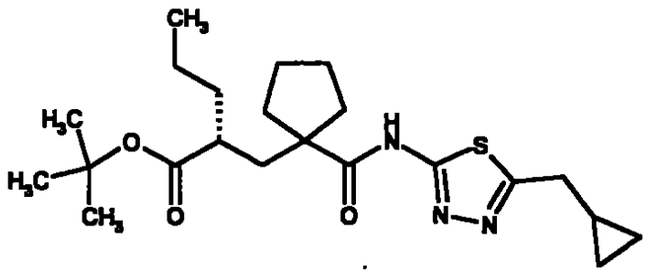
(1*R*, 2*R*,4*S*)-4-[({1-[2-(*tert*-Butoxycarbonyl)pentyl]cyclopentyl}carbonyl)amino]-2-butylcyclohexanecarboxylic acid



A mixture of the ethyl ester from preparation 48 (190mg, 0.39mmol) and 1N sodium hydroxide solution (0.85ml, 0.85mmol) in methanol (1.5ml) was stirred at room temperature for 22 hours. The reaction mixture was acidified to pH 1 using hydrochloric acid (2N), then partitioned between ethyl acetate and water. The layers were separated, and the organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to afford the title compound, 130mg, 72%; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.86 (m, 6H), 1.20-2.12 (m, 36H), 2.24 (m, 2H), 4.18 (m, 1H), 5.82 (d, 1H); LRMS : m/z 464 $(M-H)^-$

Preparation 50

tert-Butyl (2*R*)-2-[[1-({[5-(cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]methyl]pentanoate

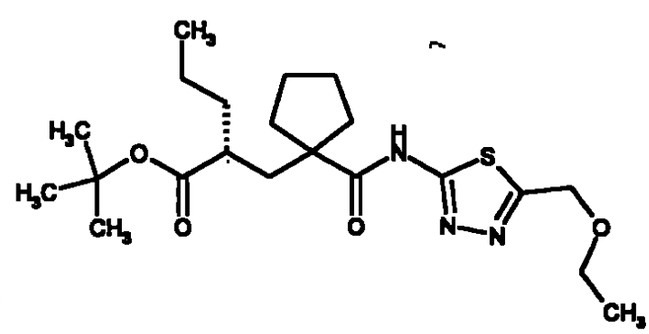


The title compound was prepared from the acid from preparation 2 and the amine from preparation 31, in 65% yield, following the procedure described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.35 (m, 2H), 0.63 (m, 2H), 0.80 (m, 3H), 1.10 (m, 1H), 1.20-1.94 (m, 20H), 2.19 (m, 4H), 2.93 (t, 2H), 3.50 (s, 1H); LRMS : m/z 422.4 (MH^+)

$[\alpha]_D$ = -14.15° (c = 0.082, methanol).

Preparation 51

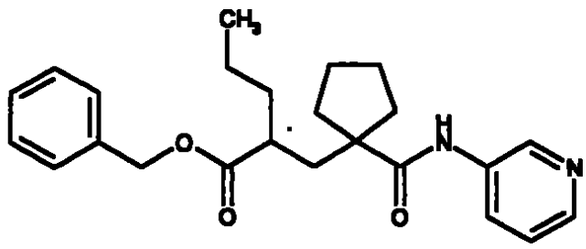
tert-Butyl (2*R*)-2-[[1-({[5-(ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)-cyclopentyl]methyl]pentanoate



The title compound was prepared from the acid from preparation 2 and 5-(ethoxymethyl)-1,3,4-thiadiazol-2-amine, in 51% yield, following the procedure described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.10-1.78 (m, 25H), 1.82 (m, 1H), 2.19 (m, 5H), 3.48 (s, 1H), 4.82 (s, 2H), 10.16 (brs, 1H); LRMS : m/z 426.4 (MH^+); $[\alpha]_D$ = -12.50° (c = 0.08, methanol).

Preparation 52

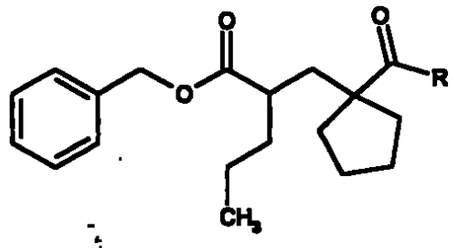
Benzyl 2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoate



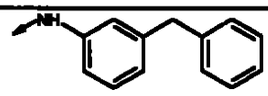
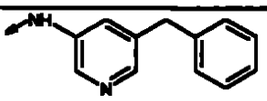
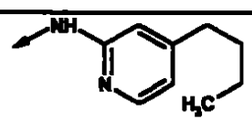
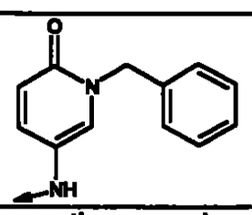
Triethylamine (0.11ml, 0.78mmol) was added to a mixture of the acid chloride from preparation 3 (200mg, 0.60mmol) and 2-aminopyridine (61mg, 0.65mmol) in dichloromethane (3ml), and the reaction stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue partitioned between sodium bicarbonate solution (5ml) and ethyl acetate (20ml), and the layers separated. The organic phase was dried ($MgSO_4$), and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant, to afford the title compound, 130mg; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.21 (m, 3H), 1.40 (m, 1H), 1.43-1.72 (m, 6H), 1.81 (d, 1H), 1.98 (m, 1H), 2.18 (m, 1H), 2.24 (m, 1H), 2.46 (m, 1H), 4.98 (m, 2H), 7.20-7.38 (m, 6H), 7.42 (s, 1H), 8.06 (d, 1H), 8.35 (d, 1H), 8.56 (s, 1H).

Preparations 53 to 56

The following compounds of general formula:



were prepared from the acid chloride from preparation 3 and the appropriate amine, following a similar procedure to that described in preparation 52.

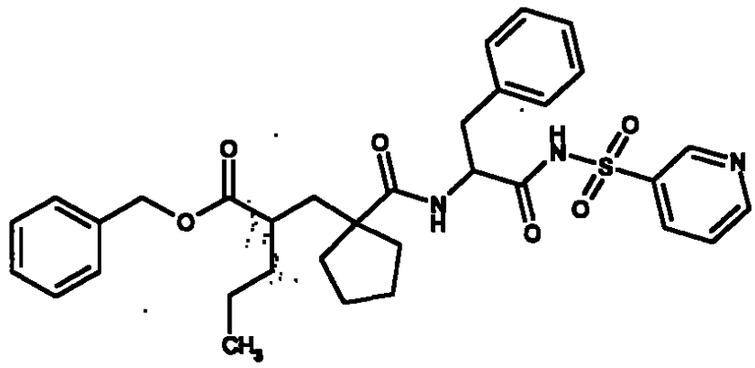
Prep	R	Yield (%)	Data
53[1]		90	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.24 (m, 2H), 1.40-1.76 (m, 7H), 1.84 (dd, 1H), 1.98 (m, 1H), 2.19 (dd, 1H), 2.28 (m, 1H), 2.56 (m, 1H), 3.98 (s, 2H), 4.99 (dd, 2H), 6.98 (d, 1H), 7.19-7.42 (m, 15H).
54		65	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.24 (m, 3H), 1.39-1.78 (m, 6H), 1.82 (dd, 1H), 1.98 (m, 2H), 2.20 (dd, 1H), 2.25 (m, 1H), 2.50 (m, 1H), 3.98 (s, 2H), 4.98 (dd, 2H), 7.18-7.40 (m, 10H), 7.45 (s, 1H), 7.98 (s, 1H), 8.23 (s, 1H), 8.42 (s, 1H).
55		30	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 0.92 (t, 3H), 1.21 (m, 2H), 1.30-1.70 (m, 12H), 1.82 (dd, 1H), 2.04 (m, 1H), 2.20 (m, 2H), 2.50 (m, 1H), 2.58 (t, 2H), 4.98 (dd, 2H), 6.83 (d, 1H), 7.30 (m, 5H), 7.90 (s, 1H), 8.08 (s, 1H), 8.15 (d, 1H).
56[2]		53	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.25 (m, 2H), 1.27-1.99 (m, 10H), 2.07-2.30 (m, 2H), 2.47 (m, 1H), 4.99 (s, 2H), 5.10 (dd, 2H), 6.59 (d, 1H), 7.15 (d, 1H), 7.34 (m, 11H), 8.10 (s, 1H).

1 = dichloromethane used as the column eluant

2 = N-methylmorpholine was used as the base

Preparation 57

Benzyl 2-({1-[({1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoate

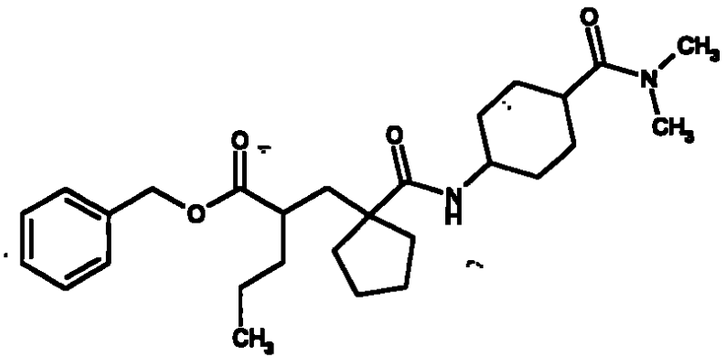


The amine hydrochloride from preparation 25 (828mg, 2.19mmol) and N-methylmorpholine (2.21g, 21.9mmol) was added to an ice-cold solution of the acid chloride from preparation 3 (737mg, 2.19mmol) in dichloromethane (50ml),

and the reaction stirred at room temperature for 24 hours. The reaction mixture was evaporated under reduced pressure, the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The organic phase was washed with brine (25ml), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:methanol (100:0 to 95:5) to give the title compound as a cream foam, 975mg, 73%; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.72 (m, 3H), 0.94-2.20 (m, 17H), 2.84 (m, 1H), 3.00 (m, 1H), 4.18 (m, 1H), 5.00 (m, 2H), 6.95 (m, 2H), 7.02 (m, 3H), 7.38 (m, 6H), 8.06 (m, 1H), 8.60 (m, 1H), 8.87 (s, 1H).

Preparation 58

cis-Benzyl 2-({1-[({4-[(dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate

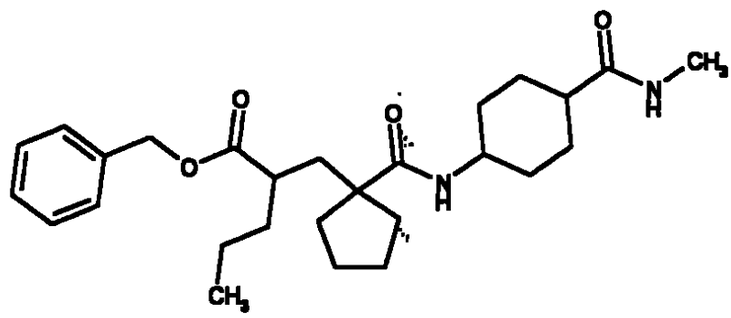


A mixture of cis-4-{[(1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentyl)carbonyl]-amino}cyclohexanecarboxylic acid (EP 274234) (200mg, 0.45mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (112mg, 0.58mmol), 1-hydroxybenzotriazole hydrate (70mg, 0.46mmol) and dimethylamine (0.56ml, 2M in tetrahydrofuran, 1.12mmol) in dichloromethane (5ml) was stirred at room temperature for 16 hours. The mixture was concentrated under reduced pressure and the residue partitioned between sodium bicarbonate solution and ethyl acetate, and the layers separated. The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant to afford the title compound, 150mg; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.22

(m, 3H), 1.32-1.88 (m, H), 2.00 (m, 4H), 2.40 (m, 1H), 2.60 (m, 1H), 2.97 (s, 3H), 3.04 (s, 3H), 4.04 (m, 1H), 5.12 (s, 2H), 5.80 (bd, 1H), 7.37 (m, 5H).

Preparation 59

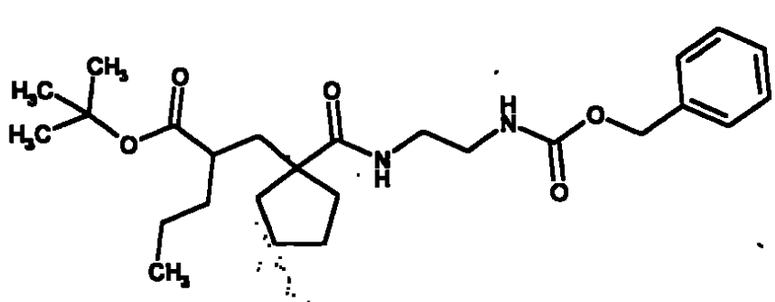
cis-Benzyl 2-({1-[({4-[(methylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate



The title compound was prepared in 49% yield from cis-4-[[(1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylic acid (EP 274234) and methylamine (2M in tetrahydrofuran), following the procedure described in preparation 58; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.17-2.12 (m, 22H), 2.21 (m, 1H), 2.41 (m, 1H), 2.80 (d, 3H), 4.00 (m, 1H), 5.12 (s, 2H), 5.61 (m, 1H), 5.79 (d, 1H), 7.38 (m, 5H).

