

Industria y Comercio

SUPERINTENDENCIA

Delegatura de Propiedad Industrial

División de Nuevas Creaciones

PCT
SOLICITUD

PATENTE DE INVENCION

04-85344

21. EXPEDIENTE No. _____

54. TITULO _____ TRIPEPTIDOS QUE POSEEN UN ÉTER DE HIDROXIPROLINA DE UNA

QUINOLINA SUSTITUIDA PARA LA INHIBICION DE NS3

(HEPATITIS C)

51. CLASIFICACION INTERNACIONAL _____

71. SOLICITANTE _____ BOEHRINGER INGELHEIM INTERNATIONAL GMBH

DOMICILIO _____ Ingelheim am Rhein, República Federal de Alemania.

74. APODERADO _____ EMILIO FERRERO

Código No. 1092

22. BOGOTA, D.C., _____

**Industria y Comercio**

SUPERINTENDENCIA

PETITORIO

SUPERINDUSTRIA Y COMERCIO

Radicación : 04085344 00000000

Folios: 170

Fecha (AMB): 2004-08-31 17:44:26

Trámite : 011 PCT-PATENT 1 REGISTRA/ 411 PRESENTAC

Dependencia: 2820 DIVISION DE NUEVAS CREACIONES

**FORMULARIO UNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL**

SOLICITUD DE:**1**☒ Patente de Invención.☐ Patente de Modelo de Utilidad.☐ Capítulo I.☒ Capítulo II.**2****SOLICITANTE
(71)**

Nombre: **BOEHRINGER INGELHEIM
INTERNATIONAL GMBH**
sociedad constituida y existente bajo las
leyes de la República Federal de Alemania

Dirección: Binger Strasse,
D-55218 Ingelheim am Rhein,
República Federal de Alemania
Domicilio: Ingelheim am Rhein,
República Federal de Alemania

IDENTIFICACIÓNC.C. ☐ NIT ☐C.E. ☐ Otro ☐

Cual _____

Número _____

3**REPRESENTANTE
O APODERADO**

Nombre: **EMILIO FERRERO**
Dirección: **Carrera 4ª. No. 72-35**
Teléfono: **347 36 11**
Fax: **211 86 50**
E-mail: **clientes@camyp.com.**
Domicilio: **Bogotá, Colombia.**

IDENTIFICACIÓNC.C. ☒ NIT ☐C.E. ☐ Otro ☐

Cual _____

Número **438.019** T.P **31.929****4****INVENTOR (ES)
(72)**

1. Montse LLINAS-BRUNET
2100 Cunard Street,
Laval, Québec H7S 2G5,
Canadá

2. Vida J. GORYS
2100 Cunard Street,
Laval, Québec H7S 2G5,
Canadá

5**PCT (86)**Solicitud Internacional No. PCT/CA03/00090Fecha: 24 de Enero de 2003Publicación Internacional No. WO 03/064456 A1Fecha: 7 de Agosto de 2003**6****Título (54)**

**TRIPEPTIDOS QUE POSEEN UN ÉTER DE HIDROXIPROLINA DE UNA QUINOLINA
SUSTITUIDA PARA LA INHIBICIÓN DE NS3 (HEPATITIS C)**

7**Clasificación Internacional (51)** C07K 5/08 A61K 31/47**8****Prioridad****(33) País de Origen****(32) Fecha****(31) Número de Solicitud**SI ☒ NO ☐CA1 de Febrero de 20022.370.396**9****Comprobante de pago No.:** 04 - 68.240**Fecha:** 30 de Agosto de 2004

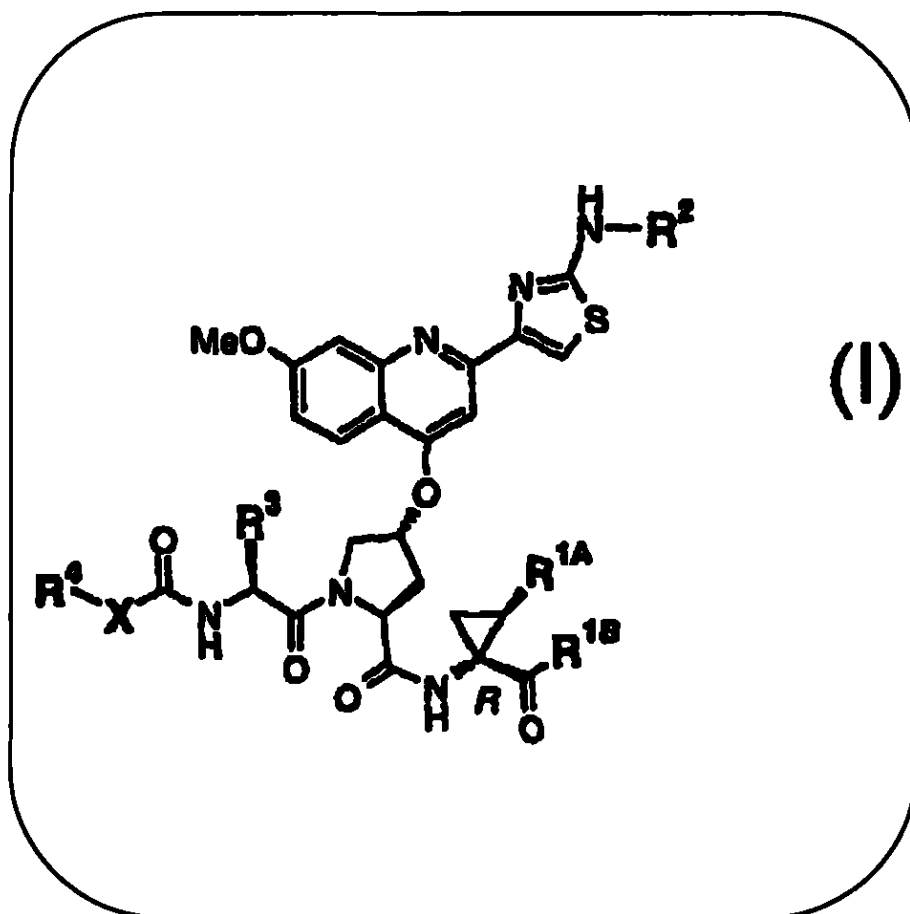
10

An x s:

- ☒ Comprobante de pago de la tasa de presentación de la solicitud por \$ 422.000.00.
- ☐ Comprobante de pago por reivindicación de prioridad.
- ☒ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☒ Poderes, si fuere el caso.
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones.
- ☒ Copia de la solicitud Internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Copia del examen preliminar efectuado por la IPEA.
- ☒ Traducción del examen preliminar 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 12x12
- ☐ 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.
- ☐ Otros

11

FIGURA CARACTERÍSTICA



12

Solicito la concesión de la patente,

NOMBRE: EMILIO FERREROFIRMA: *Emilio Ferrero*

C.C. 438.019 de Usaquén T.P. 31.929

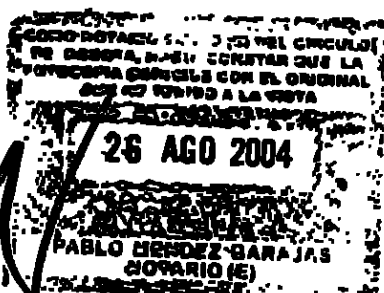
PRP

LEGALIZACION

El Señor Cónsul de Colombia debe expedir un certificado consular sobre (1) la existencia legal de la compañía que otorga el poder, (2) que ésta ejerce su objeto social, y (3) que las personas que otorgan el poder están autorizadas para darlo. A este efecto les solicitamos que muestren al Señor Cónsul las pruebas que acreditan esos hechos (1), (2) y (3).

LEGALIZATION

The Colombia Consul must issue a consular certificate, on (1) the legal existence of the company which grants Power-of-Attorney, (2) that it is in actual exercise of company purposes, and (3) that the persons who execute the Power of Attorney is authorized to do so. To this effect, please show the Consul the evidence to prove the above facts (1), (2) and (3).



NOTA PARA EL SEÑOR CONSUL DE COLOMBIA: Según los artículos 65 del C. de P.C. y 480 del C. de Co., si el poderdante es una sociedad, el Señor Cónsul debe certificar que tuvo a la vista las pruebas de su existencia, que ejerce su objeto social y que quien confiere el poder es su representante.

Vorseltige, vor mir gefertigte Namensunterschriften der Herren:

1. Dr. Dieter L a u d i e n, Dipl.-Chemiker, wohnhaft in 55127 Mainz,.
2. Dr. Gerhard H u b e r, Rechtsanwalt, wohnhaft in 55411 Bingen,

- mir bekannt -

beglaubige ich hiermit.

Gleichzeitig bescheinige ich aufgrund heutiger Einsichtnahme in das Handelsregister des Amtsgerichts Bingen, daß die Firma Boehringer Ingelheim International GmbH mit Sitz in Ingelheim am Rhein im Handelsregister des Amtsgerichts Bingen unter HR B 1063 eingetragen ist und daß die Herren Dr. Laudien und Dr. Huber als Prokuristen gemeinschaftlich zur Vertretung der Gesellschaft berechtigt sind.

Ingelheim am Rhein, den 12. Dezember 1995/hün

Dr. Weirich
Notariatsverweser
anstelle des Notars
Prof. Dr. Weirich in Ingelheim



- 910 E 1 -



COM NOTARIU PUBLICO EN CIRCULO
DE BOGOTA, MAGU CONSTA QUE LA
FUTURA COPIA CONCORDA CON EL ORIGINAL
QUE SE TIENE A LA VISTA
26 AGO 2004

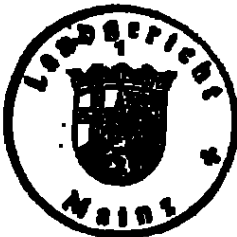
Die Echtheit vorstehender Unterschrift des Notariatsverwesers Dr. Peter Wirth - Notariatsverweser anstelle des Notars Prof. Dr. Weirich in Ingelheim und die Echtheit des oben gedruckten Dienst - ~~stempel~~ - siegels werden hierdurch bestätigt. Zugleich wird bescheinigt, daß der Vorgenannte zur Vornahme der Amtshandlung gesetzlich befugt war.

Kostenberechnung:
Gebühr für Legalisation

(Wert: DM 5.000,-)
JVKostO. v. 28.7.1987
BGBl. I S. 881
DM

13. DEZ. 1995
Mainz, den

Antonspektor
Antonspektor



Mainz, den dreizehnen Dezember neunzehnhundertfünfundneunzig
Der Präsident des Landgerichts

Dr. Hans Paul Tullberg
(Dr. Hans Paul Tullberg)



CONSULADO GENERAL DE COLOMBIA

MUNICH

Nr. 169

El Cónsul General de Colombia

CERTIFICA

Que el Dr. Hanns Paul Tüttenberg -----, quién autentica la firma del signatario en el presente documento, ejercía en la fecha allí indicada las funciones de Presidente del Tribunal Regional en Mainz.

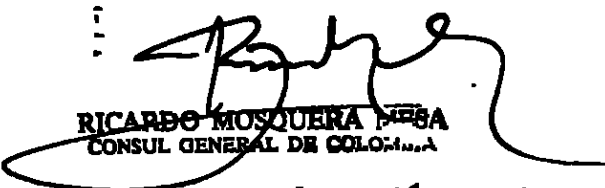
Que la firma BOEHRINGER INGELHEIM INTERNATIONAL GmbH, domiciliada en 55216 Ingelheim am Rhein, Registro Mercantil Nr. HR B 1063 del Juzgado Municipal de Bingen está constituida y ejerce su objeto social de conformidad con las leyes de la República Federal de Alemania.

Que los señores Dr. DIETER LAUDIN Y Dr. GERHARD HUBER

quien (es) firman el presente documento tiene (n) facultad legal suficiente para representar a la mencionada firma y otorgar poderes en su nombre.

Que ha tenido a la vista la documentación que prueba lo anterior.

Munich, 18 de Diciembre de 1995


RICARDO MOSQUERA MESA
CONSUL GENERAL DE COLOMBIA

Derechos para la Nación	US \$	6.-
Fondo Rotatorio	US \$	100.-
Tasa consular	US \$	1 x DM 1,60 = DM 169.60



PAGADO

26 AGO 2004

PABLO BENDEZ RAMA JAG
NOTARIO (E.)



MINISTERIO DE RELACIONES
EXTERIORES
SECRETARÍA DE ASUNTOS
EXTERIORES

NO SE ASUME
RESPONSABILIDAD DEL TEXTO
Hoy ENE - 11 A 11:44
JEFE DE LEGALIZACIONES
ANTARTE DE BOGOTÁ D.C.

COPIA DEL ACTO EN UN CIRCULO
DE LA, HAYO DUBITAR SUS LA
CONCORDIA CON EL ORIGINAL
SIN EL TIEMPO A LA VISTA
26 AGO 2004
PAULO HERNANDEZ BARAJAS
NOTARIO

1-24-5133-4027

TRADUCCION OFICIAL No. 5133

de un documento escrito en Alemán, el cual para su identificación se sella con el sello de la traductora, lo mismo que la presente.-

(A continuación del poder conferido a GERMAN CAVELIER, tpa. 832; GONZALO PERDOMO, tpa. 831; EMILIO FERRERO tpa, 31929; y LUZ CLEMENCIA DE PAEZ, tpa 23555; domiciliados en la Carrera 4 No. 72-35, Bogotá, Colombia, por la BOEHRINGER INGELHEIM INTERNATIONAL GMBH, domiciliada en D-55216 Ingelheim am Rhein, RFA, a través de sus representantes los Srs. Dr. Dieter Laudien y Dr. Gerhard Huber:)

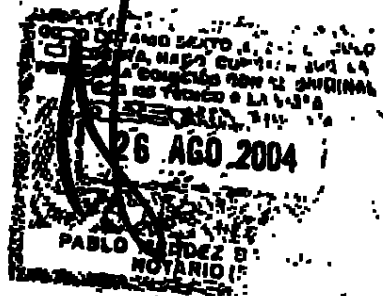
Legalización

Protocolo notarial No. 2659/95 V

Certifico la autenticidad de las firmas antecedentes puestas ante mí por los señores:

1. Dr. Dieter Laudien, Dipl. Chim., domiciliado en 55127 Mainz,
2. Dr. Gerhard Huber, abogado, domiciliado en 55411 Bingen

a quienes conozco en persona.



Iguualmente certifico, por haber examinado en la fecha el Registro de Comercio del Juzgado Municipal de Bingen, HR B 1063, que la firma Boehringer Ingelheim International GmbH, domiciliada en Ingelheim am Rhein, está inscrita bajo el No. HR B 1063 en el Registro de Comercio del Juzgado Municipal de Bingen y que los señores Dr. Laudien y Dr. Huber, en su calidad de apoderados, están facultados para representar conjuntamente a la sociedad.

Ingelheim am Rhein, 12 de diciembre de 1995/hún

Fdo.: firma ilegible

(Hilo y oblea roja en relieve:)

El notario suplente

El notario suplente - Ingelheim

del notario de Ingelheim

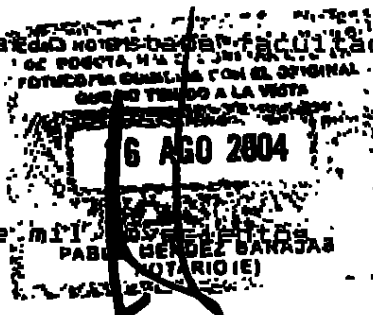
Prof. Dr. Weirich

(dos sellos redondos en tinta del Tribunal Regional de Mainz)

910 E 1

Se certifica la autenticidad de la firma antecedente del Dr. Peter Wirth, como notario suplente del Prof. Dr. Weirich, notario de Ingelheim, y la del sello oficial que la acompaña. Igualmente se certifica que el mencionado notario está facultado para realizar ese acto oficial.

Mainz, trece de diciembre de mil
noventa y cinco



El Presidente del Tribunal Regional

(E) Fdo.: firma ilegible

(Dr. Hanns Paul Tüttenberg)

Costas

Derechos de legalización
(Valor: DM 5.000,-)
JVKost0 del 26 de julio 1957
Gacetas Oficial I Pag. 861
DM 20,--
Mainz, 13 de diciembre de 1995
Fdo.: firma ilegible
Inspector

EN ESPAÑOL: La legalización de las firmas antecedentes por el
Consulado de Colombia en Munich bajo el No. 169 del 16 de
diciembre de 1995, y por el Ministerio de Relaciones Exteriores
de Colombia bajo el No. 051606 del 4 de enero de 1996.
Pagados derechos y Fondo Rotatorio US\$ 106,--

ES TRADUCCION FIEL Y COMPLETA, de la cual se deja copia en los
archivos de estas oficinas para su confrontación.

Santafé de Bogotá, 6 de enero de 1996.



TELEFAX (57) (1) 30-9890
TELEX 45479 CAVE CO
12317407375 CAVE UC

CAVELIER
ABOGADOS
EDIFICIO SISKI
CARRERA 49, No. 72-35
BOGOTA 8, COLOMBIA

CORREO ELECTRONICO MUNDIAL: CAVE
TELEFONO (57) (1) 3120100
CABLES: CAVELEYES

Señor Jefe de la
DIVISION DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Poderdante: BOEHRINGER INGELHEIM INTERNATIONAL GMBH

Poder conferido el: 12-12-95.

Yo, GERMAN CAVELIER, obrando en mi calidad de apoderado principal del poderdante arriba mencionado atentamente manifiesto a Usted que sustituyo el poder que me ha sido conferido en la persona del doctor EMILIO FERRERO, Abogado con Tarjeta Profesional No. 31.929, con todas las facultades que me fueron conferidas.

Hago la anterior sustitución conforme al artículo 66 del Código de Procedimiento Civil ya que los otros sustitutos faltan en este momento.

Señor Jefe,

Acepto:



EMILIO FERRERO

T.P.A. No 31.929

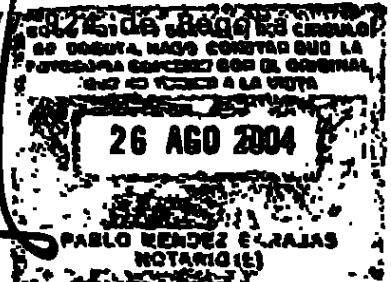
C.C. No. 438.019 de Usaquén



GERMAN CAVELIER

T.P.A. No. 832

C.C. No. 2.87



2e-780-2

NOTARIA SEXTA DE BOGOTÁ
RECONOCIMIENTO Y PRESENTACION PERSONAL

Bogotá,, D. C. 11 ENE 1996

Ante M. PABLO MENDEZ BARAJAS S.

NOTARIO SEXTO ENCARGADO DEL CIRCULO DE BOGOTÁ.

Compareció(eron) [Firma]

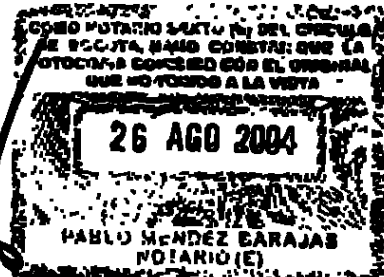
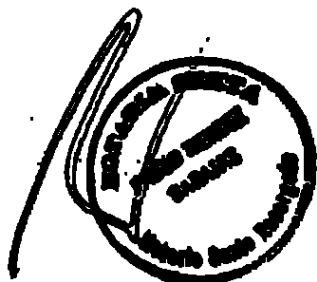
quien(es) exhibió(aron) la(s) 2877924
438019

y la(s) Libreta(s) Militar(es) [Firma]

domiciliad:(s) en Bogotá y declaró(aron) que la(s) firma(s) que
aparecen(n) en el presente documento es(son) la(s) suya(s) y
que el contenido del mismo es cierto.
En constancia se firma esta Diligencia.

[Firma]

[Firma]



COMO NOTARIO SEXTO ENCARGADO DE SANTAFE
DE BOGOTA HAGO CONSTAR QUE LA FIRMA DE

Jay Arango
del 7 de Enero

C. C. _____
COINCIDE CON LA REGISTRADA POR EL (ELLA)
EN ESTA NOTARIA

09 ENE 1996

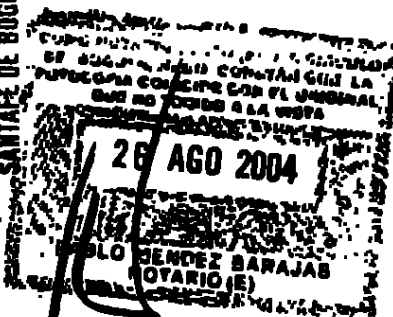
SANTAFE DE BOGOTA D.C.



NO SE ASUME
RESPONSABILIDAD DEL TEXTO

1996 ENE 10 A 11:33

JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.



MINISTERIO DE RELACIONES
EXTERIORES

CUYA FIRMA
DOCUMENTO
LAS FUNCIONES

(19) World Intellectual Property Organization
International Bureau



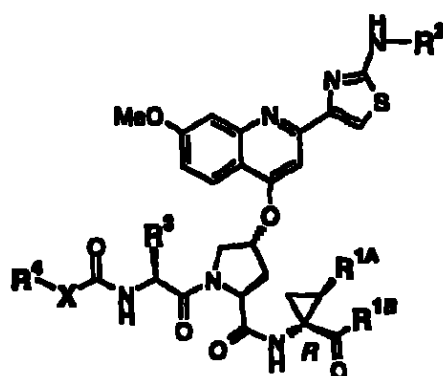
(43) International Publication Date
7 August 2003 (07.08.2003)

PCT

(10) International Publication Number
WO 03/064456 A1

- (51) International Patent Classification⁷: C07K 5/08, A61P 31/14
- (74) Agent: BERNIER, Louise, G.; Boehringer Ingelheim (Canada) Ltd., 2100 Cunard Street, Laval, Québec H7S 2G5 (CA).
- (21) International Application Number: PCT/CA03/00090
- (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, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 24 January 2003 (24.01.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 2,370,396 1 February 2002 (01.02.2002) CA
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): BOEHRINGER INGELHEIM INTERNATIONAL GMBH [DE/DE]; Binger Strasse 173, 55216 Ingelheim (DE).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): LLINAS-BRUNET, Montse [CA/CA]; 2100 Cunard Street, Laval, Québec H7S 2G5 (CA). GORYS, Vida, J. [CA/CA]; 2100 Cunard Street, Laval, Québec H7S 2G5 (CA).
- Published:
— with international search report
- 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.

(54) Title: TRIPEPTIDES HAVING A HYDROXYPROLINE ETHER OF A SUBSTITUTED QUINOLINE FOR THE INHIBITION OF NS3 (HEPATITIS C)



(I)

(57) Abstract: Disclosed herein are compounds of formula (I), wherein R^1 is hydroxy or NHSO_2R^{1A} wherein R^{1A} is (C_{1-6}) alkyl, (C_{3-7}) cycloalkyl or $\{ (C_{1-6})$ alkyl- (C_{3-7}) cycloalkyl $\}$, which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, $O-(C_{1-6})$ alkyl, amido, amino or phenyl, or R^{1A} is C_6 or C_{10} aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C_{1-6}) alkyl, $O-(C_{1-6})$ alkyl, amido, amino or phenyl; R^2 is (C_{4-6}) cycloalkyl; R^3 is *t*-butyl or (C_{3-6}) cycloalkyl and R^4 is (C_{4-6}) cycloalkyl; or a pharmaceutically acceptable salt thereof. The compounds are useful as inhibitors of HCV NS3 protease for the treatment of Hepatitis C.

WO 03/064456 A1



HEPATITIS C INHIBITOR TRI-PEPTIDES

FIELD OF THE INVENTION

The present invention relates to compounds, processes for their synthesis,
5 compositions and methods for the treatment of hepatitis C virus (HCV) infection. In particular, the present invention provides novel peptide analogs, pharmaceutical compositions containing such analogs and methods for using these analogs in the treatment of HCV infection.

10 BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) is the major etiological agent of post-transfusion and community-acquired non-A non-B hepatitis worldwide. It is estimated that over 200 million people worldwide are infected by the virus. A high percentage of carriers become chronically infected and many progress to chronic liver disease, so-called
15 chronic hepatitis C. This group is in turn at high risk for serious liver disease such as liver cirrhosis, hepatocellular carcinoma and terminal liver disease leading to death.

The mechanism by which HCV establishes viral persistence and causes a high rate
20 of chronic liver disease has not been thoroughly elucidated. It is not known how HCV interacts with and evades the host immune system. In addition, the roles of cellular and humoral immune responses in protection against HCV infection and disease have yet to be established. Immunoglobulins have been reported for prophylaxis of transfusion-associated viral hepatitis, however, the Center for
25 Disease Control does not presently recommend immunoglobulins treatment for this purpose. The lack of an effective protective immune response is hampering the development of a vaccine or adequate post-exposure prophylaxis measures, so in the near-term, hopes are firmly pinned on antiviral interventions.

30 Various clinical studies have been conducted with the goal of identifying pharmaceutical agents capable of effectively treating HCV infection in patients afflicted with chronic hepatitis C. These studies have involved the use of interferon-alpha, alone and in combination with other antiviral agents. Such studies have shown that a substantial number of the participants do not respond to these

therapies, and of those that do respond favorably, a large proportion were found to relapse after termination of treatment.

Until recently, interferon (IFN) was the only available therapy of proven benefit approved in the clinic for patients with chronic hepatitis C. However the sustained response rate is low, and interferon treatment also induces severe side-effects (i.e. retinopathy, thyroiditis, acute pancreatitis, depression) that diminish the quality of life of treated patients. Recently, interferon in combination with ribavirin has been approved for patients non-responsive to IFN alone. However, the side effects caused by IFN are not alleviated with this combination therapy. Pegylated forms of interferons such as PEG-Intron® and Pegasys® can apparently partially address these deleterious side-effects but antiviral drugs still remain the avenue of choice for oral treatment of HCV.

Therefore, a need exists for the development of effective antiviral agents for treatment of HCV infection that overcome the limitations of existing pharmaceutical therapies.

HCV is an enveloped positive strand RNA virus in the Flaviviridae family. The single strand HCV RNA genome is approximately 9500 nucleotides in length and has a single open reading frame (ORF) encoding a single large polyprotein of about 3000 amino acids. In infected cells, this polyprotein is cleaved at multiple sites by cellular and viral proteases to produce the structural and non-structural (NS) proteins. In the case of HCV, the generation of mature nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) is effected by two viral proteases. The first one, as yet poorly characterized, cleaves at the NS2-NS3 junction (henceforth referred to as NS2/3 protease); the second one is a serine protease contained within the N-terminal region of NS3 (NS3 protease) and mediates all the subsequent cleavages downstream of NS3, both in *cis*, at the NS3-NS4A cleavage site, and in *trans*, for the remaining NS4A-NS4B, NS4B-NS5A, NS5A-NS5B sites. The NS4A protein appears to serve multiple functions, acting as a cofactor for the NS3 protease and possibly assisting in the membrane localization of NS3 and other viral replicase components. The complex formation of the NS3 protease with NS4A seems necessary to the processing events, enhancing the proteolytic efficiency at all of the

sites. The NS3 protein also exhibits nucleoside triphosphatase and RNA helicase activities. NS5B is a RNA-dependent RNA polymerase that is involved in the replication of HCV.

- 5 A general strategy for the development of antiviral agents is to inactivate virally encoded enzymes that are essential for the replication of the virus.

The following is a list of patent applications published in the last few years that disclose HCV NS3 protease inhibitor peptide analogs that are structurally different
10 from the compounds of the present invention:

GB 2,337,262; JP10298151; JP 11126861; JP 11292840; JP 2001-103993;
US 6,159,938; US 6,187,905; WO 97/43310; WO 98/17679; WO 98/22496;
WO 98/46597; WO 98/46630; WO 99/38888; WO 99/50230; WO 99/64442;
15 WO 99/07733; WO 99/07734; WO 00/09543; WO 00/09558; WO 00/20400;
WO 00/59929; WO 00/31129; WO 01/02424; WO 01/07407; WO 01/16357;
WO 01/32691; WO 01/40262; WO 01/58929; WO 01/64678; WO 01/74768;
WO 01/77113; WO 01/81325; WO 02/08187; WO 02/08198; WO 02/08244;
WO 02/08251; WO 02/08256; WO 02/18369; WO 02/60926 and WO 02/79234.

20

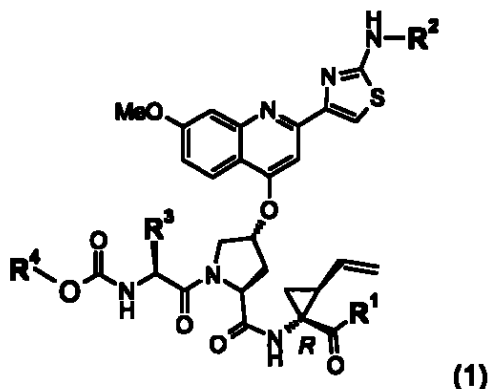
One advantage of the present invention is that it provides tripeptide compounds that are inhibitory to the NS3 protease, an enzyme essential for the replication of the hepatitis C virus. Furthermore, the compounds are able to inhibit HCV RNA replication in the replicon cell model.

25

A further advantage of one aspect of the present invention resides in the fact that the compounds specifically inhibit the NS3 protease and do not show significant inhibitory activity against other serine proteases such as human leukocyte elastase (HLE), porcine pancreatic elastase (PPE), or bovine pancreatic chymotrypsin, or
30 cysteine proteases such as human liver cathepsin B (Cat B).

SUMMARY OF THE INVENTION

Included in the scope of the invention is a compound of formula (1):



- wherein R^1 is hydroxy or NHSO_2R^{1A} wherein R^{1A} is (C_{1-6}) alkyl, (C_{3-7}) cycloalkyl or $\{(C_{1-6})$ alkyl- (C_{3-7}) cycloalkyl $\}$, which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, $O-(C_{1-6})$ alkyl, amido, amino or phenyl, or R^{1A} is C_6 or C_{10} aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C_{1-6}) alkyl, $O-(C_{1-6})$ alkyl, amido, amino or phenyl; R^2 is (C_{4-6}) cycloalkyl; R^3 is t-butyl or (C_{6-8}) cycloalkyl and R^4 is (C_{4-6}) cycloalkyl; or a pharmaceutically acceptable salt thereof.

- Included within the scope of this invention is a pharmaceutical composition comprising an anti-hepatitis C virally effective amount of a compound of formula I, or a therapeutically acceptable salt thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.

- According to one embodiment, the pharmaceutical composition of this invention further comprises interferon (pegylated or not), or ribavirin, or one or more other anti-HCV agents, or any combination of the above.

- Another important aspect of the invention involves a method of treating a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of a compound of formula I, a pharmaceutically acceptable salt thereof, or a composition as described above, alone or in combination with one or more of: Interferon (pegylated or not), or ribavirin, or one or more other anti-HCV agents, administered together or separately.

- Another important aspect of the invention involves a method of preventing a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of a compound of formula I, a pharmaceutically

acceptable salt thereof, or a composition as described above, alone or in combination with one or more of: Interferon (pegylated or not), or ribavirin, or one or more other anti-HCV agent, all of which administered together or separately.

- 5 Also within the scope of this invention is the use of a compound of formula I, as described herein, for the manufacture of a medicament for the treatment or prevention of hepatitis C viral infection.

10 The present description refers to a number of documents, the content of which is herein incorporated by reference.

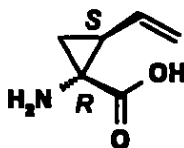
DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

- 15 As used herein, the following definitions apply unless otherwise noted:
With reference to the instances where (R) or (S) is used to designate the absolute configuration of a substituent or asymmetric center of a compound of formula 1, the designation is done in the context of the whole compound and not in the context of the substituent or asymmetric center alone.

20 The designation "P1, P2, and P3" as used herein refer to the position of the amino acid residues starting from the C-terminus end of the peptide analogs and extending towards the N-terminus (i.e. P1 refers to position 1 from the C-terminus, P2: second position from the C-terminus, etc.) (see Berger A. & Schechter I., Transactions of the
25 Royal Society London series B257, 249-264 (1970)).