Preparation 60

tert-Butyl 2-[(1-[[(2-[[(benzyloxy)carbonyl]amino}ethyl)amino]carbonyl]-cyclopentyl)methyl]pentanoate

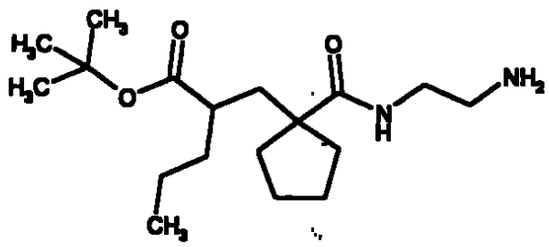


The title compound was obtained as a yellow oil in 55% yield, from the acid from preparation 1 and N-benzyloxycarbonyl-1,2-diaminoethane, following a similar procedure to that described in preparation 44; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.74 (m, 17H), 1.90 (m, 2H), 2.04 (m, 1H), 2.20

(m, 1H), 3.32 (m, 3H), 3.44 (m, 1H), 5.10 (s, 2H), 5.61 (m, 1H), 6.20 (m, 1H), 7.36 (m, 5H).

Preparation 61

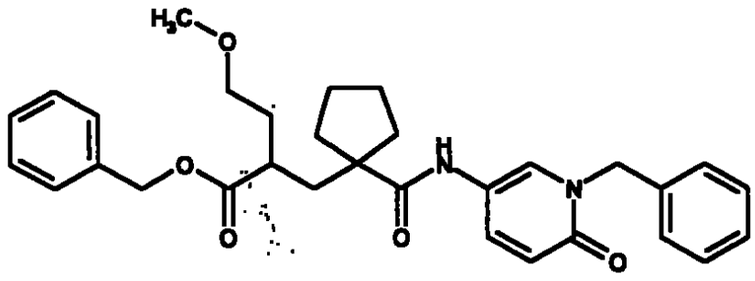
tert-Butyl 2-[(1-{[(2-aminoethyl)amino]carbonyl}cyclopentyl)methyl]pentanoate



A mixture of the carbamate from preparation 60 (1.43g, 3.10mmol) and 10% palladium on charcoal (200mg) in ethanol (8ml) was hydrogenated at room temperature and 1 atm for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to afford the title compound, 920mg, 92%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.54 (m, 12H), 1.61 (m, 5H), 1.92-2.12 (m, 3H), 2.20 (m, 1H), 2.98 (m, 2H), 3.38 (m, 1H), 3.42 (m, 1H), 3.97 (m, 2H), 6.65 (m, 1H); LRMS : m/z 326.8 (M^+).

Preparation 62

Benzyl 2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]-4-methoxybutanoate

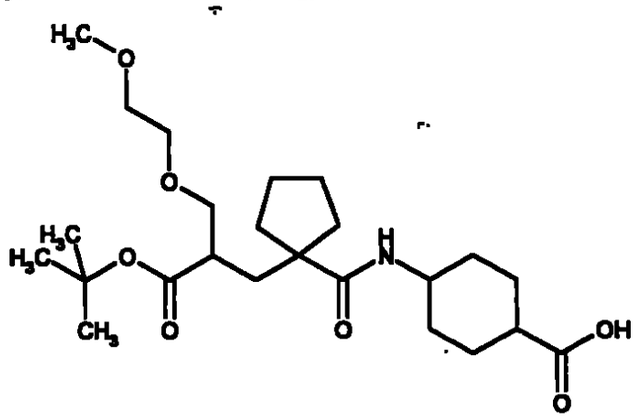


Oxalyl chloride (0.26ml, 3.0mmol) was added to an ice-cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-methoxybutyl}cyclopentanecarboxylic acid (EP 274234) (1.0g, 3.0mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (20ml), and the reaction stirred at room temperature for 2 hours. The solution

was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x10ml). The product was dissolved in dichloromethane (20ml), then cooled in an ice-bath. The amine from preparation 28 (600mg, 3mmol) and N-methylmorpholine (0.6ml, 5.45mmol) were added and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and partitioned between water and ether. The organic layer was washed with hydrochloric acid (2N), sodium bicarbonate solution, then water, dried ($MgSO_4$) and evaporated under reduced pressure. The residual green solid was purified by medium pressure column chromatography on silica gel using ethyl acetate:hexane (90:10) as eluant to afford the title compound, 880mg, 57%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.37-2.28 (m, 12H), 2.46-2.64 (m, 1H), 3.20 (s, 3H), 3.31 (m, 2H), 4.97 (dd, 2H), 5.08 (dd, 2H), 6.57 (d, 1H), 7.12 (m, 1H), 7.18-7.48 (m, 10H), 8.08 (d, 1H).

Preparation 63

4-[[(1-{3-*tert*-Butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)-carbonyl]amino}cyclohexanecarboxylic acid

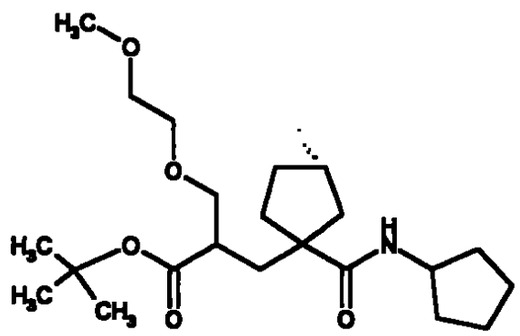


A mixture of benzyl 4-[[(1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylate (EP 274234), and 10% palladium on charcoal (250mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 18 hours. The reaction mixture was filtered through Solkafloc®, the filtrate concentrated under reduced pressure and the residue azeotroped with toluene (3x) and then dichloromethane (3x), to give the title compound, 2.0g, 96%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.48 (s, 9H),

1.53-1.84 (m, 14H), 1.94-2.10 (m, 5H), 2.60 (m, 2H), 3.40 (s, 3H), 3.41-3.63 (m, 5H), 3.96 (m, 1H), 5.90 (bd, 1H).

Preparation 64

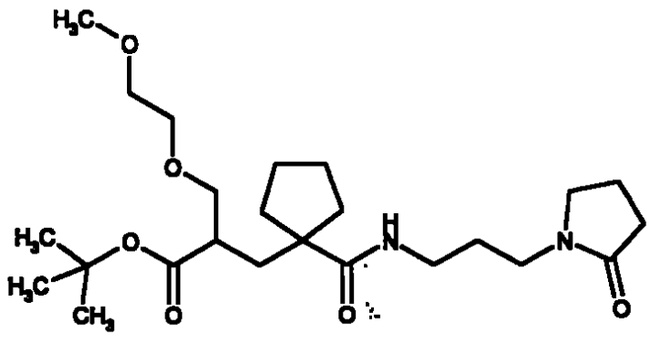
tert-Butyl 3-[1-[(cyclopentylamino)carbonyl]cyclopentyl]-2-[(2-methoxyethoxy)methyl]-propanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (197mg, 1.07mmol), 1-hydroxybenzotriazole hydrate (139mg, 1.07mmol), N-methylmorpholine (0.18ml, 1.64mmol) and cyclopentylamine (101µl, 1.07mmol) were added to a solution of 1-[3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl]-cyclopentanecarboxylic acid (EP 274234) (400mg, 1.07mmol) in dichloromethane (5ml), and the reaction stirred at room temperature for 22 hours. The reaction was quenched by the addition of water, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to afford the title compound as a clear oil, 320mg, 78%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.22-2.02 (m, 27H), 2.58 (m, 1H), 3.36 (s, 3H), 3.40 (m, 1H), 3.46 (m, 2H), 3.57 (m, 3H), 4.10-4.20 (m, 1H), 5.80 (bs, 1H).

Preparation 65

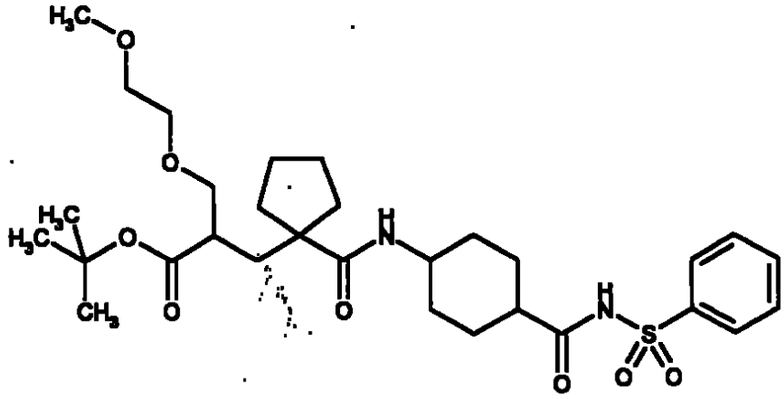
tert-Butyl 3-(2-methoxyethoxy)-2-[[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]methyl]propanoate



The title compound was obtained as a clear oil in 97% yield from 1-[3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl]-cyclopentanecarboxylic acid (EP 274234) and 1-(3-aminopropyl)-2-pyrrolidinone, following a similar procedure to that described in preparation 64, except dichloromethane:methanol (95:5) was used as the column eluant, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.50 (m, 2H), 1.60-1.70 (m, 7H), 1.78 (m, 1H), 1.90 (m, 1H), 2.20 (m, 4H), 2.40 (m, 2H), 2.58 (m, 1H), 3.14 (m, 1H), 3.20 (m, 1H), 3.38 (m, 6H), 3.42-3.60 (m, 6H), 7.00 (m, 1H).

Preparation 66

cis-tert-Butyl 3-(2-methoxyethoxy)-2-[(1-[[(4-[[(phenylsulfonyl)amino]carbonyl]-cyclohexyl)amino]carbonyl]cyclopentyl)methyl]propanoate

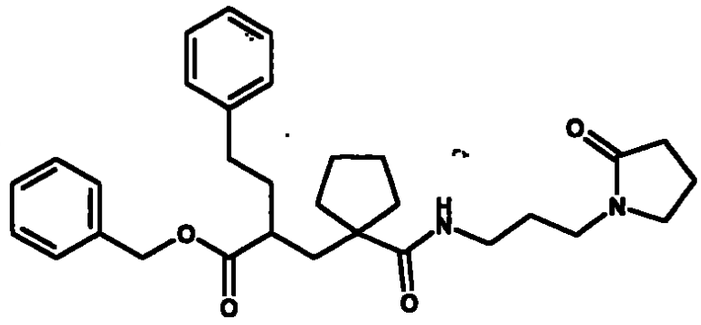


N,N'-Dicyclohexylcarbodiimide (199mg, 0.97mmol), 4-dimethylaminopyridine (118mg, 0.97mmol) and benzenesulphonamide (152mg, 0.97mmol) were added to an ice-cooled solution of the acid from preparation 63 (400mg, 0.878mmol) in

dichloromethane (12ml) and N,N-dimethylformamide (0.5ml), and the reaction stirred at room temperature for 20 hours. The mixture was concentrated under reduced pressure and the residue suspended in cold ethyl acetate. The resulting insoluble material was filtered off, the filtrate washed with hydrochloric acid (1N), and water, then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound as a white foam, 480mg, 92%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.44 (s, 9H), 1.63 (m, 13H), 1.80 (m, 2H), 1.88 (m, 1H), 1.98 (m, 2H), 2.36 (m, 1H), 2.57 (m, 1H), 3.38 (s, 3H), 3.40 (m, 1H), 3.51 (t, 2H), 3.58 (m, 3H), 3.95 (m, 1H), 5.92 (d, 1H), 7.56 (m, 2H), 7.62 (m, 1H), 8.05 (d, 2H), 8.75 (bs, 1H); LRMS : m/z 618 (MNa^+).