As used herein the term "vinyl-ACCA" refers to a compound of formula:



namely, (1R, 2S) 1-amino-2-ethenylcyclopropylcarboxylic acid.

30

The term "(C₁₋₃)alkyl" as used herein, either alone or in combination with another



substituent, means acyclic straight or branched alkyl substituents containing for 1 to 8 carbon atoms and includes, for example, methyl, ethyl, 2-methylhexyl, 1,1-dimethylhexyl (or t-butyl) and octyl.

- 5 The term "(C₃₋₇)cycloalkyl" as used herein, either alone or in combination with another substituent, means a cycloalkyl substituent containing from 3 to 7 carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

10 The term "{(C₁₋₆)alkyl-(C₃₋₆)cycloalkyl}" as used herein means a cycloalkyl radical containing from 3 to 6 carbon atoms directly linked to an alkylene radical containing 1 to 6 carbon atoms; for example, cyclopropylmethyl, cyclopentylethyl, cyclohexylmethyl, and cyclohexylethyl. In the instance where R^{1A} is a {(C₁₋₆)alkyl-(C₃₋₆)cycloalkyl}, this group is attached to the SO₂ group via the (C₁₋₆)alkyl (i.e. the alkylene portion).

15 The term "C₆ or C₁₀ aryl" as used herein, either alone or in combination with another radical, means either an aromatic monocyclic group containing 6 carbon atoms or an aromatic bicyclic group containing 10 carbon atoms. For example, aryl includes phenyl, 1-naphthyl or 2-naphthyl.

20 The term "O-(C₁₋₆) alkyl" as used herein, either alone or in combination with another radical, means the radical -O-(C₁₋₆)alkyl wherein alkyl is as defined above containing up to six carbon atoms, and includes methoxy, ethoxy, propoxy, 1-methylethoxy, butoxy and 1,1-dimethylethoxy. The latter radical is known commonly as *tert*-
25 butoxy.

The term "halo" as used herein means a halogen substituent selected from bromo, chloro, fluoro or iodo.

- 30 The term "pharmaceutically acceptable salt" means a salt of a compound of formula (1) which is, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, generally water or oil-soluble or dispersible, and effective for their intended use.

A
II

The term includes pharmaceutically-acceptable acid addition salts and pharmaceutically-acceptable base addition salts. Lists of suitable salts are found in, e.g., S.M. Birge et al., J. Pharm. Sci., 1977, 66, pp. 1-19, which is hereby incorporated by reference in its entirety.

5

The term "pharmaceutically-acceptable acid addition salt" means those salts which retain the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, sulfamic acid, nitric acid, phosphoric acid, and the like, and organic acids such as acetic acid, trichloroacetic acid, trifluoroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 2-acetoxybenzoic acid, butyric acid, camphoric acid, camphorsulfonic acid, cinnamic acid, citric acid, digluconic acid, ethanesulfonic acid, glutamic acid, glycolic acid, glycerophosphoric acid, hemisulfic acid, heptanoic acid, hexanoic acid, formic acid, fumaric acid, 2-hydroxyethanesulfonic acid (isethionic acid), lactic acid, maleic acid, hydroxymaleic acid, malic acid, malonic acid, mandelic acid, mesitylenesulfonic acid, methanesulfonic acid, naphthalenesulfonic acid, nicotinic acid, 2-naphthalenesulfonic acid, oxalic acid, pantoic acid, pectinic acid, phenylacetic acid, 3-phenylpropionic acid, picric acid, pivalic acid, propionic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, sulfanilic acid, tartaric acid, p-toluenesulfonic acid, undecanoic acid, and the like.

The term "pharmaceutically-acceptable base addition salt" means those salts which retain the biological effectiveness and properties of the free acids and which are not biologically or otherwise undesirable, formed with inorganic bases such as ammonia or hydroxide, carbonate, or bicarbonate of ammonium or a metal cation such as sodium, potassium, lithium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically-acceptable organic nontoxic bases include salts of primary, secondary, and tertiary amines, quaternary amine compounds, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion-exchange resins, such as methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine,



triethylamine, isopropylamine, tripropylamine, tributylamine, ethanolamine, diethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, tetramethylammonium compounds, tetraethylammonium compounds, pyridine, N,N-dimethylaniline, N-methylpiperidine, N-methylmorpholine, dicyclohexylamine, dibenzylamine, N,N-dibenzylphenethylamine, 1-phenamine, N,N'-dibenzylethylenediamine, polyamine resins, and the like. Particularly preferred organic nontoxic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline, and caffeine.

The term "antiviral agent" as used herein means an agent (compound or biological) that is effective to inhibit the formation and/or replication of a virus in a mammal. This includes agents that interfere with either host or viral mechanisms necessary for the formation and/or replication of a virus in a mammal. Antiviral agents include, for example, ribavirin, amantadine, VX-497 (merimepodib, Vertex Pharmaceuticals), VX-498 (Vertex Pharmaceuticals), Levovirin, Viramidine, Ceplene (maxamine), XTL-001 and XTL-002 (XTL Biopharmaceuticals).

The term other anti-HCV agent as used herein means those agents that are effective for diminishing or preventing the progression of hepatitis C related symptoms of disease. Such agents can be selected from: anti-viral agents, immunomodulatory agents, inhibitors of HCV NS3 protease, inhibitors of HCV polymerase or inhibitors of another target in the HCV life cycle.

The term "immunomodulatory agent" as used herein means those agents (compounds or biologicals) that are effective to enhance or potentiate the immune system response in a mammal. Immunomodulatory agents include, for example, class I interferons (such as α -, β - and omega interferons, tau-interferons, consensus interferons and asialo-interferons), class II interferons (such as γ -interferons) and pegylated interferons.

The term "inhibitor of HCV NS3 protease" as used herein means an agent (compound or biological) that is effective to inhibit the function of HCV NS3 protease

14

in a mammal. Inhibitors of HCV NS3 protease include, for example, those compounds described in WO 99/07733, WO 99/07734, WO 00/09558, WO 00/09543, WO 00/59929 or WO 02/060926, the Boehringer Ingelheim clinical candidate identified as BILN 2061 and the Vertex/Eli Lilly pre-development candidate identified as VX-950 or LY-570310. Particularly, compounds # 2, 3, 5, 6, 8, 10, 11, 18, 19, 29, 30, 31, 32, 33, 37, 38, 55, 59, 71, 91, 103, 104, 105, 112, 113, 114, 115, 116, 120, 122, 123, 124, 125, 126 and 127 disclosed in the table of pages 224-226 in WO 02/060926, can be used in combination with the compounds of the present invention .

10

The term "inhibitor of HCV polymerase" as used herein means an agent (compound or biological) that is effective to inhibit the function of an HCV polymerase in a mammal. This includes, for example, inhibitors of HCV NS5B polymerase. Inhibitors of HCV polymerase include non-nucleosides, for example, those compounds described in:

- 15 - US Application No. 10/198,680, which corresponds to PCT/CA02/01127, both filed 18 July 2002 (Boehringer Ingelheim),
- US Application No. 10/198,384, which corresponds to PCT/CA02/01128, both filed 18 July 2002 (Boehringer Ingelheim),
- 20 - US Application No. 10/198,259, which corresponds to PCT/CA02/01129, both filed 18 July 2002 (Boehringer Ingelheim),
- WO 02/100846 A1 and WO 02/100851 A2 (both Shire),
- WO 01/85172 A1 and WO 02/098424 A1 (both GSK),
- WO 00/06529 and WO 02/06246 A1 (both Merck),
- 25 - WO 01/47883 and WO 03/000254 (both Japan Tobacco) and
- EP 1 256 628 A2 (Agouron).

Furthermore other inhibitors of HCV polymerase also include nucleoside analogs, for example, those compounds described in:

- WO 01/90121 A2 (Idenix),
- 30 - WO 02/069903 A2 (Bocryst Pharmaceuticals Inc.), and
- WO 02/057287 A2 and WO 02/057425 A2 (both Merck/Isis).

Specific examples of inhibitors of an HCV polymerase, include JTK-002, JTK-003 and JTK-109 (Japan Tobacco).



The term "Inhibitor of another target in the HCV life cycle" as used herein means an agent (compound or biological) that is effective to inhibit the formation and/or replication of HCV in a mammal other than by inhibiting the function of the HCV NS3 protease. This includes agents that interfere with either host or HCV viral mechanisms necessary for the formation and/or replication of HCV in a mammal. Inhibitors of another target in the HCV life cycle include, for example, agents that inhibit a target selected from a helicase, an HCV NS2/3 protease and an inhibitor of internal ribosomal entry site (IRES). A specific example of inhibitors of another target in the HCV life cycle include ISIS-14803 (ISIS Pharmaceuticals).

10

The term "HIV inhibitor" as used herein means an agents (compound or biological) that is effective to inhibit the formation and/or replication of HIV in a mammal. This includes agents that interfere with either host or viral mechanisms necessary for the formation and/or replication of HIV in a mammal. HIV inhibitors include, for example, nucleosidic inhibitors, non-nucleosidic inhibitors, protease inhibitors, fusion inhibitors and integrase inhibitors.

15

The term "HAV inhibitor" as used herein means an agent (compound or biological) that is effective to inhibit the formation and/or replication of HAV in a mammal. This includes agents that interfere with either host or viral mechanisms necessary for the formation and/or replication of HAV in a mammal. HAV inhibitors include Hepatitis A vaccines, for example, Havrix® (GlaxoSmithKline), VAQTA® (Merck) and Avaxim® (Aventis Pasteur).

20

The term "HBV inhibitor" as used herein means an agent (compound or biological) that is effective to inhibit the formation and/or replication of HBV in a mammal. This includes agents that interfere with either host or viral mechanisms necessary for the formation and/or replication of HBV in a mammal. HBV inhibitors include, for example, agents that inhibit HBV viral DNA polymerase or HBV vaccines. Specific examples of HBV inhibitors include Lamivudine (Epivir-HBV®), Adefovir Dipivoxil, Entecavir, FTC (Coviracil®), DAPD (DXG), L-FMAU (Clevudine®), AM365 (Amrad), Ldt (Telbivudine), monoval-LdC (Valtorcitabine), ACH-126,443 (L-Fd4C) (Achillon), MCC478 (Eli Lilly), Racivir (RCV), Fluoro-L and D nucleosides, Robustaflavone, ICN 2001-3 (ICN), Barn 205 (Novelos), XTL-001 (XTL), Imino-Sugars (Nonyl-DNJ)

30

(Synergy), HepBzyme; and immunomodulator products such as: interferon alpha 2b, HE2000 (Hollis-Eden), Theradigm (Eplimmune), EHT899 (Enzo Biochem), Thymosin alpha-1 (Zadaxin®), HBV DNA vaccine (PowderJect), HBV DNA vaccine (Jefferson Center), HBV antigen (OraGen), BayHep B® (Bayer), Nabi-HB® (Nabi) and
 5 Anti-hepatitis B (Cangene); and HBV vaccine products such as the following: Engerix B, Recombivax HB, GenHevac B, Hepacare, Bio-Hep B, TwinRix, Comvax, Hexavac.

The term "class I interferon" as used herein means an interferon selected from a
 10 group of interferons that all bind to receptor type I. This includes both naturally and synthetically produced class I interferons. Examples of class I interferons include α -, β -, omega interferons, tau-interferons, consensus interferons, asialo-interferons.

The term "class II interferon" as used herein means an interferon selected from a
 15 group of interferons that all bind to receptor type II. Examples of class II interferons include γ -interferons.

The pharmaceutical compositions of the invention may contain one or more additional active agents selected, for example, from antiviral agents,
 20 immunomodulatory agents, other inhibitors of HCV NS3 protease, inhibitors of HCV polymerase, inhibitors of another target in the HCV life cycle, HIV inhibitors, HAV inhibitors and HBV inhibitors. Examples of such agents are provided in the Definitions section above.

25 Specific preferred examples of some of these agents are listed below:

- antiviral agents: ribavirin and amantadine;
- immunomodulatory agents: class I interferons, class II interferons and pegylated interferons;
- Inhibitor of another target in the HCV life cycle that inhibits a target selected
 30 from: NS3 helicase, HCV NS2/3 protease or internal ribosome entry site (IRES);
- HIV inhibitors: nucleosidic inhibitors, non-nucleosidic inhibitors, protease inhibitors, fusion inhibitors and integrase inhibitors; or
- HBV inhibitors: agents that inhibit HBV viral DNA polymerase or is an HBV vaccine.

As discussed above, combination therapy is contemplated wherein a compound of formula (1), or a pharmaceutically acceptable salt thereof, is co-administered with at least one additional agent selected from: an antiviral agent, an immunomodulatory agent, another inhibitor of HCV NS3 protease, an inhibitor of HCV polymerase, an inhibitor of another target in the HCV life cycle, an HIV inhibitor, an HAV inhibitor and an HBV inhibitor. Examples of such agents are provided in the Definitions section above. These additional agents may be combined with the compounds of this invention to create a single pharmaceutical dosage form. Alternatively these additional agents may be separately administered to the patient as part of a multiple dosage form, for example, using a kit. Such additional agents may be administered to the patient prior to, concurrently with, or following the administration of wherein a compound of formula (1), or a pharmaceutically acceptable salt thereof.

As used herein, the term "treatment" means the administration of a compound or composition according to the present invention to alleviate or eliminate symptoms of the hepatitis C disease and/or to reduce viral load in a patient.

As used herein, the term "prevention" means the administration of a compound or composition according to the present invention post-exposure of the individual to the virus but before the appearance of symptoms of the disease, and/or prior to the detection of the virus in the blood.

Preferred embodiments

Preferably, compounds of formula 1 as defined above wherein R¹ is hydroxy, NHSO₂Me, NHSO₂cyclopropyl or NHSO₂Ph. More preferably, R¹ is NHSO₂cyclopropyl or NHSO₂Ph. Alternatively, most preferably, R¹ is hydroxy.

Preferably, compounds of formula 1 as defined above wherein R² is cyclopentyl or cyclohexyl. Most preferably, R² is cyclopentyl.

Preferably, R³ is t-butyl or cyclohexyl. Most preferably, R³ is t-butyl.

Preferably, compounds of formula 1 as defined above wherein R⁴ is cyclobutyl or

cyclopentyl. Most preferably, R⁴ is cyclopentyl.

More preferably, a compound of formula 1 as defined above wherein R¹ is hydroxy, R² and R⁴ each is cyclopentyl and R³ is t-butyl.

5

More preferably, a compound of formula 1 wherein R¹ is hydroxy, R² is cyclobutyl, R³ is t-butyl and R⁴ is cyclopentyl.

10 More preferably, a compound of formula 1 wherein R¹ is hydroxy, R² is cyclohexyl, R³ is t-butyl and R⁴ is cyclopentyl.

More preferably R¹ is NHSO₂Ph, R² and R⁴ each is cyclopentyl and R³ t-butyl.

15 More preferably, a compound of formula 1 wherein R¹ is hydroxy, R² is cyclopentyl, R³ is t-butyl and R⁴ is cyclobutyl.

More preferably, a compound of formula 1 wherein R¹ is hydroxy, R² is cyclopentyl, R³ is t-butyl and R⁴ is cyclohexyl.

20 More preferably, a compound of formula 1 wherein R¹ is hydroxy, R² and R⁴ each is cyclopentyl and R³ is cyclohexyl.

More preferably, a compound of formula 1 wherein R¹ is hydroxy, R², R³ and R⁴ each is cyclopentyl.

25

According to an alternate embodiment, the pharmaceutical composition of this invention may additionally comprise another anti-HCV agent. Examples of anti-HCV agents include, α - (alpha), β - (beta), δ - (delta), γ - (gamma) or ω - (omega) Interferon, ribavirin and amantadine.

30

According to another alternate embodiment, the pharmaceutical composition of this invention may additionally comprise another inhibitor of HCV NS3 protease.

According to another alternate embodiment, the pharmaceutical composition of this

invention may additionally comprise an inhibitor of HCV polymerase.