Preparation 67

Benzyl 2-[[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl]-4-phenylbutanoate

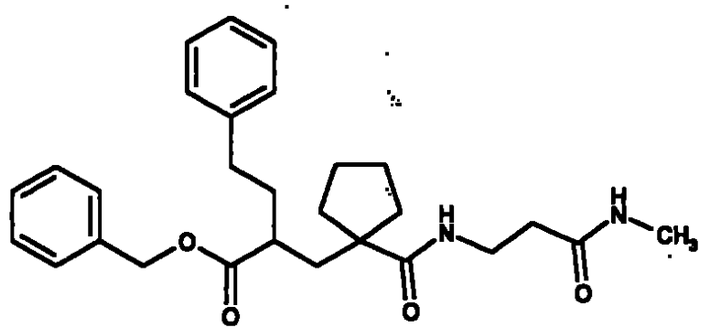


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.06g, 5.53mmol), 1-hydroxybenzotriazole hydrate (0.60g, 4.44mmol) and 4-methylmorpholine (0.56g, 5.54mmol) were added sequentially to a cooled solution of 1-[2-[(benzyloxy)carbonyl]-4-phenylbutyl]cyclopentane-carboxylic acid (EP 274234) (1.5g, 3.94mmol) in dry dichloromethane (15ml) at room temperature, followed by N-(3-aminopropyl)-2-pyrrolidinone (0.56g, 3.94mmol), and the reaction stirred at room temperature for 18 hours. The mixture was washed with water, 2N hydrochloric acid, saturated aqueous sodium bicarbonate solution, and then dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using ethyl acetate:pentane

(50:50) as the eluant to provide the title compound as a clear gum, 800mg, 40%; ^{1}H NMR ($CDCl_3$, 300MHz) d : 1.37-2.20 (m, 16H), 2.34-2.58 (m, 5H), 2.92-3.46 (m, 6H), 5.07 (d, 1H), 5.18 (d, 1H), 6.98-7.47 (m, 10H).

Preparation 68

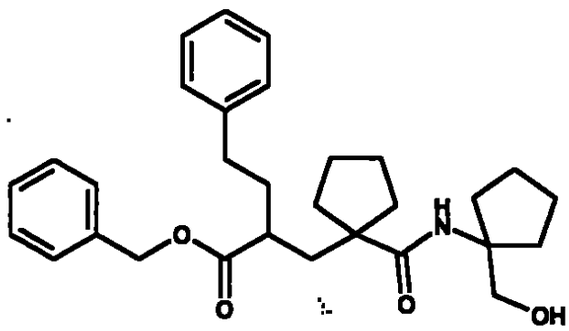
Benzyl 2-{[1-({[3-(methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (122mg, 0.64mmol), 1-hydroxybenzotriazole hydrate (86mg, 0.64mmol) and 4-methylmorpholine (173µl, 1.59mmol) were added sequentially to a cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (202mg, 0.53mmol) in N,N-dimethylformamide (5ml) at room temperature, followed by the amine hydrochloride from preparation 23 (146mg, 1.06mmol), and the reaction stirred at 90°C for 18 hours. The cooled solution was concentrated under reduced pressure and the residue partitioned between water (20ml) and ethyl acetate (100ml). The layers were separated, the organic phase washed with water (3x30ml), brine (25ml) dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (98:2) as eluant to afford the title compound as a colourless oil, 162mg, 67%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.38-1.53 (m, 2H), 1.53-1.96 (m, 8H), 2.02 (m, 2H), 2.27 (t, 2H), 2.46 (m, 3H), 2.76 (d, 3H), 3.44 (m, 2H), 5.13 (s, 2H), 5.79 (bs, 1H), 6.38 (m, 1H), 7.06 (d, 2H), 7.18 (m, 1H), 7.22 (m, 2H), 7.38 (m, 5H); LRMS : m/z 465.5 (MH^+).

Preparation 69

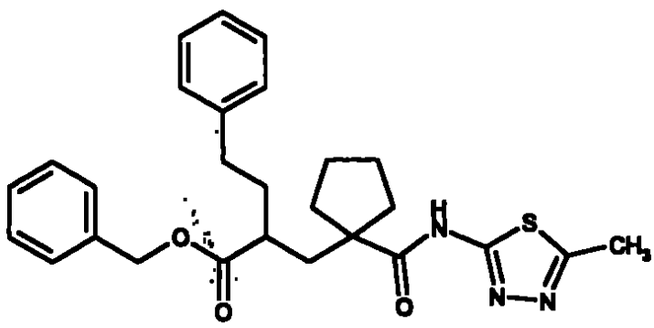
Benzyl 2-[[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl]-4-phenylbutanoate



The title compound was obtained as a crystalline solid (48%) from 1-[2-[(benzyloxy)carbonyl]-4-phenylbutyl]cyclopentane-carboxylic acid (EP 274234) and 1-amino-1-cyclopentanemethanol, following a similar procedure to that described in preparation 68, except the reaction mixture was stirred at room temperature for 18 hours, and the crude product purified by column chromatography on silica gel using ethyl acetate:pentane as eluant; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.38 (m, 2H), 1.50-1.95 (m, 16H), 2.01 (m, 2H), 2.45 (m, 3H), 3.49 (dd, 1H), 3.60 (dd, 1H), 4.58 (m, 1H), 5.10 (s, 2H), 5.67 (s, 1H), 7.01 (d, 2H), 7.14 (m, 1H), 7.20 (m, 2H), 7.36 (m, 5H); LRMS : m/z 478.3 (MH^+).

Preparation 70

Benzyl 2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoate

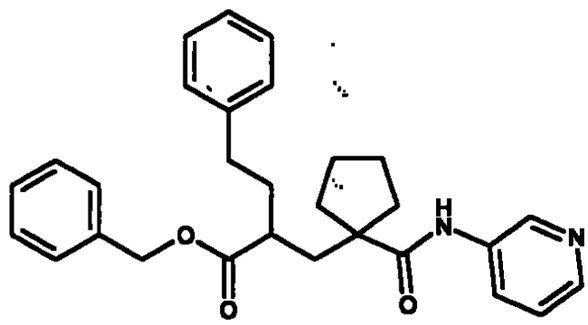


The title compound was obtained as a clear oil in 74% yield from 1-[2-[(benzyloxy)carbonyl]-4-phenylbutyl]cyclopentane-carboxylic acid (EP 274234) and 2-amino-5-methyl-1,3,4-thiadiazole, following a similar procedure to that

described in preparation 68; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.58-1.76 (m, 7H), 1.83-1.98 (m, 3H), 2.03 (m, 1H), 2.20 (m, 1H), 2.35 (m, 1H), 2.44 (m, 3H), 2.65 (s, 3H), 5.02 (dd, 2H), 7.00 (d, 2H), 7.15 (m, 1H), 7.19 (m, 2H), 7.35 (m, 5H); LRMS : m/z 478.7 (MH^+).

Preparation 71

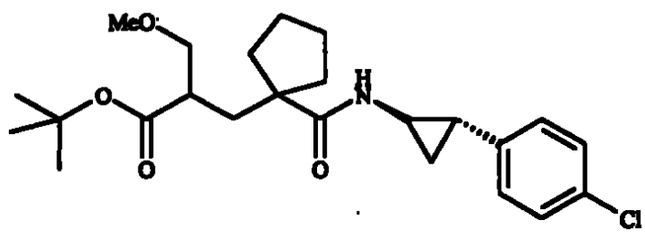
Benzyl 4-phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoate



Oxalyl chloride (2.29ml, 26.3mmol) was added to a solution of 1-[2-[(benzyloxy)carbonyl]-4-phenylbutyl]cyclopentane-carboxylic acid (EP 274234) (5.0g, 13.14mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (25ml), and the solution stirred for 2.5 hours. The mixture was evaporated under reduced pressure, the residue azeotroped with dichloromethane to give a yellow oil. This was then dissolved in dichloromethane (50ml) and a solution of this acid chloride (10ml, 2.45mmol) was added to an ice-cooled solution of triethylamine (248mg, 2.45mmol) and 3-aminopyridine (253mg, 2.70mmol) in dry dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The solution was washed with water (3x), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate:hexane (40:60) as eluant, and repeated using an elution gradient of ether:hexane (90:10 to 100:0). The product was crystallised from ethyl acetate:hexane to afford the title compound, 740mg, 66%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.38-2.07 (m, 10H), 2.10-2.37 (m, 2H), 2.42-2.63 (m, 3H), 5.02 (s, 2H), 6.94-7.44 (m, 10H), 7.50 (s, 1H), 8.03 (d, 1H), 8.36 (d, 1H), 8.52 (s, 1H).

Preparation 72

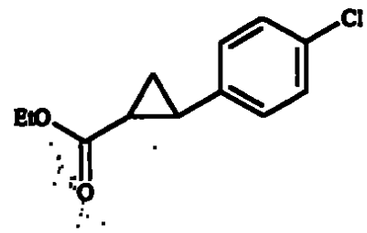
trans-tert-Butyl-3-[1-({[2-(4-chlorophenyl)cyclopropyl]amino}carbonyl)-cyclopentyl]-2-(methoxymethyl)propanoate



The product from Preparation 94 (286mg, 1mmol), *trans*-2-(4-chlorophenyl)cyclopropylamine (see Preparation 76) (203mg, 1mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (211mg, 1.1mmol), triethylamine (1ml) and HOBt (148mg, 1.1mmol) were all combined in 5mls DCM at room temperature and stirred for 16h. The reaction mixture was washed with water (20mls), dried over $MgSO_4$ and purified by column chromatography using 1:8, then 1:5 EtOAc:pentane as eluant to provide the title product as a colourless film (122mg, 40%); R_f 1:5 (EtOAc:pentane) 0.2; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.05-1.2 (m, 2H), 1.4 (s, 9H), 1.55-1.75 (m, 4H), 1.9-2.0 (m, 4H), 2.4-2.5 (m, 1H), 2.75-2.85 (m, 1H), 3.3 (s, 3H), 3.4-3.5 (m, 1H), 6.3 (m, 1H), 7.05 (d, 2H), 7.2 (d, 2H); HRMS : m/z M+H, Found 436.2242. $C_{24}H_{38}NO_4Cl$ requires 436.2249.

Preparation 73

Ethyl-2-(4-chlorophenyl)cyclopropanecarboxylate

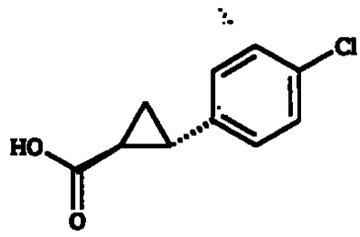


A mixture of 4-chlorostyrene (10.1ml, 96mmol) and rhodium acetate dimer (1g, 4.5mmol) in toluene (50ml) was heated to 85°C before adding ethyl diazoacetate (11.3mls, 94mmol) over 30mins and the whole then heated at 80°C for a further

1h before concentration *in vacuo*. Th residue was then purified by column chromatography using 1:2 DCM:pentane as eluant to give the *cyclopropane* as a colourless oil (7.8g, 37%); R_f 1:2 (DCM:pentane) 0.35; ^{1}HNMR (400MHz, $CDCl_3$) δ 1.15-1.3 (m, 4H), 1.5-1.7 (m, 1H), 1.8-1.9 (m, 1H), 2.4-3.55 (m, 1H), 4.2 (q, 2H), 6.95 (d, 2H), 7.20-7.28 (m, 2H); LRMS : m/z, $M+NH_4^+$ 242.

Preparation 74

trans-2-(4-Chlorophenyl)cyclopropanecarboxylic acid

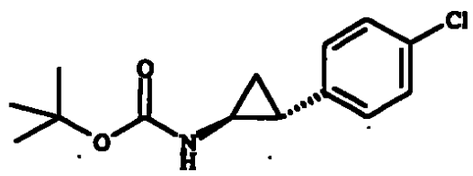


The product from preparation 73 (7.8g, 37mmol) was dissolved in EtOH (75mls) at room temperature under nitrogen and sodium methoxide (8.1g, 150mmol) was added portionwise over 15mins. After the addition was complete, the whole was then refluxed for 18h. The reaction mixture was concentrated *in vacuo*, and the resulting residue diluted with DCM and water (150mls, 2:1 mixture). The organic layer was removed, and the aqueous layer re-extracted with DCM (2x50mls). The combined organic extracts were dried over $MgSO_4$ and evaporated to provide the *trans* ester (4.96g, 62%). Acidification of the aqueous layer with concentrated HCl to pH 1 resulted in a white precipitate, which was filtered and dried under vacuum to provide the hydrolysis product (the corresponding acid) as a white powder (1.95g, 27%). Dissolution of the ester in MeOH (50mls), water (50mls) and LiOH (1.34g, 32mmol) gave a clear solution which was heated at *ca.* 70°C overnight. The reaction mixture was cooled, concentrated *in vacuo*, and acidified with concentrated HCl to pH 1. The resulting white precipitate was extracted with EtOAc (3x50mls) and the combined organic extracts were dried over $MgSO_4$ and evaporated to dryness, to provide the acid (4g, 96%). This acid was combined with the hydrolysed product from the previous step to give a total of 5.95g; ^{1}HNMR (400MHz, $CDCl_3$) δ 1.3-1.4 (m, 1H), 1.6-1.7 (m, 1H), 1.8-1.9 (m, 1H),

2.5-2.6 (m, 1H), 7.00 (d, 2H), 7.26 (d, 2H); LRMS : m/z, M-H 195.

Preparation 75

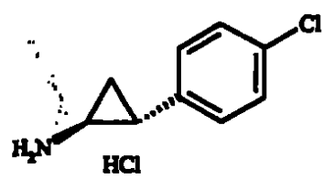
trans-tert-Butyl-2-(4-chlorophenyl)cyclopropylcarbamate



The product form preparation 74 (1.5g, 7.65mmol), DPPA (1.8ml, 8.4mmol) and triethylamine (1.25ml, 12mmol) were combined in 20mls *tert*-butanol under nitrogen and heated at *ca.* 90°C for 48h before the heating was removed and the mixture allowed to cool to room temperature. The mixture was diluted with EtOAc (20mls) and saturated Na_2CO_3 solution (20mls) and the organic layer was then removed. The aqueous layer was reextracted with EtOAc (20mls) and the combined organic layers were dried over $MgSO_4$, filtered and evaporated. The resulting residue was purified by column chromatography using 1:10, then 1:2 EtOAc:pentane as eluant to provide the carbamate as a white solid (1.7g, 83%); R_f 1:2 (EtOAc:pentane) 0.9; ^{1}HNMR (400MHz, $GDCl_3$) δ : 1.2-1.3 (m, 1H), 1.4 (s, 10H), 1.9-2.0 (m, 1H), 2.6 (br.s, 1H), 4.8 (br.s, 1H), 7.0 (d, 2H), 7.2 (d, 2H); HRMS : m/z M+Na, Found 290.0923. $C_{14}H_{18}NO_2ClNa$ requires 290.0918.

Preparation 76

2-(4-Chlorophenyl)cyclopropylamine



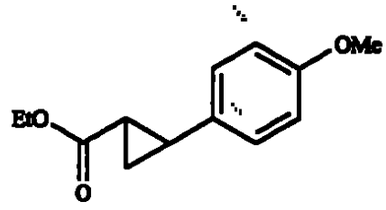
The product from Preparation 75 was taken up in EtOAc, cooled to 0°C and

454

hydrogen chloride gas was bubbled through the solution for 30mins. The solution was then concentrated *in vacuo* to give a pale yellow solid of the amine salt (1.29g, 6.3mmol); R_f 1:3 (EtOAc:pentane); ^{1}HNMR (400MHz, CD_3OD) δ : 1.3-1.4 (m, 1H), 1.4-1.5 (m, 1H), 2.3-2.4 (m, 1H), 2.8-2.9 (m, 1H), 7.15 (d, 2H), 7.3 (d, 2H).

Preparation 77

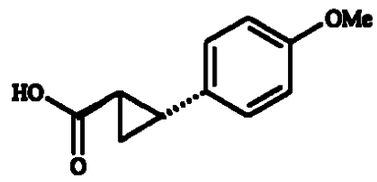
Ethyl-2-(4-methoxyphenyl)-cyclopropanecarboxylate



A mixture of 4-methoxystyrene (25.5mls, 192mmols), rhodium acetate dimer (2g, 4.5mmol) and toluene (100mls) was stirred at room temperature under nitrogen for 20mins and then heated to 85°C. After the reaction attained this temperature, ethyl diazoacetate (19.8mls, 188mmols) was added dropwise over 50mins at a rate of one drop every 2 to 3 seconds to maintain the internal reaction temperature around 95°C. After the addition was complete, the mixture was heated for 1h at 85°C and then cooled to room temperature. The mixture was filtered through arbocel and evaporated to an oil which was purified by column chromatography using DCM:pentane (1:2) as eluant to provide the cyclopropane as a pale yellow solid (13g, 31%) which was a 3:1 mixture of the *trans:cis* isomers; R_f 0.22 (DCM:pentane) 1:2; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.20-1.38 (m, 5H), 1.83 (ddd, 1H), 2.50 (ddd, 1H), 3.80 (s, 3H), 4.16 (q, 2H), 6.82 (d, 2H), 7.03 (d, 2H).

Preparation 78

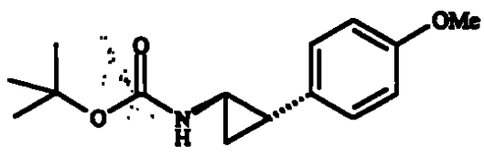
Trans-2-(4-methoxyphenyl)-cyclopropanecarboxylic acid



Sodium methoxide (14.8g, 273.8mmol) was added to a solution of the product from Preparation 77 (13g, 59mmol) in EtOH (135mls) while stirring at room temperature under a nitrogen atmosphere. After the addition was complete, the mixture was refluxed gently for 1h, by which time TLC analysis indicated that there was no *cis* isomer remaining. The reaction was cooled to room temperature and water (100mls) added in one portion. The whole was then stirred at room temperature for 63h and then evaporated to low volume to remove the MeOH before acidifying with concentrated HCl to pH 1. The suspension was extracted with EtOAc (2x100mls), and the extracts dried ($MgSO_4$) and evaporated to give a yellow solid, which was purified by column chromatography using 1:1 EtOAc:pentane to provide the acid as a pale yellow solid (8.9g, 78%); R_f 1:1 (EtOAc:pentane) 0.4; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.30-1.41 (m, 1H), 1.58-1.69 (m, 1H), 1.79-1.90 (m, 1H), 2.53-2.62 (m, 1H), 6.83 (d, 2H), 7.04 (d, 2H).

Preparation 79

Trans-tert-Butyl-2-(4-methoxyphenyl)-cyclopropylcarbamate

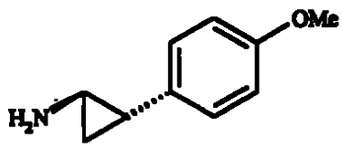


DPPA (11mls, 50.9mmol) was added to a stirred mixture of the product from Preparation 78 (8.9g, 46.3mmol), triethylamine (10.1mls, 72.7mmol) and *tert*-

BuOH (75mls). The whole was then heated at 90°C for 43h, and the *tert*-BuOH then removed by evaporation and the resulting oily residue treated with 120mls of saturated K_2CO_3 and then extracted with EtOAc (2x100mls). The combined organic extracts were then evaporated under reduced pressure to give a brown solid which was purified by column chromatography using DCM:MeOH (98:2) as eluant to provide the carbamate (5.8g, 48%) as a clear oil; ^{1}HNMR (400MHz, $CDCl_3$) δ: 1.07-1.14 (m, 2H), 1.44 (s, 9H), 1.93-2.06 (m, 1H), 2.62-2.71 (m, 1H), 3.80 (s, 3H), 4.72-4.88 (m, 1H), 6.80 (d, 2H), 7.08 (d, 2H).

Preparation 80

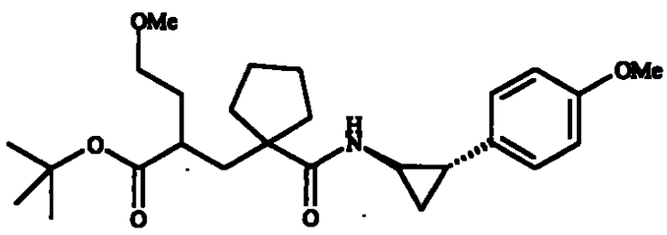
Trans-2-(4-methoxyphenyl)-cyclopropylamine



TFA (20mls) was added to a stirred mixture of the product from Preparation 79 (5.8g, 22.0mmol) and DCM (15mls) at room temperature under nitrogen. The reaction was stirred for 16h, after which time the solvent was removed under reduced pressure, and the resulting oil treated with saturated aqueous K_2CO_3 until a pH of 10 was reached (*ca.* 150mls required). This opaque solution was extracted with EtOAc (2x150mls) and the extracts were then dried over $MgSO_4$ and evaporated to give a biege solid. This solid was purified by column chromatography using 99:1, the 95:5 DCM:MeOH as eluant to give the amino cyclopropane as a white solid (3.2g, 89%); R_f DCM:MeOH (19:1) 0.18 ^{1}HNMR (400MHz, $CDCl_3$) δ : 0.87-1.04 (m, 2H), 1.79-1.90 (m, 1H), 2.43-2.54 (m, 1H), 3.80 (s, 3H), 6.80 (d, 2H), 6.98 (d, 2H).

Preparation 81

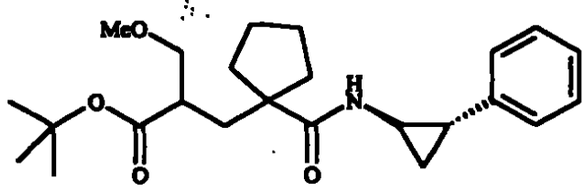
Tert-Butyl-4-methoxy-2-[[1-({[2-(4-methoxyphenyl)cyclopropyl]amino}carbonyl)-cyclopentyl]methyl]butanoate



1-[2-[(*tert*-Butoxy)carbonyl]-4-methoxybutyl]cyclopentane carboxylic acid (see for preparation EP274234) (210mg, 0.66mmol), triethylamine (1ml), HOBt (140mg, 0.73mmol) and the product from Preparation 80 (107mg, 0.66mmol) were combined sequentially in DCM at room temperature under nitrogen. WSCDI (98mg, 0.73mmol) was then added to the mixture and the whole stirred overnight for some 16h. The reaction was diluted with water, the organic layer was separated and then washed with brine, dried over $MgSO_4$, filtered and evaporated to a yellow oil. This was purified by column chromatography using 1:3 EtOAc:pentane as eluant to provide the amide (113mg, 38%) as a colourless film; R_f 1:10 (EtOAc:pentane) 0.2; ^{1}NMR (400MHz, $CDCl_3$) δ : 1.0-1.10 (m, 2H), 1.40 (s, 9H), 1.35-1.45 (m, 2H), 1.50-1.80 (m, 7H), 1.85-2.10 (m, 4H), 2.25-2.35 (m, 1H), 2.70-2.80 (m, 1H), 3.2 (s, 3H), 3.25-3.35 (m, 2H), 3.7 (s, 3H), 6.05 (br.s, 1H), 6.70 (d, 2H), 7.05 (d, 2H); LRMS: m/z 446 (M+H).

Preparation 82

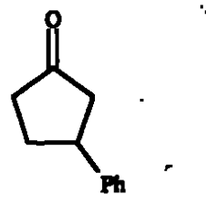
Tert-Butyl-3-methoxy-2-[(1-[[(2-phenylcyclopropyl)amino]carbonyl]cyclopentyl)-methyl]propanoate



The product from Preparation 94 (200mg, 0.66mmol), triethylamine (1ml), HOBt (98mg, 0.73mmol) and 1-*S*-amino-2-*R*-phenyl cyclopropane (*J. Med. Chem.*, **1986**, 29, 2044) (123mg, 0.73mmol) were combined sequentially in DCM (6mls) at room temperature under nitrogen. WSCDI (98mg, 0.73mmol) was then added to the mixture and the whole stirred overnight for some 16h. The reaction was diluted with water, the organic layer was separated and then washed with brine, dried over $MgSO_4$, filtered and evaporated to a yellow oil. This was purified by column chromatography using 1:3 EtOAc:pentane as eluant to provide the amide (164mg, 62%) as a colourless film; R_f 1:10 (EtOAc:pentane) 0.2; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.0-1.1 (m, 2H), 1.4 (s, 9H), 1.5-1.8 (m, 6H), 1.8-2.05 (m, 4H), 2.3-2.4 (m, 1H), 2.7-2.8 (m, 1H), 3.2 (s, 3H), 3.25-3.35 (m, 1H), 3.7 (s, 3H), 6.05 (s, 1H), 6.7 (d, 2H), 7.05 (d, 2H); LRMS : m/z M+H, 448.

Preparation 83

3-Phenyl cyclopentanone

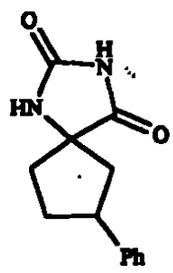


Phenyl magnesium bromide (0.27moles) was taken up in 200ml of dry ether, and cooled to 0°C under a nitrogen atmosphere. Copper (I) iodide (25.5g, 0.13moles) was added in one portion, and the suspension stirred at 0°C for 20mins. Cyclopenten-2-one was then added dropwise over 10-15mins and the resulting solution stirred at 0°C for 10mins, and then allowed to warm to room temperature over the course of 1h. The reaction mixture was added to 100mls of a mixture of saturated ammonium chloride solution and concentrated ammonia, the pH of which was initially measured at 9. The whole was stirred at room temperature for 30mins, and then filtered, and the layers of the filtrate were then separated. The aqueous layer was extracted with ether (2x70mls) and the extracts combined with the original organic layer. The bulked ether layers were

then washed with brine, dried over $MgSO_4$, filtered and evaporated to a pale yellow oil. This oil was then chromatographed using 1:3 ether/pentane to provide the phenyl cyclopentanone as a clear liquid (2.9g, 14%); R_f EtOAc:pentane (1:2) 0.65; ^{1}HNMR (300MHz, $CDCl_3$) δ : 1.91-2.10 (m, 1H), 2.18-2.59 (m, 4H), 2.67 (dd, 1H), 3.38-3.52 (m, 1H), 7.19-7.47 (m, 5H).