According to yet another alternate embodiment, the pharmaceutical composition of this invention may additionally comprise an inhibitor of other targets in the HCV life cycle, including but not limited to, helicase, NS2/3 protease or internal ribosome entry site (IRES).

The pharmaceutical composition of this invention may be administered orally, parenterally or via an implanted reservoir. Oral administration or administration by injection is preferred. The pharmaceutical composition of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, and intralesional injection or infusion techniques.

The pharmaceutical composition may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example Tween 80) and suspending agents.

The pharmaceutical composition of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

Other suitable vehicles or carriers for the above noted formulations and compositions can be found in standard pharmaceutical texts, e.g. in "Remington's Pharmaceutical Sciences", The Science and Practice of Pharmacy, 19th Ed. Mack Publishing Company, Easton, Penn., (1995).

5

Dosage levels of between about 0.01 and about 100mg/kg body weight per day, preferably between about 0.1 and about 50mg/kg body weight per day of the protease inhibitor compound described herein are useful in a monotherapy for the prevention and treatment of HCV mediated disease. Typically, the pharmaceutical composition of this invention will be administered from about 1 to about 5 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Preferably, such preparations contain from about 20% to about 80% active compound.

As the skilled artisan will appreciate, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the infection, the patient's disposition to the infection and the judgment of the treating physician. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the peptide. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. In general, the compound is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious side effects.

30

When the composition of this invention comprise a combination of a compound of formula 1 and one or more additional therapeutic or prophylactic agent, both the compound and the additional agent should be present at dosage levels of between about 10 to 100%, and more preferably between about 10 and 80% of the dosage

normally administered in a monotherapy regimen.

When these compounds or their pharmaceutically acceptable salts are formulated together with a pharmaceutically acceptable carrier, the resulting composition may be administered *in vivo* to mammals, such as man, to inhibit HCV NS3 protease or to treat or prevent HCV virus infection. Such treatment may also be achieved using a compound of this invention in combination with agents which include, but are not limited to: α -, β -, δ -, ω -, tau, or γ -interferon, ribavirin, amantadine; other inhibitors of HCV NS3 protease; inhibitors of HCV polymerase, inhibitors of other targets in the HCV life cycle, which include but not limited to, helicase, NS2/3 protease, or internal ribosome entry site (IRES); or combinations thereof. The additional agents may be combined with compounds of this invention to create a single dosage form. Alternatively these additional agents may be separately administered to a mammal as part of a multiple dosage form.

Accordingly, another embodiment of this invention provides a method of inhibiting HCV NS3 protease activity in a mammal by administering a compound of the formula 1.

In a preferred embodiment, this method is useful in decreasing the NS3 protease activity of the hepatitis C virus infecting a mammal.

If the pharmaceutical composition comprises only a compound of this invention as the active component, such method may additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV NS3 protease inhibitor, an inhibitor of HCV polymerase or an inhibitor of other targets in the HCV life cycle such as helicase, NS2/3 protease or IRES. Such additional agent may be administered to the mammal prior to, concurrently with, or following the administration of the composition of this invention.

A compound of formula 1 set forth herein may also be used as a laboratory reagent. A compound of this invention may also be used to treat or prevent viral contamination of materials and therefore reduce the risk of viral infection of laboratory or medical personnel or patients who come in contact with such materials

(e.g. blood, tissue, surgical instruments and garments, laboratory instruments and garments, and blood collection apparatuses and materials).

A compound of formula 1 set forth herein may also be used as a research reagent.

- 5 A compound of formula 1 may also be used as positive control to validate surrogate cell-based assays or *in vitro* or *in vivo* viral replication assays.

Further details of the invention are illustrated in the following examples which are understood to be non-limiting with respect to the appended claims.

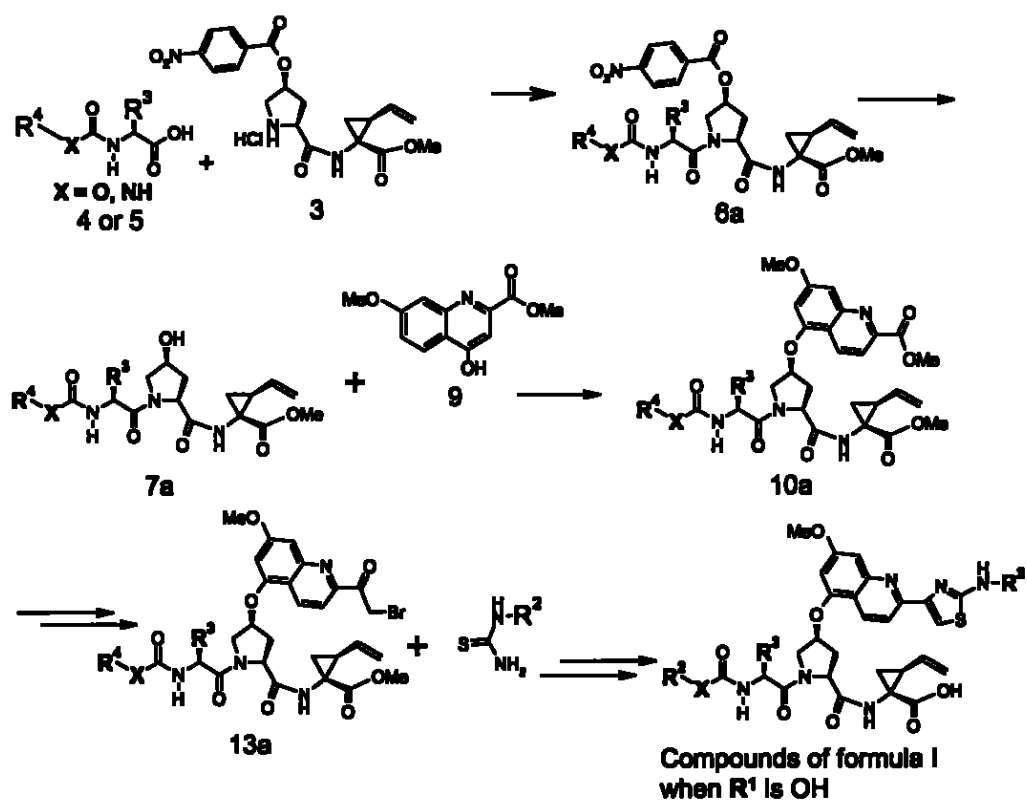
Methodology

- 10 In general, the compounds of formula 1, and intermediates therefore, are prepared by known methods using reaction conditions which are known to be suitable for the reactants. Several such methods are disclosed in WO 00/09543, WO 00/09558 and US Pat. No. 6,323,180, incorporated herein by reference.

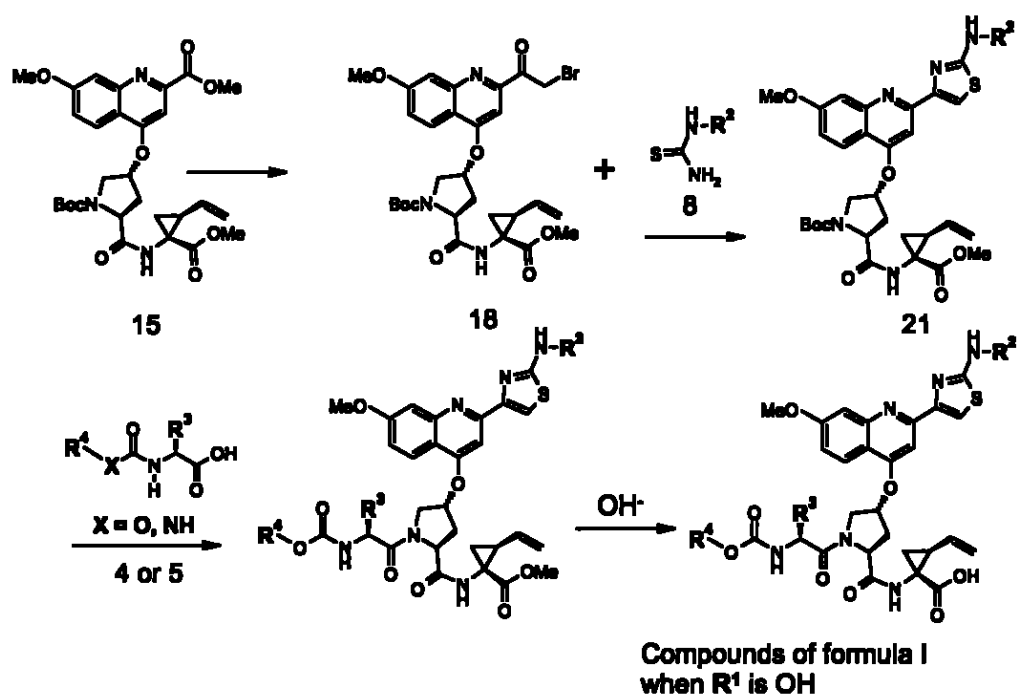
- 15 Compounds of formula I wherein R^1 is $NHSO_2R^{1A}$ as defined herein are prepared by coupling the corresponding acid of formula I (i.e. R^1 is hydroxy) with an appropriate sulfonamide of formula $R^{1A}SO_2NH_2$ in the presence of a coupling agent under standard conditions. Although several commonly used coupling agents can be employed, TBTU and HATU have been found to be practical. The sulfonamides are available commercially or can be prepared by known methods.

- 20 The following schemes illustrate two convenient processes using known methods for preparing the compounds of formula 1 when R^1 is OH.

Scheme 1:



Scheme 2:



EXAMPLES

Temperatures are given in degrees Celsius. Solution percentages express a weight to volume relationship, and solution ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer; the chemical shifts (δ) are reported in parts per million. Flash chromatography was carried out on silica gel (SiO₂) according to Still's flash chromatography technique (W.C. Still et al., J. Org. Chem., (1978), 43, 2923).

10

Abbreviations used in the examples include:

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; DCM: dichloromethane; DIAD: diisopropylazodicarboxylate; DIEA: diisopropylethylamine; DIPEA: diisopropylethylamine; DMF: N, N-dimethylformamide; DMAP: 4-(dimethylamino) pyridine; EtOAc: ethyl acetate; HATU: [O-7-azabenzotriazol-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate; HPLC: high performance liquid chromatography; MS: mass spectrometry (MALDI-TOF: Matrix Assisted Laser Desorption Ionization-Time of Flight, FAB: Fast Atom Bombardment); Me: methyl; MeOH: methanol; Ph: phenyl; R.T.: room temperature (18 to 22°); *tert*-butyl or t-butyl: 1,1-dimethylethyl; Tbg: *tert*-butyl glycine; *tert*-leucine; TBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA: trifluoroacetic acid; and THF: tetrahydrofuran.

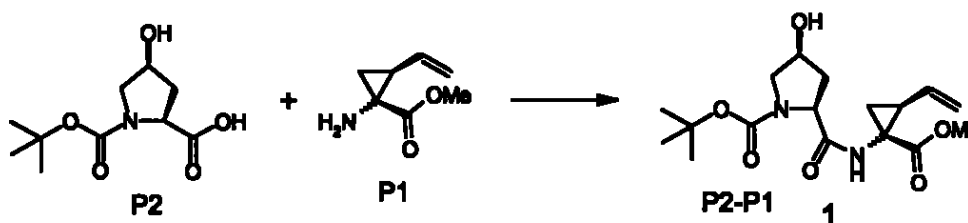
20

Synthesis of compounds of formula (I):

The dipeptide intermediate 15 (Scheme 2) and 2-carbomethoxy-4-hydroxy-7-methoxyquinoline 9 (Scheme 1) were synthesized according to the methods described in WO 00/09543.

25

Synthesis of dipeptide 1

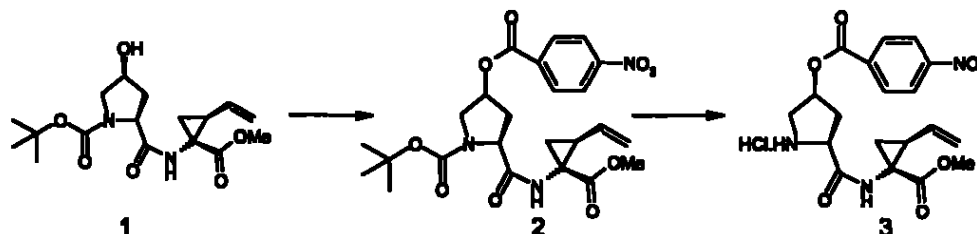


30 A mixture of Boc-hydroxyproline (50.0g, 216mmol), vinyl-ACCA methyl ester

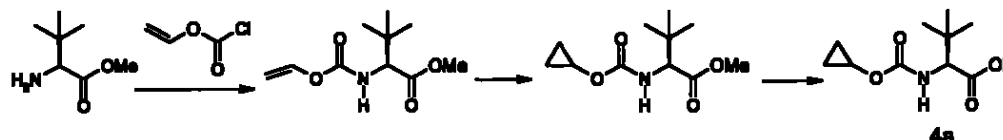
30

(42.25g, 238mmol, 1.1equiv.), TBTU (76.36g, 238mmol, 1.1equiv.) and DIPEA (113mL, 649mmol, 3equiv.) in DMF (800mL) was stirred at R.T. under a nitrogen atmosphere. After 3.5h, the solvent was evaporated and the residue extracted with EtOAc. The extract was washed with hydrochloric acid (10%), saturated sodium bicarbonate and brine. The organic phase was then dried over magnesium sulfate, filtered and evaporated to afford an oil. After drying overnight under high vacuum, dipeptide 1 was obtained as a yellow foam (72.0 g, 94%, purity >95% by HPLC).

Preparation of dipeptide 3

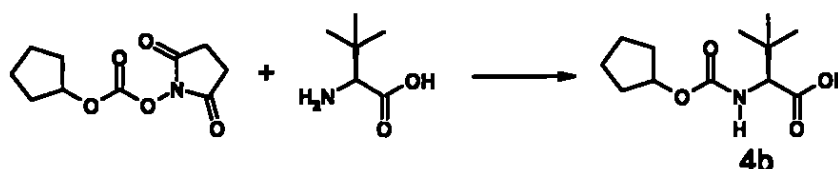


Dipeptide 1 (72.0g, 203mmol), triphenylphosphine (63.94g, 243.8mmol, 1.2equiv.) and 4-nitrobenzoic acid (41.08g, 245.8mmol, 1.2equiv) were dissolved in dry THF (1.4L). The stirred solution was cooled to 0°C under a nitrogen atmosphere. Diethyl azodicarboxylate (38.4mL, 244mmol, 1.2equiv.) was then added dropwise over 45 min and the reaction allowed to warm to R.T. After 4h, the solvent was evaporated. The residue was divided into four portions. Each of these was purified by chromatography over fine silica gel (10–40µm mesh, column diameter 12cm, column length 16cm) using a gradient of 2 :1 hexane/EtOAc to 1:1 hexane/EtOAc to pure EtOAc. In this manner, the Boc-dipeptide ester 2 was obtained as an amorphous white solid after evaporation of the solvents and drying of the residues under high vacuum at 70°C for 1h (108.1g, quantitative-105%). A solution of 4N hydrogen chloride in dioxane was added to the Boc-dipeptide ester 2 (108g, 243mmol) resulting in a colorless solution. The solution was stirred at R.T. for 1h. The solvent was evaporated and the residue placed under high vacuum for 3h affording the hydrochloride salt of compound 3 as an amorphous solid. The solid was used as such.

Synthesis of carbamates 4**Preparation of carbamate 4a:**

At 0°C, was added to a solution of *tert*-butyl glycine methyl ester (590mg, 3.25mmol) in THF (8mL). vinylchloroformate (0.55mL, 6.47mmol) and triethylamine (1.15mL, 8.25mmol). The temperature was allowed to rise to R.T. The solution was stirred for 16h. The solution was concentrated and the residue dissolved in EtOAc. The EtOAc solution was washed with a 10% aqueous solution of citric acid (2x), a saturated aqueous solution of NaHCO₃ (2x) and brine, dried and concentrated to yield the corresponding vinyl carbamate (608mg) as a colorless oil. The oil was dissolved in DCM (4mL), cooled at 0°C, and diiodomethane (0.15mL, 1.86mmol) and diethylzinc (95μL, 0.93mmol) were added. A white solid appeared at first but dissolved with time (~ 1h). The suspension/solution was stirred at R.T. for 5h. A saturated solution of ammonium chloride was added and the solution extracted with EtOAc (2x). The organic extract was dried (MgSO₄) and concentrated. The residue was purified by flash column chromatography. Elution with hexane:EtOAc 95:5 gave the corresponding methyl ester as a colorless oil (96mg, 90% yield).

A solution the methyl ester (93mg; 0.41mmol) in THF (5mL), MeOH (1mL) and an aqueous solution of LiOH (45mg ; 1.81mmol) in water (2mL) was stirred for 4h. The solution is dilute with water and extracted with EtOAc (2x). The aqueous solution was acidified by the addition of 1N HCl, and the acidic solution was extracted with EtOAc (2x). The combined organic extracts were dried (MgSO₄), filtered and evaporated to obtain the desired carbamate 4a as a white solid (53mg; 60% yield)

Preparation of carbamate 4b

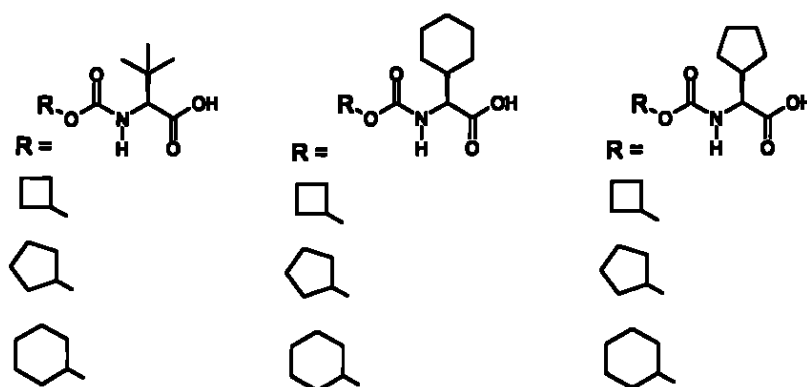
THF (350mL) was added to a flask containing carbonic acid cyclopentyl ester 2,5-dioxo-pyrrolidin-1-yl ester (9.00g; 39.6mmol) and *tert*-butyl glycine (6.24g;

- 47.5mmol) resulting in a suspension. Distilled water (100mL) was added with vigorous stirring. A small amount of solid remained undissolved. Triethylamine (16.6mL; 119mmol) was then added resulting in a homogenous solution which was stirred at R.T. After 2.5h, the THF was evaporated and the aqueous residue diluted with water (100mL). The reaction was rendered basic by the addition of 1 N NaOH (25mL - final pH >10). The solution was washed with EtOAc (2 x 200mL) and the aqueous phase acidified with 1 N HCl (ca. 70mL - final pH <2). The turbid solution was extracted with EtOAc (200 + 150mL). The extract was dried (MgSO₄) and evaporated to give carbamate 4b as a white solid (8.68g).

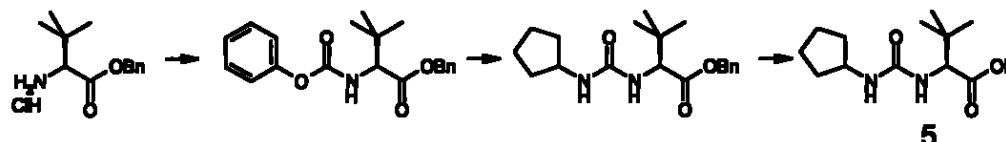
10

Preparation of other carbamates

- Using the procedure described above and using appropriate combinations of *tert*-butyl glycine, cyclopentyl glycine, or cyclohexyl glycine and carbonic acid cyclobutyl, cyclopentyl, or cyclohexyl ester 2,5-dioxo-pyrrolidin-1-yl ester, carbamates of the following formulas were prepared:



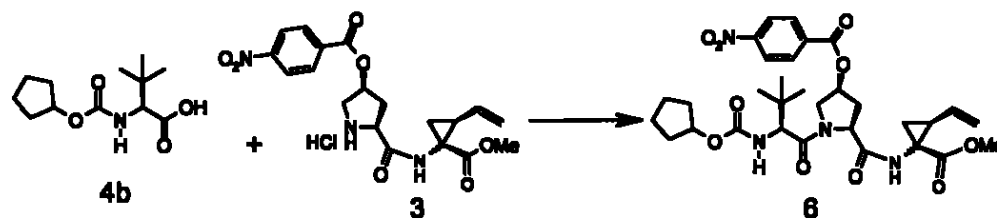
Preparation of Ureas 5



- 20 A solution of *tert*-butyl glycine benzyl ester hydrochloride salt (2.55g; 9.89mmol) in THF (20mL) and pyridine (2.0mL; 24.73mmol) was cooled to 0° C. Phenyl chloroformate (1.30mL; 10.19mmol) was added dropwise to the cooled solution. The resulting suspension was stirred for 5min at 0° C, then at R.T. for 1.5h. The reaction

- mixture was diluted with EtOAc, washed with 10% citric acid (2x) water (2x) saturated NaHCO₃ (2x), water (2x) and brine (1x), dried (MgSO₄), filtered and evaporated to gain the crude compound as a nearly colorless oil (3.73g ; >100%; assume 9.89mmol). The crude product (1.01g; 2.97mmol) was dissolved in DMSO (6.5mL) and cyclopentylamine was added dropwise. The reaction mixture was stirred at R.T. for 45min. The reaction mixture was diluted with EtOAc. The organic phase was washed with washed with 10% citric acid (2x) water (2x) saturated NaHCO₃ (2x), water (2x) and brine (1x), dried (MgSO₄), filtered and evaporated to give the crude cyclopentyl urea -Tbg-OBn product as a nearly colorless oil. The crude material was purified by flash column chromatography with silica using hexane:EtOAc 9:1 to remove the less polar impurities and 7:3 to elute the purified product as a thick colourless oil (938mg; 95%). The ester benzyl ester product (938mg; 2.82mmol) was deprotected under a hydrogen filled balloon at R.T. in absolute ethanol (15mL) solution by stirring the solution with 10% Pd/C (93.6mg) for 5.5h. The reaction mixture was filtered through a 0.45micron filter and evaporated to dryness to provide urea 5 as a white solid (668.8mg ; 98%)
- ¹H NMR (400 MHz,DMSO-d₆): δ 12.39 (s, 1H) , 6.09 (d, J = 7.4 Hz, 1H) , 5.93 (d, J = 9.4 Hz, 1H), 3.90 (d, J = 9.4 Hz, 1H), 3.87-3.77 (m, 1H), 1.84-1.72 (m, 2H), 1.63-1.42 (m, 4H), 1.30-1.19 (m, 2H), 0.89 (s, 9H).
- M.S.(electrospray) : 241.0 (M-H)⁻ 243.0 (M+H)⁺ . Reverse Phase HPLC Homogeneity (0.06% TFA; CH₃CN : H₂O) : 99%.

Synthesis of tripeptide 6

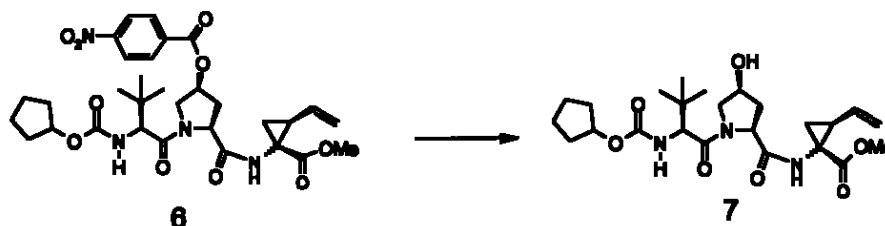


- Carbamate 4b (6.15g, 22.5mmol) and TBTU (7.72g, 24.7mmol) were suspended in DCM and the suspension was stirred rapidly. DIPEA (3.92mL, 22.5mmol) was added at R.T. and after 10 min, the reaction was nearly homogeneous. A solution of dipeptide 3 (10.39g, 23.6mmol) in anhydrous DCM (100mL) containing DIPEA (4.11mL, 23.62mmol) was then poured into the reaction. The resulting yellow solution was allowed to stir for 14h. The solvent was then evaporated yielding a

yellow syrup which was extracted with EtOAc (300 + 150mL) and washed with 0.05N HCl (2 x 200mL), saturated Na₂CO₃ (300mL) and brine (150mL). The combined extracts were dried over MgSO₄ and evaporated to yield the tripeptide **6** as a pale yellow foam (15.68g, quantitative).

5

Synthesis of tripeptide 7:

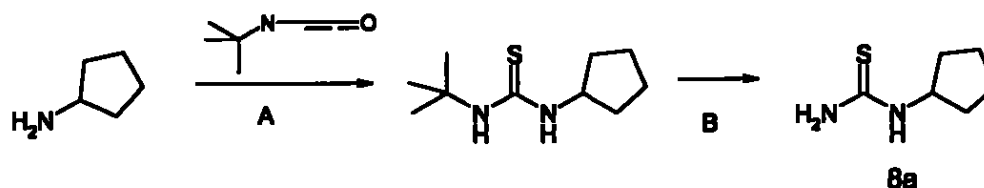


The tripeptide **6** (15.68g) was dissolved in THF (200mL) and water (30mL) was added. The resulting solution was cooled to 0°C and a solution of lithium hydroxide monohydrate (1.18g, 28.12mmol) was added over 3 min with vigorous stirring. After 3h at 0°C, the excess base was neutralized with 1N HCl (final pH ca. 6) and the THF evaporated, resulting in an aqueous suspension (yellow gum). The mixture was extracted with EtOAc (2 x 200mL) and washed with saturated NaHCO₃ (2 x 300mL). The combined extracts were dried over MgSO₄ and evaporated to yield a pale yellow foam. Flash chromatography of the foam over silica gel using EtOAc as eluent afforded **7** as a white amorphous solid (9.77g, 91%).

15

Preparation of Thioureas 8

Synthesis of thiourea 8a:



20

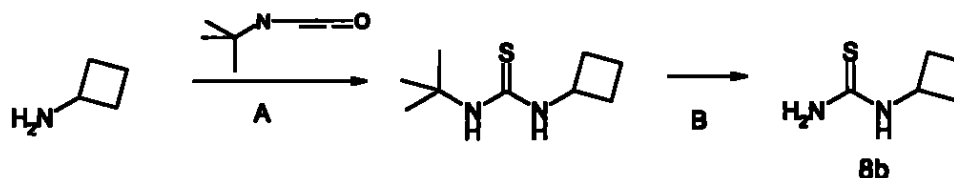
To a solution of *tert*-butyl isothiocyanate (5.0mL; 39.4mmol) in DCM (200mL) was added cyclopentylamine (4.67mL; 47.3mmol) followed by DIEA and the reaction mixture was stirred at R.T. for 2h. The mixture was diluted with EtOAc, washed with a 10% aqueous solution of citric acid (2x), saturated NaHCO₃ (2x), H₂O (2x) and brine (1x). The organic layer was dried over anhydrous MgSO₄, filtered and evaporated to yield *N-tert*-butyl – *N'*-cyclopentyl thiourea as a white solid (3.70g;

25

- 47% yield). The *N*-*tert*-butyl-*N'*-cyclopentyl thiourea (3.70g) was dissolved in concentrated HCl (46mL). The dark yellow solution was heated at a gentle reflux. After 40min the reaction mixture was allowed to cool to R.T. and thereafter cooled in ice and rendered basic to pH 9.5 with solid and a saturated aqueous solution of
- 5 NaHCO_3 . The product was extracted into EtOAc (3x). The combined EtOAc extracts were washed with H_2O (2x) and brine (1x). The organic layer was dried (MgSO_4), filtered and concentrated to yield a beige solid (2.46g crude). Trituration of the crude material in hexane/ EtOAc 95 / 5 provided, after filtration, the *N*-cyclopentylthiourea
- 8a as a white solid (2.38; 90% yield).
- 10 ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.58 (bs, 1H), 7.19 (bs, 1H), 6.76 (bs, 1H), 4.34 (bs, 1H), 1.92-1.79 (m, 2H), 1.66-1.55 (m, 2H), 1.55-1.30 (m, 4H). MS; m/z : 144.9 ($M + H$) $^+$, m/z : 142.8 ($M - H$) $^-$.

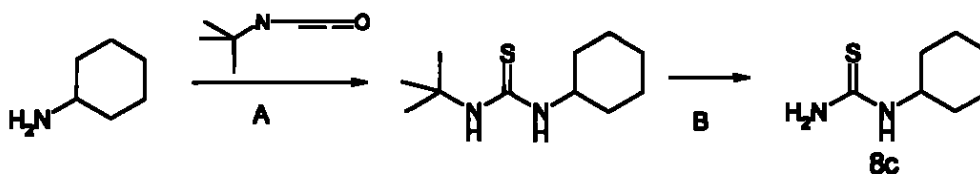
Preparation of thiourea 8b

- 15 Using the procedure describe above and using commercially available cyclobutylamine instead of cyclopentylamine yielded thiourea 8b:

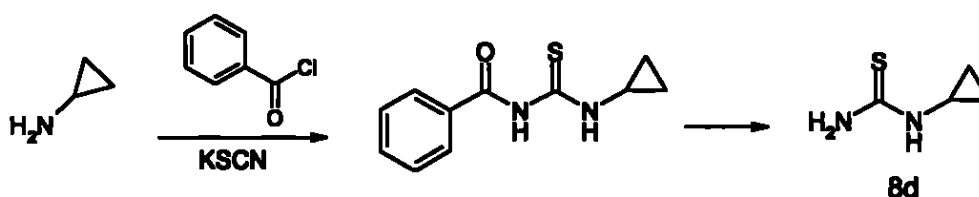


Preparation of thiourea 8c

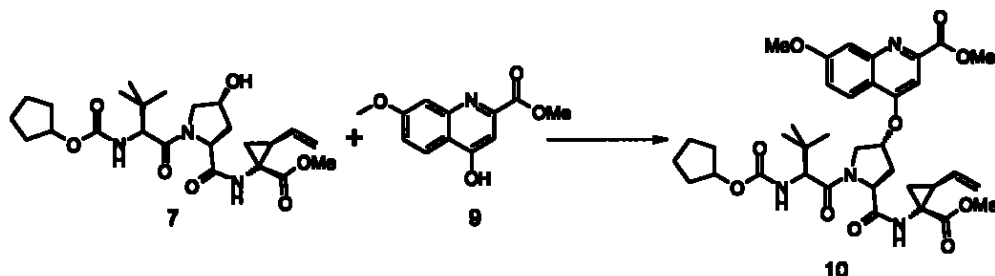
- 20 Using the procedure describe above and using commercially available cyclohexylamine instead of cyclopentylamine yielded thiourea 8c.