Preparation 84

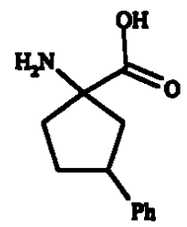
7-Phenyl-1,3-diazaspiro[4.4]nonane-2,4-dione



3-Phenyl cyclopentanone (5.8g), KCN (2.75g) and ammonium carbonate (9.1g) were heated in 80mls of 50% aqueous EtOH for 7h, and then at room temperature for 48h. The mixture was filtered, and the solid washed thoroughly with water (3x50mls). The filtrate was concentrated, the pH adjusted to 2 using concentrated HCl and the resulting suspension filtered off and washed with water (3x50mls). The bulked solids were recrystallised from EtOH and water (300ml:100ml) to provide 6.32g of the hydantoin as 1:1 mixture of diastereoisomeric pairs; R_f 0.6 in EtOAc; ^{1}HNMR (300MHz, $CDCl_3$) δ : 1.60-2.58 (m, 6H), 3.08-3.37 (m, 1H), 6.83-7.44 (m, 5H), 8.33 (d, 1H), 10.60 (s, 1H); Anal. Found: C, 68.09; H, 6.21; N, 12.25%. $C_{13}H_{14}N_2O_2$ requires C, 67.81; H, 6.13; N, 12.17%.

Preparation 85

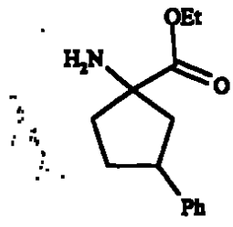
1-Amino-3-phenyl-cyclopentane carboxylic acid



The hydantoin from Preparation 84 (6.2g), barium hydroxide octahydrate (17.2g) and 100mls of water were heated together in a bomb at 160°C for 7h, and then allowed to stand overnight at room temperature. The reaction mixture was acidified to pH 1 using concentrated H_2SO_4. The resulting suspension was then filtered and the solid was washed with water (100mls). The filtrate was then basified to *ca.* pH 6 using concentrated ammonia solution, the suspension cooled in a ice bath, and then filtered. The solid was washed with water, and dried under vacuum to provide 2.9g of the amino acid as a white solid, MPt 265°C (dec.); R_f 0.5 in methyl *iso*butyl ketone: acetic acid: water (2:1:1); ^{1}HNMR (300MHz, $CDCl_3$) δ : 2.06-3.14 (m, 6H), 3.42-3.73 (m, 1H), 7.12-7.44 (m, 5H); Anal. Found C, 69.54; H, 7.26; N, 6.73%. $C_{12}H_{15}NO_2$ requires C, 70.22; H, 7.37; N, 6.82%.

Preparation 86

Ethyl-1-amino-3-phenyl-cyclopentanecarboxylate

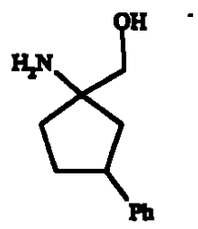


The product from Preparation 85 (500mg, 2.4mmol) was taken up in 70mls of EtOH saturated with hydrogen chloride at 0°C and then stirred at room

temperature for 16h. Nitrogen gas was then bubbled through the solution for 10mins, and the solvent evaporated to give a biege solid. This was treated with saturated aq. $NaHCO_3$ solution (10mls) and extracted with EtOAc (2x10mls). The combined organic extracts were dried and evaporated to give the *ester* as a clear oil (360mg, 63%); R_f DCM:MeOH (97:3) 0.23; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.31 (t, 3H), 1.50-2.37 (m, 4H), 2.38-2.44 (m, 1H), 2.63 (dd, 1H), 3.22-3.36 and 3.43-3.57 (m, 1H), 4.20 (q, 2H), 7.20-7.35 (m, 5H).

Preparation 87

(1-Amino-3-phenylcyclopentyl)methanol

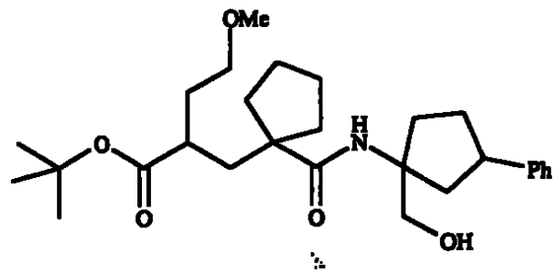


Sodium borohydride (190mg) was added portionwise to a stirred solution of the product from Preparation 86 (390mg, 1.67mmol) in 8mls of a 50% solution of aqueous EtOH and then heated at 50°C for 3h. The reaction mixture was then evaporated to give a suspension of an oil in water. This oil was extracted with EtOAc (20mls), and evaporated under reduced pressure to provide the amino alcohol as a white solid (295mg); R_f (1:2 ether:pentane) 0.65; ^{1}HNMR (400MHz, $CDCl_3$) δ : 1.30-2.36 (m, 6H), 3.02-3.17 (m, 1H), 3.33-3.50 (m, 2H), 7.14-7.37 (m, 5H).

458

Preparation 88

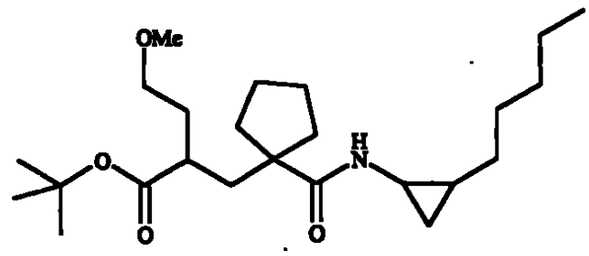
Tert-Butyl-2-{[1-({[1-(hydroxymethyl)-3-phenylcyclopentyl]amino}carbonyl)-cyclopentyl]methyl}-4-methoxybutanoate



Prepared by a similar method to that described in preparation 33, wherein 1-{2-[(*tert*-butoxy)carbonyl]-4-methoxybutyl}cyclopentane carboxylic acid (see for preparation EP274234) is coupled to the product from preparation 87; R_f EtOAc: pentane (1:4) 0.25; ^{1}HNMR (400MHz, $CDCl_3$) δ : 0.80-0.88 (m, 3H), 1.16-2.60 (m, 21H), 1.42 (s, 9H), 3.04-3.32 (m, 1H), 3.57-3.84 (m, 2H), 4.56-4.77 (m, 1H), 5.94 (br.t, 1H), 7.16-7.28 (m, 5H).

Preparation 89

Tert-Butyl-4-methoxy-2-{(1-{[(2-pentylcyclopropyl)amino]carbonyl}cyclopentyl)-methyl}butanoate

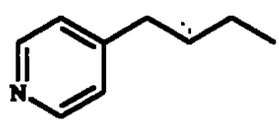


1-{2-[(*tert*-Butoxy)carbonyl]-4-methoxybutyl}cyclopentane carboxylic acid (see for preparation EP274234) (105mg, 0.33mmol), triethylamine (0.5ml), HOBt (49mg, 0.36mmol) and 1-amino-2-pentyl-cyclopropane (100mg, 0.33mmol) were combined sequentially in DCM at room temperature under nitrogen. WSCDI (70mg, 0.36mmol) was then added to the mixture and the whole stirred overnight for some 16h. The reaction was diluted with water, the organic layer was separated and then washed with brine, dried over $MgSO_4$, filtered and

evaporated to a yellow oil. This was purified by column chromatography using 1:3 EtOAc:pentane as eluant to provide the amide (96mg, 71%) as a colourless film; R_f 1:6 (EtOAc:pentane) 0.2; ^{1}H NMR (400MHz, $CDCl_3$) δ : 0.4-0.6 (m, 2H), 0.7-0.9 (m, 4H), 1.05-1.15 (m, 1H), 1.2-1.3 (m, 4H), 1.3-1.5 (m, 14H), 1.5-2.0 (m, 10H), 2.2-2.4 (m, 2H), 3.2-3.35 (m, 5H), 5.7-5.9 (br.s, 1H); LRMS : m/z, M+H, 410.

Preparation 90

4-Butylpyridine



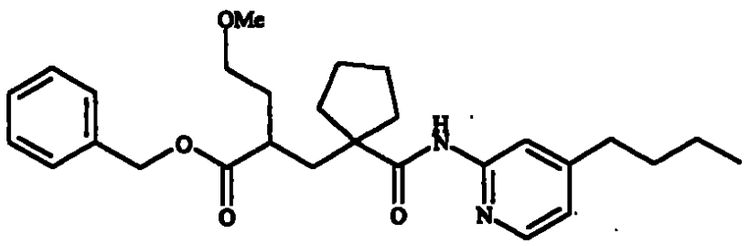
Lithium di*iso*propylamide was formed by the addition of 43mls of a 2.5M solution in hexanes of butyllithium to a stirred solution of di*iso*propylamine (10.9g) in dry THF (100mls) at -30°C under nitrogen. After 1h of stirring at this temperature, the solution was cooled to -78°C and a solution of 4-methyl pyridine was added (10g) in 20mls dry THF, followed by continued stirring at -78°C for 1h. Iodopropane (20g) was added dropwise over 45mins as a solution in 20mls dry THF, followed by continued stirring of the whole mixture for 1h. 20mls Of a saturated aqueous solution of ammonium chloride was added, and the reaction was then extracted with ether (2x100mls). The combined ether extracts were washed with water (75mls), dried over $MgSO_4$ and evaporated to give a clear oil. This oil was purified by distillation under water aspiration vacuum (*ca.* 30mmHg) and the butyl pyridine was collected as the fraction which distilled over at 84-90°C; ^{1}H NMR (400MHz, $CDCl_3$) δ : 0.93 (t, 3H, Me), 1.30-1.42 (m, 2H), 1.57-1.66 (m, 2H), 2.60 (t, 2H), 7.06 (d, 2H), 8.48 (d, 2H).

2-Amino-4-butyl pyridine was prepared by the method described in the Journal of the Chemical Society, 1946, p936.

1159

Preparation 91

Benzyl-2-[(1-[[(4-butyl-2-pyridinyl)-amino]carbonyl]cyclopentyl)methyl]-4-methoxy-butanoate

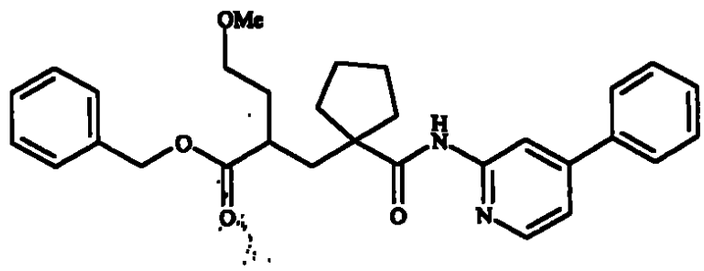


This compound was prepared by a similar procedure to that described in preparation 62; ^{1}HNMR ($CDCl_3$, 400MHz) δ : 0.89 (t, 3H, Me), 1.36 (q, 2H, C<u>H</u>$_2$), 1,47-2.26 (m, 14H), 2.55-2.68 (m, 3H), 3.17 (s, 3H, OMe), 3.24 (t, 2H, C<u>H</u>$_2$OMe), 4.91 (d, 1H, C<u>H</u>Ph), 5.00 (d, 1H, C<u>H</u>Ph), 6.83 (d, 1H, Ar), 7.27-7.35 (m, 5H), 7.94 (brs, 1H, N<u>H</u>), 8.07 (s, 1H, Ar), 8.13 (d, 1H, Ar).

2-Amino-4-phenyl pyridine was prepared by the method described in the Journal of Medicinal Chemistry, 1978, p874.