Preparation of thiourea 8d



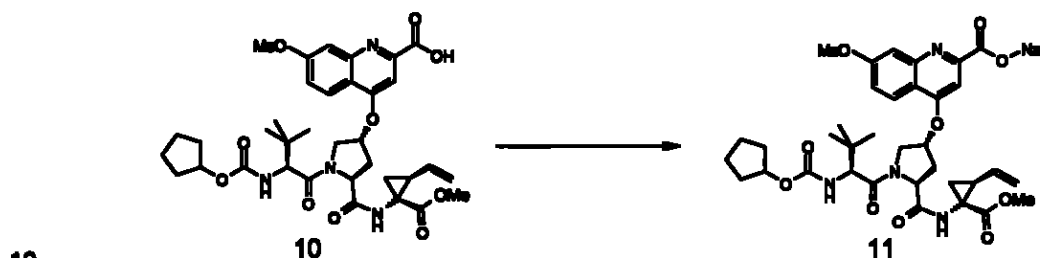
- To a solution of the KSCN (4.60g; 47.33mmol) in acetone (35mL), at 0°C, was added dropwise benzoylchloride (5.0mL; 43.03mmol). The milky solution was stirred in an ice bath for 1.5h, then, cyclopropylamine (3.2mL; 46.0mmol) was added dropwise. The reaction mixture was stirred for 1.5h at 0°C, then, more cyclopropylamine (0.50mL, 7.22mmol) was added and the reaction mixture stirred at R.T. for an additional 30min. The reaction mixture was poured into ice/H₂O (300mL), stirred for 5min. and the light yellow solid was filtered, washed several times with H₂O and dried to provide N-benzyloxy-N'-cyclopropyl thiourea (6.62g). This thiourea was suspended in a solution of 2N NaOH (50mL) and heated at reflux for 15min. The solution was cooled to R.T., saturated with solid NaCl and extracted with EtOAc (3x). The combined EtOAc extracts were washed with H₂O (2x) and brine (1x), dried (MgSO₄), filtered and evaporated to give the crude product as an off-white solid. The solid was triturated in hexane/EtOAc 5/5 to provide the N-cyclopropyl thiourea **8d** as a white crystalline solid (2.5g ; 50% yield).
- ¹H NMR (400 MHz, DMSO-d₆): δ 7.92 (bs, 1H), 7.61 (bs, 1H), 7.13 (bs, 1H), 2.39 (bs, 1H), 0.67-0.63 (m, 2H), 0.51-0.44 (m, 2H).
MS; es⁺ 116.9 (M + H)⁺, es⁻ 114.8 (M - H)⁻

EXAMPLE 1**20 Preparation of Compound 100****Step 1: Synthesis of tripeptide 10:**

- To tripeptide **1** (1.0g; 2.09mmol) dissolved in THF (35mL), hydroxyquinoline **9** (729mg; 3.13mmol) and triphenylphosphine (1.1g ; 4.2mmol) were added. The yellow suspension was cooled in an ice bath and DIAD (821μL, 4.2mmol) was added dropwise. The solution was stirred at ice bath temperature for 30 min, and at R.T. for 16h. The solution was evaporated to dryness and the residue was dissolved in EtOAc, washed with a saturated sodium bicarbonate solution (2x), water (2x) and brine (1x), dried (MgSO₄), filtered and evaporated to obtain a yellow oil which

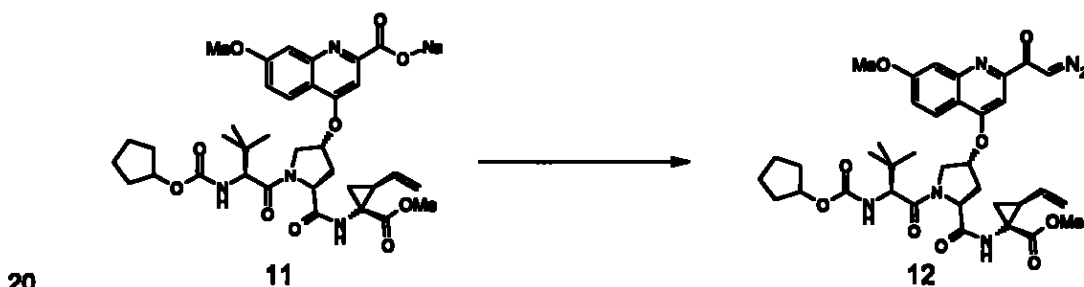
precipitated on standing. The crude solid was suspended in DCM and the insoluble material was filtered off. The solution was concentrated and the residue purified by flash chromatography in Hexane : EtOAc; 5 : 5 to remove all less polar impurities and in CHCl_3 :EtOAc; 80:20 till all the $\text{Ph}_3\text{P}=\text{O}$ has eluted. The desired compound was eluted with CHCl_3 :EtOAc; 65:35 as a white solid (1g; 70% yield).
 M.S.(electrospray) : 693.3 (M-H)⁻ 695.4 (M+H)⁺ 717.4 (M+Na)⁺ .
 Reverse Phase HPLC Homogeneity (0.06% TFA ; CH_3CN : H_2O) : 99%.

Step 2 : Selective monohydrolysis of ester 10:



Tripeptide 10 (1g; 1.44mmol) was dissolved in THF (10mL) and MeOH (5mL), water (5mL) and a 1N NaOH aqueous solution (1.5mL) were added and the solution stirred at R.T. for 2h. The mixture was evaporated to dryness and then co-evaporating with MeOH:toluene (1:1; 4x), toluene (2x) and diethyl ether (2x) to obtain the product (water-free) as a white flaky solid (1.04g; 100% yield).
 M.S.(electrospray) : 679.3 (M-H)⁻ 681.3 (M+H)⁺ 703.3 (M+Na)⁺ .
 Reverse Phase HPLC Homogeneity (0.06% TFA ; CH_3CN : H_2O) : 95%.

Step 3 : Synthesis of diazoketone 12:



Sodium salt 11 (assume 1.44mmol) was dissolved in THF (16mL), triethylamine (301 μL ; 2.16mmol) was added and the solution cooled to 0°C .

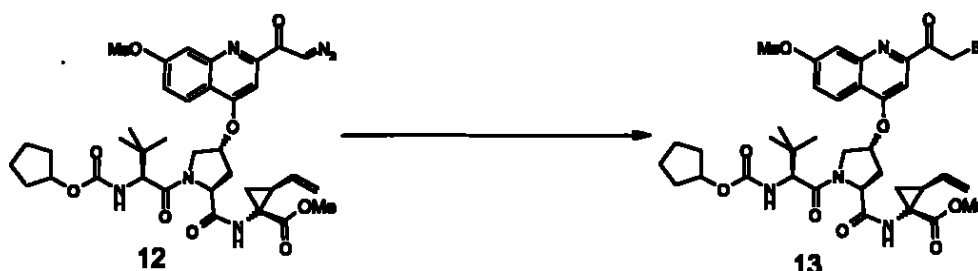
Isobutylchloroformate (280 μL ; 2.16mmol) was added dropwise and the white suspension was stirred at 0°C for 75min, followed by the addition of a solution of

- diazomethane (0.67M in diethyl ether; 13mL; 8.64mmol). The reaction mixture is stirred 1h at 0°C, 45min at R.T. and evaporated to provide a thick suspension. This suspension was dissolved in EtOAc and water. The organic solution was washed with saturated NaHCO₃ (2x), water (2x) and brine (1x), dried (MgSO₄), filtered and evaporated to give the diazoketone product as an ivory solid (crude material used for next step; assume 1.44mmol).

M.S.(electrospray) : 703.3 (M-H)⁻ 705.3 (M+H)⁺ . Reverse Phase HPLC

Homogeneity (0.06% TFA ; CH₃CN : H₂O) : 91%.

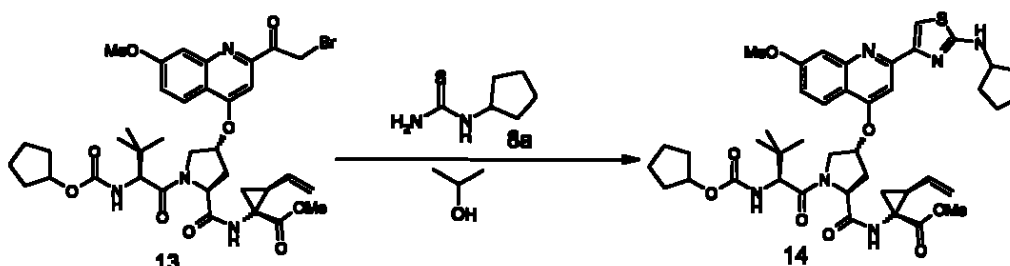
10 **Step 4 : Synthesis of bromoketone 13:**



- At 0°C, to a solution of diazoketone 12 (1.44mmol) in THF (24mL) was added dropwise an HBr solution (1.0mL) and the mixture was stirred for 1h. The solution was diluted with EtOAc, washed with a saturated NaHCO₃ solution (2x), water (2x) and brine (1x), dry (MgSO₄), filtered and evaporated to give the desired bromoketone as an ivory-beige solid (1.1g; assume 1.44mmol).

M.S.(electrospray) : 757.3 (M) 759.3 (M+2)

Step 5 : Synthesis thiazolyl tripeptide 14:

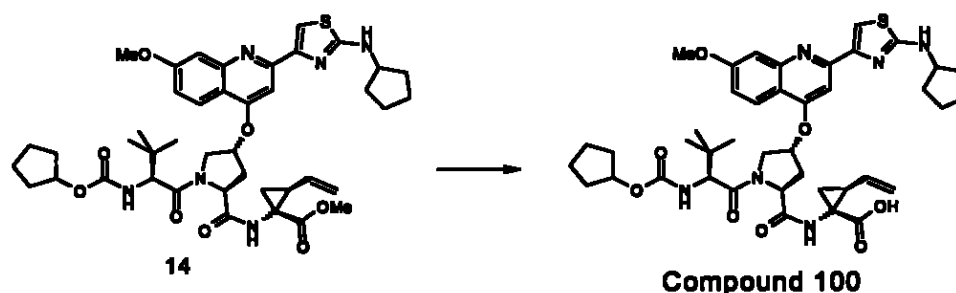


- 20 α -Bromoketone 13 (0.40mmol) and N-cyclopentylthiourea (68.5mg; 0.48mmol) were dissolved in isopropanol (15mL) and the yellow solution was heated at 70°C for 75min. The solution was allowed to cool to R.T., and evaporated to dryness. The residue was dissolved in EtOAc. The solution was washed with saturated NaHCO₃

(2x), water (2x) and brine (1x), dried (MgSO₄), filtered and concentrated to give the product as an orange-brown foam. Flash column chromatography in hexane:EtOAc 7:3 removed less polar impurities and 6 : 4 retrieved the desired compound as a light yellow foam (218mg; 69%).

- 5 M.S.(electrospray) : 801.4 (M-H)⁻ 803.4 (M+H)⁺ 825 (M+Na)⁺ .
Reverse Phase HPLC Homogeneity (0.06% TFA ; CH₃CN : H₂O) : 99%.

Step 6: Hydrolysis of ester 14:



- 10 A solution of methyl ester 14 (145mg; 0.181mmol) in THF (3mL), MeOH (1.5mL) and an aqueous solution of LiOH (75.8mg; 1.81mmol) in water (1.5mL) was stirred for 18h. The organic solution was concentrated to provide an off-white suspension which was diluted with EtOAc and brine to obtain a total solution. The pH was adjusted to 6 by the addition of 1N HCl and the organic layer was extracted further
- 15 with EtOAc (2x). The combined organic extracts were washed with water (2x), brine (1x), dried (MgSO₄), filtered and evaporated to give the desired compound as a yellow solid (138.2mg; 97% yield).

Conversion to Na Salt:

- 20 Compound 100 (138.2mg; 0.175mmol) was dissolved in MeOH (30mL) and 1 equivalent 0.01N NaOH (17.5mL) was added. The clear yellow solution was concentrated to remove MeOH and diluted with water, frozen and lyophilized to obtain the product (Na salt) as a yellow amorphous solid (139mg; theoretical yield : 142mg; MW Na salt : 810.95)
- 25 M.S.(electrospray) : 787.2 (M-H)⁻ 789.3 (M+H)⁺ 811.3 (M+Na)⁺ . Reverse Phase HPLC Homogeneity (0.06% TFA ; CH₃CN : H₂O) : 98%.
¹H NMR (400 MHz,DMSO-d₆): ca, 5:1 mixture of rotamers ;δ 8.14 (bs, 1H) , 8.02 (d, J = 9.2 Hz, 1H) , 7.89 (d, J = 6.7 Hz, 1H), 7.49-7.36 (m, 2H), 7.27 (bs, 1H),

✓

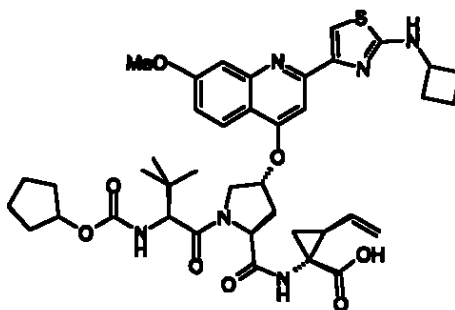
7.06-6.96 (m, 2H), 6.10-5.90 (m, 1H), 5.33 (s, 1H), 5.01 (d, J = 16.8 Hz, 1H), 4.84 (d, J = 10.6 Hz, 1H), 4.79-4.65 (m, 1H), 4.47-4.40 (m, 1H), 4.30 (d, J = 11.5 Hz, 1H), 4.15 (d, J = 8.8 Hz, 1H), 4.00-3.85 (m, 2H), 3.90 (s, 3H), 2.37-2.26 (m, 1H), 2.15-1.91 (m, 2H), 1.80-1.23 (m, 18H), 0.96 & 0.86 (2x s, 9H).

5

EXAMPLE 2**Preparation of Compound 101**

Using the same procedure described in example 1 and using N-cyclobutyl thiourea 8b instead of using N-cyclopentyl thiourea 8a in step 5 and resulted in compound

10 101 as the TFA salt:



Compound 101

¹H NMR (400 MHz, DMSO-d₆): ca, 90:10 mixture of rotamers, major isomer description: 8.59 (s, 1H), 8.45-8.39 (m, 1H), 8.25 (bs, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.84 (bs, 1H), 7.74 (s, 1H), 7.32-7.26 (m, 1H), 7.01 (d, J = 8.3 Hz, 1H), 5.78-5.66 (m, 2H), 5.20 (dd, J = 17.0, 1.6 Hz, 1H), 5.09-5.04 (m, 1H), 4.53-4.36 (m, 4H), 4.05-3.92 (m, 2H), 3.97 (s, 3H), 2.63-2.55 (m, 1H), 2.44-2.29 (m, 3H), 2.07-1.95 (m, 3H), 1.79-1.23 (m, 12H), 0.96 (s, 9H).

15

M.S.(electrospray) : 773.4 (M-H)⁻ 775.4 (M+H)⁺ . Reverse Phase HPLC

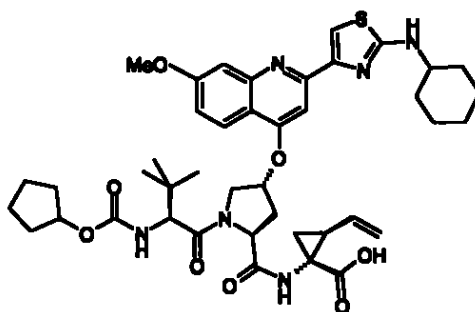
Homogeneity (0.06% TFA; CH₃CN : H₂O) : 98%

20

EXAMPLE 3**Preparation of Compound 102**

Using the same procedure described in example 1 and using N-cyclohexyl thiourea 8c instead of using N-cyclopentyl thiourea 8a in step 5 and resulted in compound

25 102 as the TFA salt:



Compound 102

¹H NMR (400 MHz, DMSO-d₆): ca, 90:10 mixture of rotamers, major isomer

description: δ 8.61 (s, 1H), 8.28-8.17 (m, 2H), 8.13-8.04 (m, 1H), 7.81 (bs, 1H), 7.73 (bs, 1H), 7.32-7.24 (m, 1H), 7.07 (d, J = 8.2 Hz, 1H), 5.78-5.65 (m, 2H), 5.23-5.15 (m, 1H), 5.09-5.03 (m, 1H), 4.51-4.43 (m, 3H), 4.05-3.77 (m, 3H), 3.97 (s, 3H), 2.64-2.55 (m, 1H), 2.38-2.27 (m, 1H), 2.05-1.95 (m, 3H), 1.79-1.71 (m, 2H), 1.66-1.19 (m, 16H), 0.96 (s, 9H).