Preparation 92

Benzyl-2-[(1-[[(4-phenyl-2-pyridinyl)-amino]carbonyl]cyclopentyl)methyl]-4-methoxy-butanoate

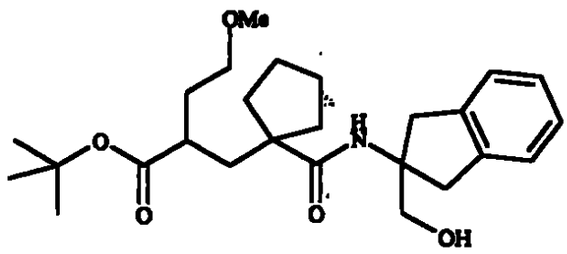


Prepared by a similar procedure to that described in preparation 62; ^{1}HNMR ($CDCl_3$, 400MHz) δ : 1.43-2.34 (m, 10H), 2.60-2.68 (m, 1H), 3.17 (s, 3H, OMe), 3.26 (t, 2H, C<u>H</u>$_2$), 4.93 (d, 1H, C<u>H</u>Ph), 5.02 (d, 1H, C<u>H</u>Ph), 7.18-7.32 (m, 5H, Ph),

7.38-7.46 (m, 3H), 7.61-7.69 (m, 2H), 8.02 (brs, 1H, NH), 8.29 (d, 1H, Ar), 8.57 (s, 1H, Ar).

Preparation 93

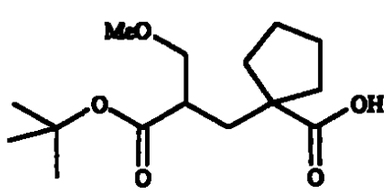
Tert-Butyl-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl]-4-methoxybutanoate



Prepared by a similar procedure to that described in preparation 33; ^{1}HNMR ($CDCl_3$, 400MHz) δ : 1.40 (s, 9H), 1.44-2.00 (m, 12H), 2.37-2.43 (m, 1H), 2.99 (d, 1H), 3.08 (d, 1H), 3.20-3.38 (m, 7H), 3.65 (dd, 1H), 3.84 (dd, 1H), 4.40 (t, 1H), 6.00 (s, 1H), 7.10-7.18 (m, 4H).

Preparation 94

3-(1-Carboxycyclopentyl)-2-(methoxymethyl)propanoic acid *tert*-butyl ester



3-(1-Carboxycyclopentyl)-propanoic acid *tert*-butyl ester (10g, 41.3mmol) was taken up in THF at -78°C and lithium di*iso*propylamide (43ml, 86.7mmol, 2M solution in THF) added dropwise. The mixture was stirred at -78°C for 40min, after which time chloromethyl methyl ether (4.7ml, 62mmol) was added dropwise. The solution was then allowed to warm slowly to room temperature overnight

and was quenched by the addition of 2N HCl (100ml). The organics were extracted with EtOAc (2x100ml), dried ($MgSO_4$) and purified by column chromatography using 2%, then 3%, and then 5% MeOH in DCM to provide the *title compound* as a yellow oil (6.2g, 53%); ^{1}HNMR ($CDCl_3$, 400MHz) δ 1.40 (9H, s), 1.40-1.50 (4H, m), 1.20-1.80 (1H, m), 1.80-1.90 (1H, m), 2.00 (1H, dd), 2.00-2.05 (3H, m), 2.20 (1H, dd), 2.50-2.60 (1H, m), 3.30 (1H, s), 3.30-3.40 (1H, m), 3.40 (1H, t); LRMS: m/z, MNH_4^+ 304.

NEP Assay

The Preparation and Assay of Soluble Neutral Endopeptidase (NEP) from Canine, Rat, Rabbit and Human Kidney Cortex.

Soluble NEP is obtained from the kidney cortex and activity is assayed by measuring the rate of cleavage of the NEP substrate Abz-D-Arg-Arg-Leu-EDDnp to generate its fluorescent product, Abz-D-Arg-Arg.

Experimental Procedure:-

1 Materials

All water is double de ionised.

1.1 Tissues:

Human Kidney	IIAM (Pennsylvania. U.S.A.)
Rat Kidney	In house tissue supply
Rabbit Kidney	In house tissue supply
Canine Kidney	In house tissue supply

1.2 Homogenisation medium:

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.3 Tris buffer (NEP buffer):

50ml of 50mM Tris pH 7.4 (Sigma T2663) is diluted in 950ml of water.

1.4 Substrate (Abz-D-Arg-Arg-Leu-EDDnp):

Made to order from SNPE, and is stored as a powder at –20°C. A 2mM

stock is made by gently re-suspending the substrate in Tris buffer, this should not be vortexed or sonicated. 600µl aliquots of the 2mM stock are stored at –20 for up to one month. (Medeiros, M.A.S., Franca, M.S.F. et al., (1997), Brazilian Journal of Medical and Biological Research, 30, 1157-1162).

1.5 Total product:

Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined. The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.6 Stock solution:

A 300µM stock of Phosphoramidon (Sigma R7385) is made up in NEP buffer and stored in 50µl aliquots at -20.

1.7 Dimethyl sulphoxide (DMSO).

1.8 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.9 Black 96 well flat bottom assay plates (Costar 3915).

1.10 Topseal A (Packard 6005185).

1.11 Centrifuge tubes

2 Specific Equipment

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 Fluostar galaxy.

2.5 Weebart 1589 shaking incubator.

3 Methods

3.1 Tissue Preparation

3.2 Dog, rat, rabbit, and human NEP is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is

461

dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approximately 10 volumes of homogenisation buffer (1.2) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.8) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer containing magnesium chloride (9mg MgCl in 5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,630rpm) for 12 minutes in a Beckman centrifuge (2.3) before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge (2.1) and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer containing magnesium chloride (0.9mg MgCl in 0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer (2.2). This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4 <u>Determination of NEP Activity</u>

The activity of the previously aliquoted NEP is measured by its ability to cleave the NEP specific peptide substrate.

4.1 A 4% DMSO/NEP buffer solution is made (4mls DMSO in 96mls NEP buffer).

4.2 Substrate, total product, enzyme, and Phosphoramidon stocks are left on ice to thaw.

4.3 50µl of 4% DMSO/NEP buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:40 to make a 50µM solution. 100µl of 50µM substrate is added to each well (final concentration 25µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of NEP buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:80 to make a 25µM solution. 200µl of 25µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37oC in a shaking incubator for 60 minutes.

4.8 The 300µM Phosphoramidon stock is diluted 1:100 to 300nM. The reaction is stopped by the addition of 100µl 300nM Phosphoramidon and incubated at 37°C in a shaking incubator for 20 minutes before being read on the Fluostar (ex320/em420).

5 <u>NEP Inhibition Assays</u>

5.1 Substrate, total product, enzyme and Phoshoramidon stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in NEP buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO solution (4mls DMSO in 96mls NEP buffer).

5.3 50µl of compound in duplicate is added to the 96 well plate and 50µl of 4% DMSO/NEP buffer is added to control and blank wells (see appendix for plate layout). Alternatively see appendix for robotic dilutions.

5.4 The 2mM substrate stock is diluted 1:40 in NEP buffer to make a 50µM solution (275µl 2mM substrate to 10.73ml buffer is enough for 1 plate).

5.5 The enzyme stock diluted in NEP buffer (determined from activity checks).

5.6 The 2mM total product stock is diluted 1:80 in NEP buffer to make a 25µM solution. 200µl is added to the first four wells of a separate plate.

5.7 The 300µM Phosphoramidon stock is diluted 1:1000 to make a 300nM stock (11µl Phosphoramidon to 10.99ml NEP buffer.

5.8 To each well in the 96 well plate the following is added:

Table: Reagents to be added to 96 well plate.

462

467

	Compound/ DMSO	Tris Buffer	Substrate	NEP enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.9 The reaction is initiated by the addition of the NEP enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.10 The reaction is stopped with 100µl 300nM Phosphoramidon and incubated at 37°C for 20 minutes in a shaking incubator before being read on the Fluostar (ex320/em420).

6 Calculations

The activity of the NEP enzyme is determined in the presence and absence of compound and expressed as a percentage.

% Control activity (turnover of enzyme) =

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

% Activity with inhibitor =

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

Activity expressed as % of control =

$$\frac{\text{\% Activity with inhibitor}}{\text{\% Control activity}} \times 100$$

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC50 values calculated using LabStats fit-curve in Excel.

The specific examples herein all had an IC50 against NEP of less than 5000nM.

In addition (in a preferred embodiment) many of the examples tested also had a selectivity for NEP over ACE of at least 300 fold.

ACE Assay

The Preparation and Assay of Soluble Angiotensin Converting Enzyme (Ace), from Porcine and Human Kidney Cortex.

Soluble ACE activity is obtained from the kidney cortex and assayed by measuring the rate of cleavage of the ACE substrate Abz-Gly-p-nitro-Phe-Pro-OH to generate its fluorescent product, Abz-Gly.

1 Materials

All water is double de ionised.

1.1 Human Kidney: IIAM (Pennsylvania. U.S.A.) or UK Human Tissue Bank (UK HTB)

1.2 Porcine kidney ACE Sigma (A2580)

1.3 Homogenisation buffer-1

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.4 Homogenisation buffer-2

100mM Mannitol, 20mM Tris @ pH7.1 and 10mM $MgCl_2 6H_2O$ (Fisher M0600/53)

To 500ml of the homogenisation buffer 1 (1.4) 1.017g of $MgCl_2$ is added.

1.5 Tris buffer (ACE buffer).

50mM Tris and 300mM NaCl @ pH 7.4

50ml of 50mM Tris pH 7.4 (Sigma T2663) and 17.52g NaCl (Fisher S/3160/60) are made up to 1000ml in water.

463

1.6 Substrate (Abz-D-Gly-p-nitro-Phe-Pro-OH) (Bachem M-1100)
ACE substrate is stored as a powder at –20°C. A 2mM stock is made by gently re-suspending the substrate in ACE buffer, this must not be vortexed or sonicated. 400µl aliquots of the 2mM stock are stored at –20°C for up to one month.

1.7 Total product
Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined (see calculations). The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.8 Stop solution.
0.5M EDTA (Promega CAS[6081/92/6]) is diluted 1:250 in ACE buffer to make a 2mM solution.

1.9 Dimethyl sulphoxide (DMSO).

1.10 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.11 Black 96 well flat bottom assay plates (Costar 3915 or Packard).

1.12 Topseal A (Packard 6005185).

1.13 Centrifuge tubes

2 Specific Equipment

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 BMG Fluostar Galaxy.

2.5 Wesbart 1589 shaking incubator.

3 Methods

3.1 Tissue Preparation

3.2 Human ACE is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approximately 10 volumes of homogenisation buffer-1 (1.4) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.11) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer-2 (1.5) (5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,630rpm) for 12 minutes in a Beckman centrifuge before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer-2 (0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer. This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4 <u>Determination Of ACE Activity</u>

The activity of the previously aliquoted ACE is measured by its ability to cleave the ACE specific peptide substrate.

Porcine ACE (1.2) is defrosted and resuspended in ACE buffer (1.6) at 0.004U/µl, this is frozen down in 50µl aliquots.

4.1 A 4% DMSO/ACE buffer solution is made (4mls DMSO in 96mls ACE buffer).

4.2 Substrate (1.7), total product (1.8) and enzyme (1.1, 1.2, 1.3), are left on ice to thaw.

4.3 50µl of 4% DMSO/ACE buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:100 to make a 20µM solution. 100µl of 20µM substrate is added to each well (final concentration in the assay

464

10µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of ACE buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:200 to make 10µM solution. 200µl 10µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37°C in a shaking incubator for 60 minutes.

4.8 The enzyme reaction is stopped by the addition of 100µl 2mM EDTA in ACE buffer and incubated at 37°C in a shaking incubator for 20 minutes before being read on the BMG Fluostar Galaxy (ex320/em420).

5 <u>ACE Inhibition Assays</u>

5.1 Substrate, total product, and enzyme stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in ACE buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO/ACE buffer solution (4mls DMSO in 96mls ACE buffer).

5.3 50µl of compound, in duplicate, is added to the 96 well plate and 50µl of 4% DMSO/ACE buffer is added to control and blank wells (see appendix-1 for plate layout).

5.4 Steps 5.2 and 5.3 can be carried out either by hand or using the Packard multiprobe robots (see appendix-2 for details)

5.5 The 2mM substrate stock is diluted 1:100 in ACE buffer to make a 20µM solution (10µM final concentration in the assay) (110µl of 2mM substrate added to 10.89ml buffer is enough for 1 plate).

5.6 The enzyme stock is diluted in ACE buffer, as determined from activity checks (4.0).

5.7 The 2mM total product stock is diluted 1:200 in ACE buffer to make a 10µM solution. 200µl is added to the first four wells of a separate plate.

5.8 The 0.5mM EDTA stock is diluted 1:250 to make a 2mM stock (44µl EDTA to 10.96ml ACE buffer).

5.9 To each well of the 96 well plate the following reagents are added:

Table 1: Reagents added to 96 well plate.

	Compound/ DMSO	Tris Buffer	Substrate	ACE enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.10 50µl of the highest concentration of each compound used in the assay is added in duplicate to the same 96 well plate as the totals (5.7). 150µl of ACE buffer is added to determine any compound fluorescence.