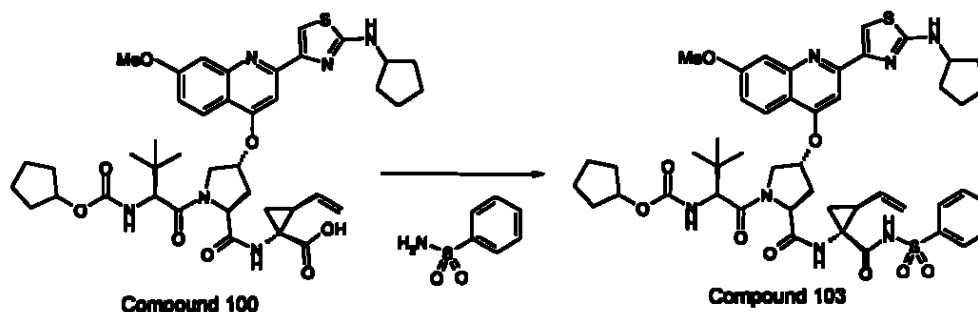
M.S.(electrospray) : 801.4 (M-H)⁻ 803.4 (M+H)⁺ . Reverse Phase HPLC

Homogeneity (0.06% TFA; CH₃CN : H₂O) : 99%

10

EXAMPLE 4

Preparation of compound 103



Compound 100 (30mg, 0.038mmol) was combined with HATU (17mg, 0.045mmol)

and dissolved in anhydrous DMF (4mL). The solution was stirred at R.T. before
DIPEA (26μL, 0.15mmol) was added dropwise over ca. 1min. The mixture was
stirred for 60min. at R.T. and analyzed by analytical HPLC for the formation of the
activated ester. Following this, a solution of benzenesulfonamide (23mg, 0.15mmol),
DMAP (17mg, 0.14mmol) and DBU (22μL, 0.15mmol) was added in DMF (1mL).
The reaction mixture was stirred 24h at R.T. before being poured into EtOAc (50mL)
and washed with sat. NaHCO₃, and sat. brine solutions. The organic phase was

20

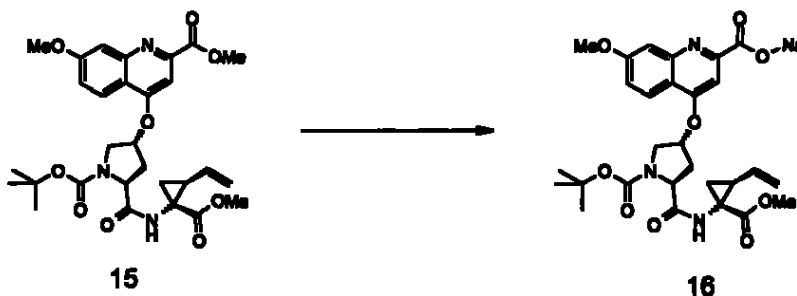
dried over MgSO_4 , filtered and concentrated. The residue was reconstituted in DMSO and purified by preparative HPLC. Lyophilization gave the final product (17 mg, 48%) as a pale yellow amorphous solid.

- $^1\text{H-NMR}$ (400MHz, DMSO-d_6), δ 10.89 (s, 1H), 8.84 (s, 1H), 8.20 (d, $J = 8.8$ Hz, 1H), 8.18-8.08 (m, 2H), 7.89 (d, $J = 7.6$ Hz, 2H), 7.70 (dd, $J = 7.6, 7.6$ Hz, 2H), 7.58 (dd, $J = 7.7, 7.7$ Hz, 2H), 7.28 (bs, 1H), 7.11 (d, $J = 6.6$ Hz, 1H), 5.75 (bs, 1H), 5.37-5.25 (m, 1H), 5.12 (d, $J = 17$ Hz, 1H), 4.92 (d, $J = 12$ Hz, 1H), 4.58-4.42 (m, 3H), 4.24 (bs, 1H), 4.05 (d, $J = 7.8$ Hz, 1H), 3.97 (s, 3H), 3.93 (d, $J = 7.8$ Hz, 1H), 2.69-2.61 (m, 1H), 2.35-2.21 (m, 1H), 2.13-1.98 (m, 3H), 1.78-1.68 (m, 4H), 1.68-1.51 (m, 8H), 1.50-1.41 (m, 4H), 1.29-1.22 (m, 1H), 0.99 (s, 9H).
- MS (electrospray): 928.5 ($\text{M} + \text{H}$) $^+$, and 926.5 ($\text{M} - \text{H}$) $^-$.
- RP-HPLC: $R_t = 7.3$ minutes (homogeneity = 99%).

- Using the synthetic sequence shown in Scheme 2 the following compounds were prepared:

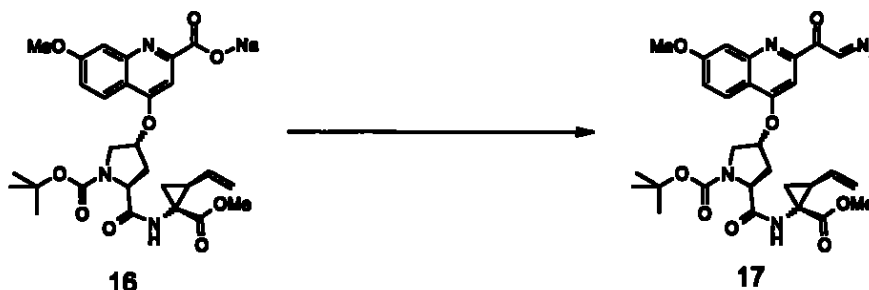
EXAMPLE 5

Step 1: synthesis of dipeptide 16:



- Dipeptide 15 (4.0g ; 7.02mmol) was dissolved in THF (20mL) and MeOH (10mL), water (10mL) and a 1N NaOH aqueous solution (1.05 equivalents; 7.4mL) was added. The solution stirred at R.T. for 2.75h. The mixture was evaporated to dryness. The residue was diluted with water, frozen and lyophilized to provide sodium salt 16 as a white amorphous solid (4.28g).
- M.S.(electrospray) : 554.2 (M-H) $^-$ 556.3 (M+H) $^+$ 578.2 (M+Na) $^+$.
- Reverse Phase HPLC Homogeneity (0.06% TFA ; $\text{CH}_3\text{CN} : \text{H}_2\text{O}$) : 96%.

Step 2: synthesis of dipeptide diazoketone 17:



Sodium salt 16 (assume 7.02mmol) was dissolved in THF (78mL); triethylamine (1.37mL; 9.83mmol) was added and the solution cooled to 0°C .

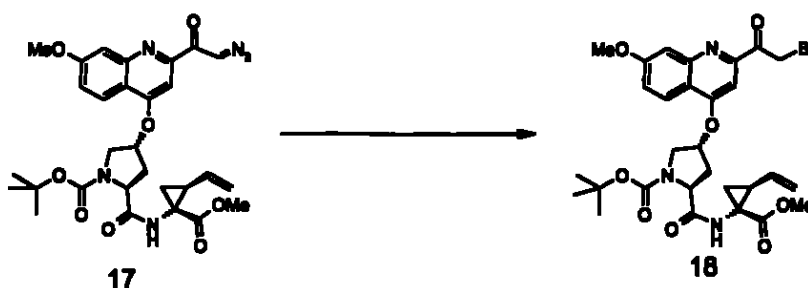
- 5 Isobutylchloroformate (1.28mL; 9.83mmol) was added dropwise and the white suspension was stirred at 0°C for 2h, , followed by the addition of a solution of diazomethane (0.67M in diethyl ether; 63mL; 42.13mmol). The reaction mixture was stirred 1h at 0°C, 1.25h at R.T. and evaporated to provide a thick suspension. This suspension was dissolved in EtOAc and water. The organic solution was washed
- 10 with saturated NaHCO₃ (2x), water (2x) and brine (1x), dried (MgSO₄), filtered and evaporated to give the diazoketone 17 as an beige solid (crude material used for next step; assume 7.02mmol).

M.S.(electrospray) : 578.2 (M-H)⁻ 580.3 (M+H)⁺ . Reverse Phase HPLC

Homogeneity (0.06% TFA ; CH₃CN : H₂O) : 90%.

15

Step 3: synthesis of dipeptide bromoketone 18:

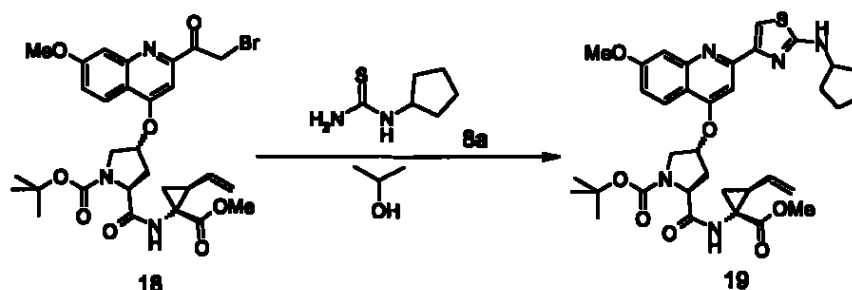


At 0°C, to a solution of diazoketone (assume 1.44mmol) in THF (116mL) was added dropwise a 48% aqueous HBr solution (5.1mL) and the mixture was stirred for 2h.

- 20 The solution was diluted with EtOAc, washed with a saturated NaHCO₃ solution(2x), water (2x) and brine (1x), dry (MgSO₄), filtered and evaporated to give the desired bromoketone as a beige solid (4.25g; 6.72mmol).

M.S.(electrospray) : 632 (M) 634.2 (M+2)

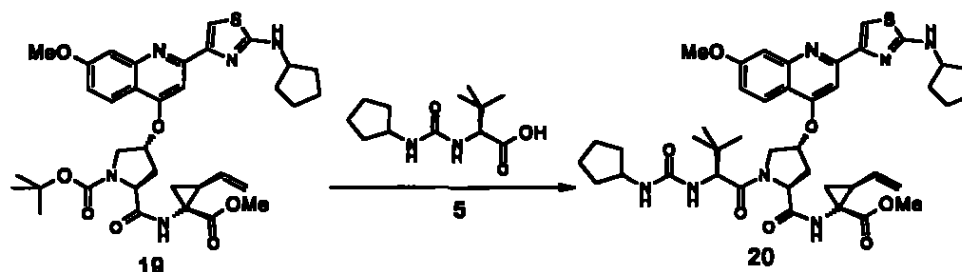
Step 4: synthesis of dipeptide 19:



α -Bromoketone 18 (512mg; 0.81mmol) and N-cyclopentylthiourea (128.4mg;

- 5 0.89mmol) were dissolved in isopropanol (20mL). The resulting yellow solution was heated at 70°C for 1.5h. The solution was allowed to cool to R.T., and evaporated to dryness. The residue was diluted with EtOAc. The EtOAc solution was washed with saturated NaHCO₃ (2x), water (2x) and brine (1x), dried (MgSO₄), filtered and concentrated to give the product as an orange-brown foam. Flash column
- 10 chromatography in hexane:EtOAc 7:3 removed less polar impurities and 6 : 4 retrieved the desired compound as a light yellow solid (411.5mg; 75%).
- M.S.(electrospray) : 676.3 (M-H)⁻ 678.3 (M+H)⁺
- Reverse Phase HPLC Homogeneity (0.06% TFA ; CH₃CN : H₂O) : 99%.

15 Step 5: synthesis of tripeptide 20:



- The Boc-dipeptide (32.6g ; 0.048mmol) was dissolved in 4N HCl/dioxane (3mL) and stirred at R.T. After 2h, the reaction mixture was worked-up by evaporating to dryness. The HCl salt thus obtained as an off-white solid. The HCl salt was
- 20 subjected to high vacuum for 30min. To a solution of the HCl salt in DCM (2mL) and DIEA (33 μ L ; 0.192mmol), was added urea 5 (13.96mg ; 0.058mmol), followed by HATU coupling agent (21.90mg ; 0.058mmol). The reaction mixture was stirred at R.T. for 3h. The reaction mixture was diluted with EtOAc, washed with saturated

65

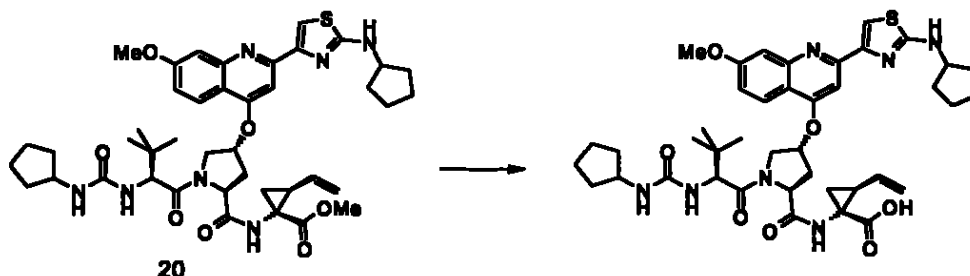
NaHCO₃ (2x), water (2x) and brine (1x), dried (MgSO₄), filtered and evaporated to give the crude product as a thick yellow oil (assume 0.048mmol).

M.S. (electrospray) : 800.4 (M-H)⁻ 802.4 (M+H)⁺ . Reverse Phase HPLC

Homogeneity (0.06% TFA ; CH₃CN : H₂O) : 95%.

5

Step 6: Hydrolysis of tripeptide methyl ester 20:



A solution of methyl ester 20 (77.80mg; 0.097mmol) in THF (2mL), and MeOH (1mL), and an aqueous solution of LiOH (40.7mg; 0.97mmol) in water (1mL), was stirred for 16h. The organic solution was concentrated to provide an off-white suspension. The crude material was purified by preparatory HPLC (YMC Combiscreen ODS-AQ, 50 x20mm ID S-5micron, 120A ; λ=220nm) using a linear gradient and 0.06% TFA CH₃CN / H₂O . The pure fractions were combined, concentrated and converted to the sodium salt.

15

Conversion to Na Salt:

The concentrated fractions were diluted with EtOAc and a few mls of brine, (basified to pH ~13 with 5N NaOH, then, neutralized to pH 5.5 – 6.0 with 1N HCl). The product was extracted with EtOAc (3x), washed with water (2x) and brine (1x), dried (MgSO₄), filtered and evaporated to dryness to provide the neutral product as a yellow solid (52.7mg ; 70%). The neutral product (49.4mg ; 0.0627mmol) was dissolved in MeOH (10mL) and 1 equivalent 0.01N NaOH (6.27mL) was added. The clear yellow solution was concentrated to remove MeOH and diluted with water, frozen and lyophilized to give the product (Na salt) as a yellow amorphous solid (50.8mg; theoretical yield : 50.8mg; MW Na salt : 809.75)

25

¹H NMR (400 MHz, DMSO-d₆): ca, 8:1 mixture of rotamers ; δ 8.20 (bs, 1H) , 7.96 (d, J = 8.8 Hz, 1H) , 7.86 (d, J = 5.7 Hz, 1H), 7.51-7.47 (m, 2H), 7.26 (s, 1H), 6.97 (d, J = 8.6 Hz, 1H), 6.19-6.02 (m, 1H), 5.33 (bs, 1H), 4.99 (d, J = 16.8 Hz, 1H), 4.77 (d, J = 10.0 Hz, 1H), 4.52-4.41 (m, 2H), 4.34 (d, J = 11.0 Hz, 1H), , 4.04-3.96

(m, 2H), 3.89 (s, 3H), 3.79-3.68 (m, 1H), 3.68-3.15 (under water peak, 2H), 2.45-2.36 (m, 1H), 2.05-1.92 (m, 2H), 1.82-1.35 (m, 16H), 1.35-1.12 (m, 2H), 0.91 & 0.84 (2x s, 9H).

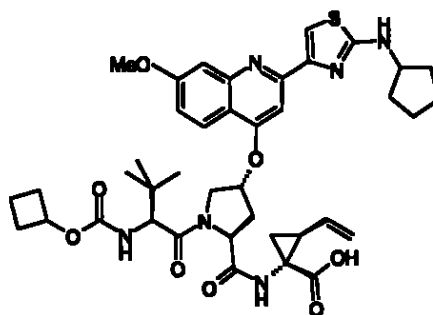
M.S.(electrospray) : 786.4 (M-H)⁻ 788.3 (M+H)⁺ 810 (M+Na)⁺ . Reverse Phase

5 HPLC Homogeneity (0.06% TFA ; CH₃CN : H₂O) : 99%.

EXAMPLE 6

Compound 104:

Using the same procedure as described in example 6 and using the cyclobutyl carbamate of *tert*-butyl glycine in step 5 Instead of urea 5 and purifying the crude carboxylic acid after step 6 by preparative HPLC afforded the title compound as a TFA salt:



Compound 104

¹H NMR (400 MHz,DMSO-d₆): ca, 7:1 mixture of rotamers ;δ 8.09 (bs, 1H) , 8.02 (d, J = 9.0Hz, 1H) , 7.88 (d, J = 6.5 Hz, 1H), 7.45 (s, 1H), 7.40 (s, 1H), 7.28 (d, J = 2.3 Hz, 1H), 7.16-7.08 (m, 1H), 7.07-7.00 (m, 1H), 6.10-5.95 (m, 1H), 5.32 (bs, 1H), 5.00 (d, J = 17.2 Hz, 1H), 4.82 (d, J = 11.4 Hz, 1H), 4.64-4.52 (m, 1H), 4.48-4.41 (m, 1H), 4.29 (d, J = 11.7 Hz, 1H), , 4.12 (d, J = 8.6 Hz, 1H), 3.97-3.85 (m, 2H), 3.91 (s, 3H), 2.36-2.27 (m, 1H), 2.18-2.04 (m, 2H), 2.03-1.81 (m, 6H), 1.77-1.43 (m, 9H), 1.41-1.34 (m, 1H), 0.96 & 0.85 (2x s, 9H).