5.11 The reaction is initiated by the addition of the ACE enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.12 The reaction is stopped by the addition of 100µl 2mM EDTA and incubated at 37°C for 20 minutes in a shaking incubator, before being read on the BMG Fluostar Galaxy (ex320/em420).

6 Calculations

The activity of the ACE enzyme is determined in the presence and absence of compound and expressed as a percentage. (FU = Fluorescence units)

(i) % Control activity (turnover of enzyme) =

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(ii) % Activity with Inhibitor =

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(iii) Activity expressed as % of control =

$$\frac{\text{\% Activity with inhibitor}}{\text{\% Control activity}} \times 100$$

or $$\frac{\text{Mean FU of compound – Mean FU of blanks}}{\text{Mean FU of controls – Mean FU of blanks}} \times 100$$

(iv) % Inhibition = 100 - % control

(v) For fluorescent compounds the mean FU of blanks containing compound (5.10) is deducted from the mean FU of compound values used to calculate the % Activity.

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC_{50} values calculated using LabStats fit-curve in Excel.

Animal Model of arousal response

A particularly preferred compound of the invention (selected from the list of 21 compounds given herebefore) was administered according to the following protocol to show an increase in genital blood flow in the rabbit (it has previously been shown by Ottensen that an increase in vaginal blood flow increase vaginal lubrication-. Ottesen, B., Pedersen, B., Nielsen, J. *et al.* (1987). Vasoactive intestinal polypeptide (VIP) provokes vaginal lubrication in normal women. *Peptides*, 8, 797-800.

"The genital organs consist of an internal and external group. The internal organs are situated within the pelvis and consist of ovaries, the uterine tubes, uterus and the vagina. The external organsare superficial to the urogenital diaphragm and below the pelvic arch. They comprise the mons pubis, the labia majora and minora pudendi, the clitoris, the vestibule, the bulb of the vestibule, and the

greater vestibular glands" (Gray's Anatomy, C.D. Clemente, 13th American Edition).

Methods

Anaesthetic Protocol

Female New Zealand rabbits (~2.5kg) were pre-medicated with a combination of Medetomidine (Domitor®) 0.5ml/kg *i.m.*, and Ketamine (Vetalar®) 0.25ml/kg *i.m.* whilst maintaining oxygen intake via a face mask. The rabbits were tracheotomised using a Portex™ uncuffed endotracheal tube 3 ID., connected to ventilator and maintained a ventilation rate of 30-40 breaths per minute, with an approximate tidal volume of 18-20 ml, and a maximum airway pressure of 10 cm H_2O. Anaesthesia was then switched to Isoflurane and ventilation continued with O_2 at 2l/min. The right marginal ear vein was cannulated using a 23G or 24G catheter, and Lactated Ringer solution perfused at 0.5ml/min. The rabbit was maintained at 3% Isoflurane during invasive surgery, dropping to 2% for maintenance anaesthesia.

Cannulation of Vessels

The left groin area of the rabbit was shaved and a vertical incision was made approximately 5cm in length along the thigh. The femoral vein and artery were exposed, isolated and then cannulated with a PVC catheter (17G) for the infusion of drugs and compounds. Cannulation was repeated for the femoral artery, inserting the catheter to a depth of 10cm to ensure that the catheter reached the abdominal aorta. This arterial catheter was linked to a Gould system to record blood pressure. Samples for blood gas analysis were also be taken via the arterial catheter. Systolic and diastolic pressures were measured, and the mean arterial pressure calculated using the formula (diastolic x2 + systolic) ÷3. Heart rate was measured via the pulse oxymeter and *Po-ne-mah* data acquisition software system (Ponemah Physiology Platform, Gould Instrument Systems Inc).

466

Stimulation of the Pelvic Nerve

A ventral midline incision was made into the abdominal cavity. The incision was about 5cm in length just above the pubis. The fat and muscle was *bluntly* dissected away to reveal the hypogastric nerve which runs down the body cavity. It was essential to keep close to the side curve of the pubis wall in order to avoid damaging the femoral vein and artery which lie above the pubis. The sciatic and pelvic nerves lie deeper and were located after further dissection on the dorsal side of the rabbit. Once the sciatic nerve is identified, the pelvic nerve was easily located. The term *pelvic nerve* is loosely applied; anatomy books on the subject fail to identify the nerves in sufficient detail. However, stimulation of the nerve causes an increase in vaginal and clitoral blood flow, and innervation of the pelvic region. The pelvic nerve was freed away from surrounding tissue and a *Harvard* bipolar stimulating electrode was placed around the nerve. The nerve was slightly lifted to give some tension, then the electrode was secured in position. Approximately 1ml of light paraffin oil was placed around the nerve and electrode. This acts as a protective lubricant to the nerve and prevents blood contamination of the electrode. The electrode was connected to a *Grass* S88 Stimulator. The pelvic nerve was stimulated using the following parameters:- 5V, pulse width 0.5ms, duration of stimulus 10 seconds and a frequency range of 2 to 16Hz. Reproducible responses were obtained when the nerve was stimulated every 15-20 minutes.

A frequency response curve was determined at the start of each experiment in order to determine the optimum frequency to use as a sub-maximal response, normally 4Hz. The compound(s) to be tested were infused, via the femoral vein, using a *Harvard* 22 infusion pump allowing a continuous 15 minute stimulation cycle.

Positioning of the Laser Doppler Probes

A ventral midline incision was made, at the caudal end of the pubis, to expose the pubic area. Remove any connective tissue, and expose the tunica of the clitoris, ensuring that the wall is free from small blood vessels. The external

vaginal wall was also exposed by removing any connective tissue. One laser Doppler flow probe was inserted 3cm into the vagina, so that half the probe shaft is still visible. A second probe was positioned so that it lies just above the external clitoral wall. The position of these probes was then adjusted until a signal was obtained. A second probe was placed just above the surface of a blood vessel on the external vaginal wall. Both probes were clamped in position. Vaginal and clitoral blood flow was recorded either as numbers directly from the Flowmeter using *Po-ne-mah* data acquisition software (Ponemah Physiology Platform, Gould Instrument Systems Inc), or indirectly from Gould chart recorder trace. Calibration is set at the beginning of the experiment (0-125ml/min/100g tissue).

Infusion of Inhibitors

A particularly preferred NEP (Neutral Endopeptidase EC3.4.24.11) inhibitor chosen from the list of 21 given hereinbefore was made up in saline or 5% glucose solution (200µl 50% glucose in 1.8ml water for injection). The inhibitor and vehicle controls were infused using a Harvard 22 pump, infusing at 500µl/min via a 3-way tap into the femoral vein. After the infusion, the catheter was flushed with heparinised saline (Hepsaline) so that no NEP inhibitor was left in the catheter.

Results and Discussion

Animal model of sexual arousal

The major cause of FSAD is decreased genital blood flow and this manifests itself as reduced vaginal, labial and clitoral engorgement. Treatment of women with FSAD is achievable by restoration of the *normal* sexual arousal response. This can be achieved by enhancing genital blood flow.

We have developed a robust reproducible model of the physiology of sexual arousal. Using this anaesthetised rabbit model, we are capable of measuring small changes in genital blood flow using Laser Doppler technology. Stimulation

467

of the pelvic nerve is used to simulate the neuronal effects of sexual arousal. FSAD is associated with and may result from reduced genital blood flow.

The selective NEP inhibitor tested, at clinically relevant doses, significantly enhanced pelvic nerve stimulated increases in genital blood flow (See Figure 1). The NEP inhibitor enhanced the peak increase in vaginal blood flow by up to 92% (n=3) and clitoral blood flow by 131% (n=3) compared to time matched control increases.

We have developed an animal model that mimics the physiological arousal response observed during female sexual arousal and directly reflects the clinical data obtained in human volunteers. The model uses Laser Doppler technologies to record small changes in vaginal and clitoral blood flow induced by pelvic nerve stimulation or vasoactive neurotransmitters. During sexual arousal, there is an increase in genital blood flow resulting from increased innervation from the pelvic nerve. The pelvic nerve-stimulated increase in vaginal and clitoral blood flow, observed in the animal model, represents the endogenous vascular effects observed during female sexual arousal. Therefore this model can be used to firstly, identify the mechanisms involved in the regulation of vaginal and clitoral blood flow and secondly, use the model to validate novel approaches for the enhancement of genital blood flow.

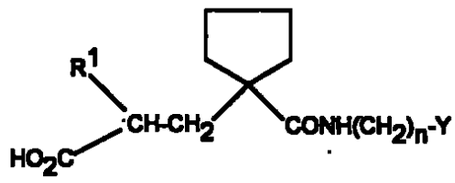
Figure 1 shows effect of administering a selective NEP inhibitoron the genital blood flow in a rabbit. The selective inhibitor of NEP, EC 3.4.24.11, enhanced pelvic nerve stimulated (PNS) increases in genital blood flow in the anaesthetised rabbit model of sexual arousal. Repetitive PNS at 15 minute intervals induced reproducible increases in genital blood flow (Hatched Bars). Administration of a NEP inhibitor (Grey bar) enhanced the peak increase in clitoral and vaginal blood flow induced by submaximal stimulation frequencies (eg 4Hz) compared to increases observed during time matched control stimulations or vehicle controls (Hatched bar). The following simultaneous enhancements were observed following a 1.0mg/kg iv bolus – a 131% increase in clitoral and a 92% increase in

vaginal blood flow (n=3). Data expressed as mean ± sem; all changes were monitored using laser Doppler technologies.

5

Claims

1 The use of a compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, in the preparation of a medicament for the treatment of female sexual dysfunction;



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6} alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

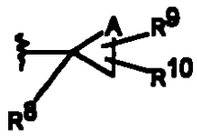
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or $-NR^2R^3$ wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

the $-(CH_2)_n-$ linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

Y is the group



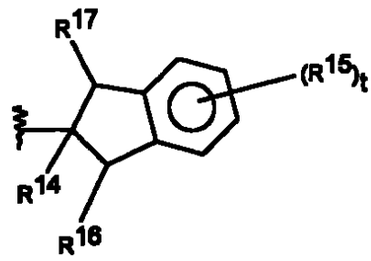
wherein A is $-(CH_2)_q-$ where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated;

R^8 is H, C_{1-8}alkyl, $-CH_2OH$, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-8}alkyl, phenyl (optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy) or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

Y is the group, $-C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

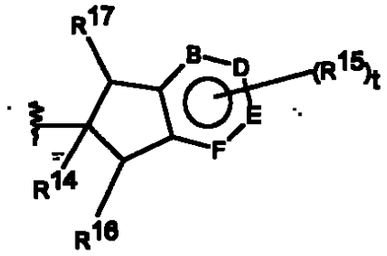
469

Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl,

heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl; and

u is 0, 1, 2 or 3.

2 A compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, wherein R^1, n and Y are as defined in claim 1 with the proviso that Y is not the group $-C(O)NR^{11}R^{12}$ and when R^1 is propyl or phenylethyl, R^{14} is not $-CH_2OH$.

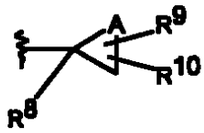
3 A compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, wherein R^1, n and Y are as defined in claim 1 with the proviso that Y is not the group $-C(O)NR^{11}R^{12}$ and R^{14} is not H or $-CH_2OH$.

4 A compound as defined in any one of claims 1 to 3 wherein R^1 is C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl, C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl or C_{1-6}alkyl substituted with aryl.

5 A compound as defined in claim 4 wherein R^1 is C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl or C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl.

6 A compound as defined in claim 5 wherein R^1 is C_{1-4}alkyl or C_{1-6}alkoxy(C_{1-3})alkyl.

7 A compound as defined in any preceding claim wherein when Y is the group



and the carbocyclic ring is fully saturated, then one of R^9 or R^{10} is $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl.

8 A compound as defined in claim 7 wherein the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl.

9 A compound as defined in claims 7 or 8 wherein R^9 and R^{10} are attached to adjacent carbon atoms in the ring.

10 A compound as defined in any one of claims 7 to 9 wherein R^8 is CH_2OH.

11 A compound as defined in any one of claims 1 to 6 wherein when Y is the group $-NR^{18}S(O)_uR^{19}$.

12 A compound as defined in any one of claims 1 to 6 or 11 wherein R^{19} is benzyl or phenyl.

13 A compound as defined in any one of claims 1 to 6 or 11 or 12 wherein u

is 2.

14 A compound as defined in any one of claims 1 to 6 wherein Y is the optionally substituted 5-7 membered heterocyclic ring.

15 A compound as defined in claim 14 wherein the 5-7 membered heterocyclic ring is an optionally substituted aromatic ring.

16 A compound as defined in claim 15 wherein said aromatic ring is pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazolyl, thiazolyl, thiadiazolyl, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, pyridonyl, quinoxalinyl or quinazolinyl each of which may be substituted as defined in claim 1.

17 A compound as defined in claim 16 wherein the aromatic ring is oxadiazole, pyridone or thiadiazole each of which may be substituted as defined in claim 1.

18 A compound as defined in claim 17 wherein the aromatic ring is 1,2,5-oxadiazole, 1,3,4-oxadiazole, 2-pyridone or 1,3,4-thiadiazole each of which may be substituted as defined in claim 1.

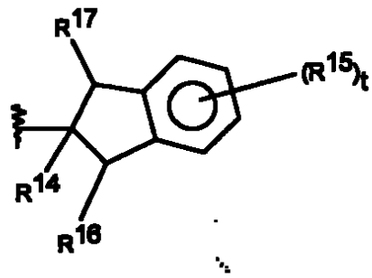
19 A compound as defined in any one of claims 14 to 18 wherein the 5-7 membered heterocyclic ring is substituted by one or more C_{1-6}alkyl, phenyl or phenylC_{1-4}alkyl.

20 A compound as defined in claim 19 wherein the 5-7 membered heterocyclic ring is substituted by C_{1-4}alkyl or benzyl.

21 A compound as defined in any one of claims 17 to 20 wherein when Y is a pyridone said pyridone is *N*-substituted pyridone.

22 A compound as defined in claims 14 wherein Y is a lactam linked at the nitrogen.

23 A compound as defined in any one of claims 1 to 6 wherein Y is



wherein R^{14} is CH_2OH or $C(O)NR^{11}R^{12}$.

24 A compound as defined in any one of claims 1 to 6 or 23 wherein R^{16} and R^{17} are hydrogen.

25 A compound as defined in any one of claims 1 to 6, 23 or 24 wherein t is 0.

26 A compound as defined in any preceding claim wherein the chiral carbon attached to R^1 is the R-enantiomer.

27 A compound according to claims 1 or 2 selected from the group consisting of:

2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl]-cyclopentyl)methyl]-4-methoxybutanoic acid (Example 35);

2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid (Example 40);

(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid (Example 44);

2-[(1-[[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid (Example 43);

cis-3-(2-methoxyethoxy)-2-[(1-[[(4-[[(phenylsulfonyl)amino]carbonyl}-cyclohexyl)amino]carbonyl}cyclopentyl)methyl]propanoic acid (Example 38);

(+)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 31);

(+)-2-[(1-[[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid (Example 30);

2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21);

2-[(1-[[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]pentanoic acid (Example 22);

2-[[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 9);

trans-3-[1-({[2-(4-chlorophenyl)cyclopropyl]amino}carbonyl)-cyclopentyl]-2-(methoxymethyl)propanoic acid (Example 46);

trans-3-[1-({[2-(4-methoxyphenyl)cyclopropyl]amino}carbonyl)-cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 47);

trans-3-[1-({[2-pentylcyclopropyl]amino}carbonyl)-cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 48);

3-[1-({[5-benzyl-[1,3,4]-thiadiazol-2-yl]amino}carbonyl)-cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 49);

3-[1-({[4-butylpyridin-2-yl]amino}carbonyl)-cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 50);

3-[1-({[4-phenylpyridin-2-yl]amino}carbonyl)-cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 51);

3-[1-({[1-hydroxymethyl-3-phenylcyclopentyl]amino}carbonyl)-cyclopentyl]-2-(methoxyethyl)propanoic acid (Example 52);

2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}-4-methoxybutanoic acid (Example 53);

(+/-)-*trans*-3-[1-({[2-phenylcyclopropyl]amino}carbonyl)cyclopentyl]-2-

(methoxyethyl)propanoic acid (Example 54);

(*R*)- 2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}-4-methoxybutanoic acid (Example 55); and

(*S*)-2-[[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}-carbonyl)cyclopentyl]methyl}-4-methoxybutanoic acid (Example 56).

28 The use of a compound as defined in claims 2 or 3 and claims dependent thereon in the preparation of a medicament for the treatment or prophylaxis of a condition for which a beneficial therapeutic response can be obtained by the inhibition of neutral endopeptidase.

29 The use according to claim 28 for the treatment or prophylaxis of sexual dysfunction.

30 The use according to claims 1 or 29 wherein the sexual dysfunction treated is female sexual dysfunction.

31 The use according to claim 30 wherein the female sexual dysfunction(s) treated includes at least female sexual arousal dysfunction.

32 The use according to any one of claims 1, or 28 to 31 wherein the medicament is administered systemically.

33 The use according to claim 32 wherein the medicament is administered orally.

34 A compound as defined in claims 2 or 3 for use in medicine.

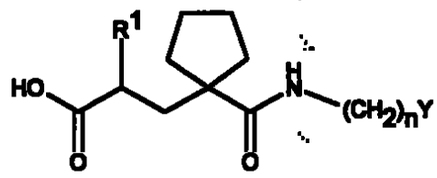
35 A pharmaceutical formulation including a compound as defined in any one of claims 1 to 27 together with a pharmaceutically acceptable excipient.

36 A method for the treatment or prophylaxis of sexual dysfunction including

administering to the patient a therapeutically effective amount of a compound as defined in any one of claims 1 to 27.

37 A sexual dysfunction pharmaceutical formulation including a therapeutically effective amount of a compound as defined in any one of claims 1 to 27 together with a pharmaceutically acceptable excipient.

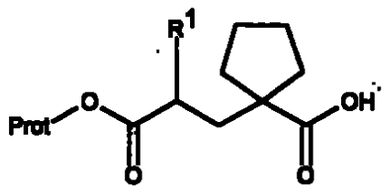
38 A process for preparing of a compound of formula I or salts thereof



(I)

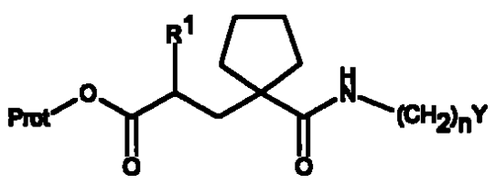
wherein R^1, n and Y are as defined in claim 2, comprising the steps of:

a) reacting a compound of formula II



(II)

wherein Prot is a suitable protecting group, with a compound of formula $Y(CH_2)_nNH_2$ (III), to give a compound of formula IV,



(IV) ;

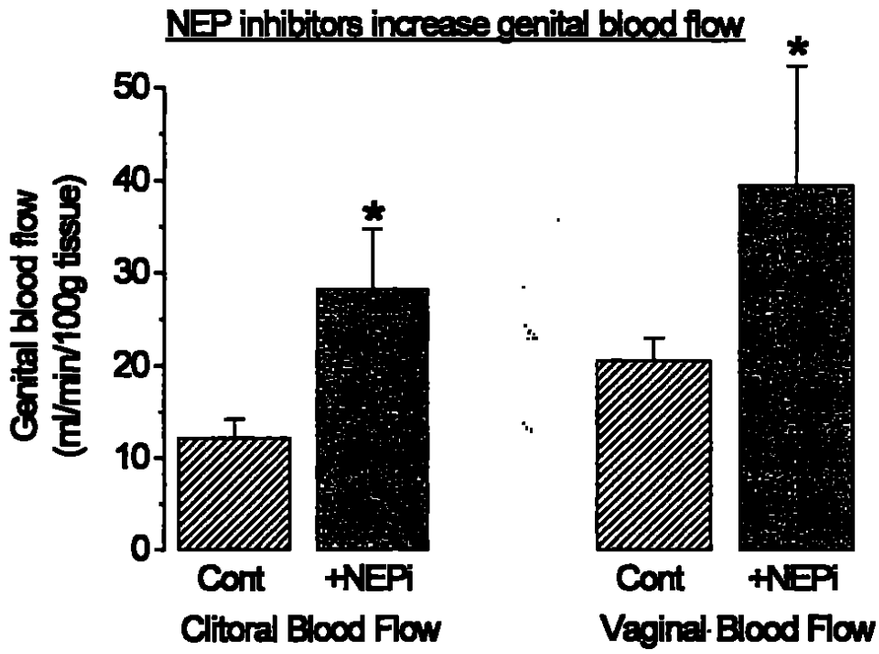
then

b) reacting the compound of formula IV under suitable deprotecting conditions to give the compound of formula I; then

c) optionally forming a salt.

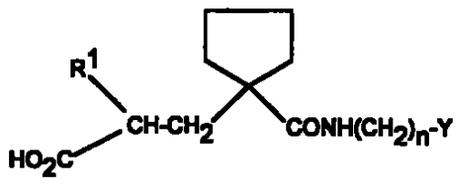
473

Figure 1


NEP inhibitors increase genital blood flow
Genital blood flow (ml/min/100g tissue)
50
40
30
20
10
0
*
*
Cont
+NEPi
Cont
+NEPi
Clitoral Blood Flow
Vaginal Blood Flow

Abstract

The use of a compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, in the preparation of a medicament for the treatment of sexual dysfunction;



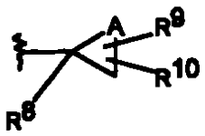
(I)

wherein R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6} alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, -NR^2R^3, -NR^4COR^5, -$NR^4SO_2R^5$, -$CONR^2R^3$, -$S(O)_pR^6$, -COR^7 and -CO_2(C_{1-4}alkyl); or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, -NR^2R^3 or -$NR^4SO_2R^5$; wherein R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group; R^4 is H or C_{1-4}alkyl;

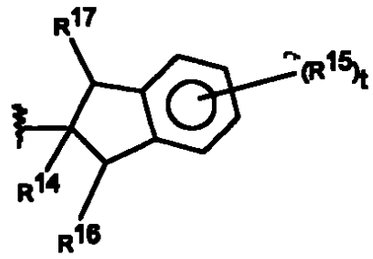
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or -NR^2R^3 wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3; the -$(CH_2)_n$- linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl; Y is

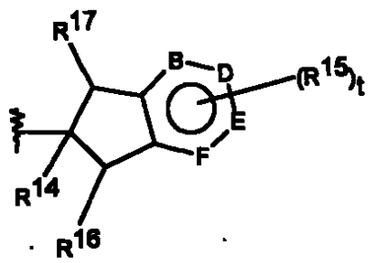
the group



wherein A is $-(CH_2)_q-$ where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, $-CH_2OH$, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$; C_{1-6}alkyl, phenyl (optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, halo or C_{1-4}alkoxy, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or Y is the group, $-C(O)\ NR^{11}\ R^{12}$ wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by: C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl (preferably pyridyl); and u is 0, 1, 2 or 3.

0101584.1


THE PATENT OFFICE
K
22 JAN 2001
LONDON


THE PATENT OFFICE
K
22 JAN 2001
LONDON

SUPERINDUSTRIA Y COMERCIO
Radicacion : 02102451 00000000 Folios: 480
Fecha (MMD): 2002-11-12 17:29:16
Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

451

476

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADO
PATENTE DE INVENCIÓN ✓ MODELO DE UTILIDAD	( )	(✓)
- Indicación de que se solicita la concesión de una patente.	( )	(✓)
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	( )	(✓)
- Descripción de la invención.	( )	(✓)
- Dibujos de ser estos pertinentes.	( )	(✓)
- Comprobante de Pago de las tasas establecidas.		
COMPLETA (✓)	INCOMPLETA ( )	( )
DISEÑO INDUSTRIAL ( )		
- Indicación de que se solicita el registro de Diseño Industrial	( )	( )
- Datos de identificacion del solicitante o de la persona que presenta	( )	( )
- la solicitud.		
- Representación gráfica y fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	( )	( )
Comprobante de pago de las tasas establecidas	( )	( )
COMPLETA ( )	INCOMPLETA ( )	( )
ESQUEMA DE TRAZADO ( )		
- Indicación de que solicita el registro de un Esquema de trazado.	( )	( )
- Datos de identificación del solicitante o de la persona que presenta La solicitud.	( )	( )
Representación gráfica del esquema de trazado	( )	( )
Comprobante de pago de las tasas establecidas.	( )	( )
COMPLETA ( )	INCOMPLETA ( )	( )

Firma Responsable: [signature]

Fecha:

Industria y Comercio
SUPERINTENDENCIA

477 487

2020
Bogotá, D.C.,

Doctor:
Carlos R. Olarte
Apoderado
PFIZER INC.

Oficio N° 06371

ASUNTO:		
	Radicación N°	02-102451
	Solicitud internacional	PCT/IB01/01205
	Fecha inicio fase nacional	06/02/2003
	Trámite	011
	Evento	379
	Actuación	330
	Folios	001

Como resultado del examen de forma, realizado con fundamento el el artículo 36 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

☐ La solicitud no contiene la traducción de los anexos del reporte de examen preliminar internacional (Art. 36.2.b) del tratado PCT).

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

El anterior requerimiento fue notificado por fijación en lista de fecha 08 AGO 2003.

202049