M.S.(electrospray) : 773.3 (M-H)⁻ 775.4 (M+H)⁺ . Reverse Phase HPLC

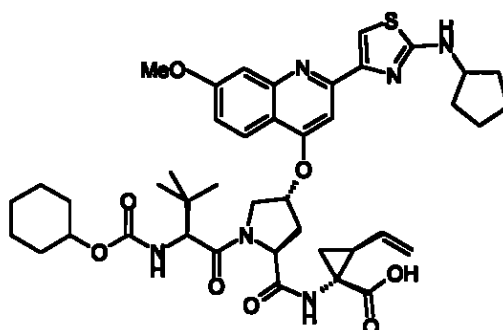
Homogeneity (0.06% TFA; CH₃CN : H₂O) : 99%.

EXAMPLE 7

Compound 105:

Using the same procedure as described in example 6 and using the cyclohexyl carbamate of *tert*-butyl glycine in step 5 Instead of urea 5 afforded the title

compound as the sodium salt:

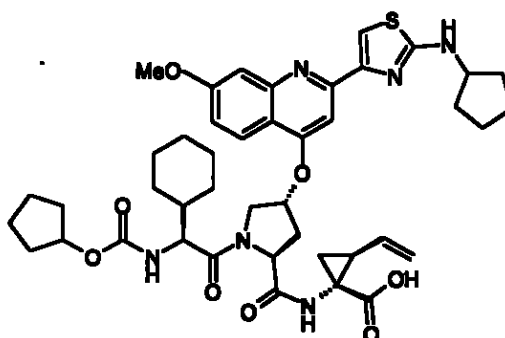


Compound 105

- ¹H NMR (400 MHz,DMSO-d₆): ca, 5:1 mixture of rotamers; δ 8.29 (bs, 1H) , 8.02 (d, J = 9.0Hz, 1H) , 7.89 (d, J = 6.5 Hz, 1H), 7.45 (s, 1H), 7.40 (s, 1H), 7.27 (d, J = 2.2 Hz, 1H), 7.05 (d, J = 8.6 Hz, 1H), 7.00 (dd, J = 2.0 , 9.0 Hz, 1H), 5.89 (bs, 1H), 5.34 (s, 1H), 5.08 (d, J = 17.0 Hz, 1H), 4.91 (d, J = 9.4 Hz, 1H), 4.43 (dd, J = 8.4 , 16.8 Hz, 1H), 4.36-4.24 (m, 1H), 4.14 (d, J = 8.6 Hz, 1H), , 4.00-3.86 (m, 3H), 3.89 (s, 3H), 2.35-2.23 (m, 1H), 2.04-1.91 (m, 5H), 1.79-1.41 (m, 10H), 1.39-1.08 (m, 7H), 0.97 & 0.86 (2x s, 9H).
- 10 M.S.(electrospray) : 801.4 (M-H)⁻ 803.4 (M+H)⁺ . Reverse Phase HPLC Homogeneity (0.06% TFA; CH₃CN : H₂O) : 98%.

EXAMPLE 8

Compound 106:



- 15 Using the same procedure as described in example 6 and using the cyclopentyl carbamate of cyclohexyl glycine in step 5 instead of urea 5 afforded the title compound as the sodium salt:
- ¹H NMR (400 MHz,DMSO-d₆): ca, 1 : 4 mixture of rotamers ;δ 8.22 & 8.04 (2xs, 1H) , 8.00 (d, J = 9.2Hz, 1H) , 7.91 (d, J = 6.5 Hz, 1H), 7.46 (s, 1H), 7.41 (s, 1H), 7.27
- 20

(d, J = 2.4 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.01 (dd, J = 2.4, 9.0 Hz, 1H), 6.09-5.98 (m, 1H), 5.34 (s, 1H), 4.98 (dd, J = 1.6, 17.4 Hz, 1H), 4.80 (d, J = 11.9 Hz, 1H), 4.73-4.67 (m, 1H), 4.43-4.31 (m, 2H), 4.05-3.95 (m, 2H), 3.95-3.84 (m, 1H), 3.90 (s, 3H), 2.38-2.29 (m, 1H), 2.03-1.92 (m, 2H), 1.83-1.21 (m, 24H), 1.21-0.83 (m, 5H)

M.S.(electrospray) : 813.4 (M-H)⁻ 815.4 (M+H)⁺ . Reverse Phase HPLC
Homogeneity (0.06% TFA; CH₃CN : H₂O) : 99%.

EXAMPLE 9

10 Compound 107:

Using the same procedure as described in example 6 and using the cyclopentyl carbamate of cyclopentyl glycine instead of urea 5 in step 5, afforded the title compound which was converted to its corresponding Na salt.

15 EXAMPLE 10

NS3-NS4A protease assay

The enzymatic assay used to evaluate the present compound is described in WO 00/09543 and WO 00/59929.

20 EXAMPLE 11

Cell Based HCV RNA Replication Assay

Cell Culture

Huh7 cells that stably maintain a subgenomic HCV replicon were established as previously described (Lohman et al., 1999. Science 285: 110-113) and designated as the S22.3 cell-line. S22.3 cells are maintained in Dulbecco's Modified Earle Medium (DMEM) supplemented with 10% FBS and 1mg/mL neomycin (Standard Medium). During the assay, DMEM medium supplemented with 10% FBS, containing 0.5% DMSO and lacking neomycin was used (Assay Medium). 16 hours prior to compound addition, S22.3 cells are trypsinized and diluted to 50 000 cells/ml

25 In Standard Medium. 200µL (10 000 cells) are distributed into each well of a 96-well plate. The plate was then incubated at 37° with 5% CO₂ until the next day.

30

Reagents and Materials:

Product	Company	Catalog #	Storage
DMEM	Wisent Inc.	10013CV	4°C
DMSO	Sigma	D-2650	RT
Dulbecco's PBS	Gibco-BRL	14190-136	RT
Fetal Bovine Serum	Bio-Whittaker	14-901F	-20°C/4°C
Neomycin (G418)	Gibco-BRL	10131-027	-20°C/4°C
Trypsin-EDTA	Gibco-BRL	25300-054	-20°C/4°C
96-well plates	Costar	3997	RT
PVDF 0.22µm Filter Unit	Millipore	SLGV025LS	RT
Deep-Well Titer Plate Polypropylene	Beckman	267007	RT

Preparation of Test Compound

10µL of test compound (In 100% DMSO) was added to 2 ml of Assay Medium for a
5 final DMSO concentration of 0.5% and the solution was sonicated for 15 min and
filtered through a 0.22µm Millipore Filter Unit. 900µl was transferred into row A of a
Polypropylene Deep-Well Titer Plate. Rows B to H, contain 400µL aliquots of Assay
Medium (containing 0.5% DMSO), and are used to prepare serial dilutions (1/2) by
transferring 400µl from row to row (no compound was included in row H).

10

Application of test compound to cells

Cell culture medium was aspirated from the 96-well plate containing the S22.3 cells.
175µL of assay medium with the appropriate dilution of test compound was
transferred from each well of the compound plate to the corresponding well of the
15 cell culture plate (row H was used as the "No inhibition control"). The cell culture
plate was incubated at 37° with 5% CO₂ for 72h.

Extraction of Total Cellular RNA

Following the 72h incubation period, the total cellular RNA was extracted from the
20 S22.3 cells of the 96-well plate using the RNeasy 96 kit (Qiagen®, RNeasy
Handbook, 1999.). Briefly, assay medium was completely removed from cells and
100 µL of RLT buffer (Qiagen®) containing 143 mM β-mercaptoethanol was added

to each well of the 96-well cell-culture plate. The microplate was gently shaken for 20 sec. 100 μ L of 70% ethanol was then added to each microplate well, and mixed by pipetting. The lysate was removed and applied to the wells of a RNeasy 96 (Qiagen®) plate that was placed on top of a Qiagen® Square-Well Block. The

5 RNeasy 96 plate was sealed with tape and the Square-Well Block with the RNeasy 96 plate was loaded into the holder and placed in a rotor bucket of a 4K15C centrifuge. The sample was centrifuged at 6000 rpm ($\sim 5600 \times g$) for 4 min at room temperature. The tape was removed from the plate and 0.8 ml of Buffer RW1 (Qiagen® RNeasy 96 kit) was added to each well of the RNeasy 96 plate. The

10 RNeasy 96 plate was sealed with a new piece of tape and centrifuged at 6000 rpm for 4 min at room temperature. The RNeasy 96 plate was placed on top of another clean Square-Well Block, the tape removed and 0.8 ml of Buffer RPE (Qiagen® RNeasy 96 kit) was added to each well of the RNeasy 96 plate. The RNeasy 96 plate was sealed with a new piece of tape and centrifuged at 6000 rpm for 4 min at

15 room temperature. The tape was removed and another 0.8 ml of Buffer RPE (Qiagen® RNeasy 96 kit) was added to each well of the RNeasy 96 plate. The RNeasy 96 plate was sealed with a new piece of tape and centrifuged at 6000 rpm for 10 min at room temperature. Tape was removed, the RNeasy 96 plate was placed on top of a rack containing 1.2-mL collection microtubes. The RNA was

20 eluted by adding 50 μ L of RNase-free water to each well, sealing plate with a new piece of tape and incubated for 1 min at room temperature. The plate was then centrifuged at 6000 rpm for 4 min at room temperature. The elution step was repeated with a second volume of 50 μ L RNase-free water. The microtubes with total cellular RNA are stored at -70° .

25

Quantification of Total Cellular RNA

RNA was quantified on the STORM® system (Molecular Dynamics®) using the RiboGreen® RNA Quantification Kit (Molecular Probes®). Briefly, the RiboGreen reagent was diluted 200-fold in TE (10mM Tris-HCl pH =7.5, 1mM EDTA).

30 Generally, 50 μ L of reagent was diluted in 10mL TE. A Standard Curve of ribosomal RNA was diluted in TE to 2 μ g/mL and pre-determined amounts (100, 50, 40, 20, 10, 5, 2 and 0 μ L) of the ribosomal RNA solution are then transferred in a new 96-well plate (COSTAR # 3997) and the volume was completed to 100 μ L with TE. Generally, column 1 of the 96-well plate was used for the standard curve and the

other wells are used for the RNA samples to be quantified. 10µL of each RNA sample that was to be quantified, was transferred to the corresponding well of the 96-well plate and 90µL of TE was added. One volume (100µL) of diluted RiboGreen reagent was added to each well of the 96-well plate and incubated for 2 to 5 minutes at room temperature, protected from light (a 10 µL RNA sample in a 200 uL final volume generates a 20 X dilution). The fluorescence intensity of each well was measured on the STORM® system (Molecular Dynamics®). A standard curve was created on the basis of the known quantities of the ribosomal RNA and the resulting fluorescent intensities. The RNA concentration in the experimental samples was determined from the standard curve and corrected for the 20X dilution.

Reagents and Materials:

Product	Company	Catalog #	Storage
DEPC	Sigma	D5758	4°C
EDTA	Sigma	E5134	RT
Trizma-Base	Sigma	T8524	RT
Trizma-HCl	Sigma	T7149	RT
Collection Tube Strips	Qiagen	19582	RT
Ribogreen RNA Quantitation Kit	Molecular Probe	R11490	-20°C
Rneasy 96 Kit	Qiagen	74183	RT
Square-Well Blocks	Qiagen	19573	RT

Real-Time RT-PCR

The Real-Time RT-PCR was performed on the ABI Prism 7700 Sequence Detection System using the TaqMan EZ RT-PCR Kit from (Perkin-Elmer Applied Biosystems®). RT-PCR was optimized for the quantification of the 5' IRES of HCV RNA by using the Taqman technology (Roche Molecular Diagnostics Systems) similar to the technique previously described (Martell et al., 1999. J. Clin. Microbiol. 37: 327-332). The system exploits the 5'-3' nucleolytic activity of AmpliTaq DNA polymerase. Briefly, the method utilizes a dual-labeled fluorogenic hybridization probe (PUTR Probe) that specifically anneals to the template between the PCR primers (primers 8125 and 7028). The 5' end of the probe contains a fluorescent reporter (6-carboxyfluorescein [FAM]) and the 3' end contains a fluorescent

quencher (6-carboxytetramethylrhodamine [TAMRA]). The FAM reporter's emission spectrum was suppressed by the quencher on the intact hybridization probe. Nuclease degradation of the hybridization probe releases the reporter, resulting in an increase in fluorescence emission. The ABI Prism 7700 sequence detector

5 measures the increase in fluorescence emission continuously during the PCR amplification such that the amplified product was directly proportion to the signal. The amplification plot was analysed early in the reaction at a point that represents the logarithmic phase of product accumulation. A point representing a defined detection threshold of the increase in the fluorescent signal associated with the

10 exponential growth of the PCR product for the sequence detector was defined as the cycle threshold (C_T). C_T values are inversely proportional to the quantity of input HCV RNA; such that under identical PCR conditions, the larger the starting concentration of HCV RNA, the lower the C_T . A standard curve was created automatically by the ABI Prism 7700 detection system by plotting the C_T against

15 each standard dilution of known HCV RNA concentration.

Reference samples for the standard curve are included on each RT-PCR plate. HCV Replicon RNA was synthesized (by T7 transcription) *in vitro*, purified and quantified by OD₂₆₀. Considering that 1µg of this RNA = 2.15×10^{11} RNA copies, dilutions are

20 made in order to have 10^8 , 10^7 , 10^6 , 10^5 , 10^4 , 10^3 or 10^2 genomic RNA copies / 5µL. Total cellular Huh-7 RNA was also incorporated with each dilution (50ng / 5µL). 5µL of each reference standard (HCV Replicon + Huh-7 RNA) was combined with 45µL of Reagent Mix, and used in the Real-Time RT-PCR reaction.

25 The Real-Time RT-PCR reaction was set-up for the experimental samples that were purified on RNeasy 96 –well plates by combining 5µl of each total cellular RNA sample with 45µL of Reagent Mix.

Reagents and Materials:

Product	COMPANY	Catalog #	Storage
TaqMan EZ RT-PCR Kit	PE Applied Biosystems	N808-0236	-20°C
MicroAmp Optical Caps	PE Applied Biosystems	N801-0935	RT
MicroAmp Optical 96-Well Reaction Plate	PE Applied Biosystems	N801-0560	RT

Reagent Mbx preparation:

Component	Volume for one sample (μL)	Volume for One Plate (μL) (91 samples + Dead Volume)	Final conc.
Rnase-free water	16.5	1617	
5X TaqMan EZ buffer	10	980	1X
Mn(OAc) ₂ (25mM)	6	588	3mM
dATP (10mM)	1.5	147	300μM
dCTP (10mM)	1.5	147	300μM
dGTP (10mM)	1.5	147	300μM
dUTP (20mM)	1.5	147	600μM
Forward Primer (10μM)	1	98	200nM
Reverse Primer (10μM)	1	98	200nM
PUTR probe (5μM)	2	196	200nM
rTth DNA polymerase (2.5 U/μL)	2	196	0.1 U/μL
AmpErase UNG (1U/μL)	0.5	49	0.01 U/μL
Total Volume	45	4410	

Forward Primer Sequence (SEQ ID. 1): 5' - ACG CAG AAA GCG TCT AGC CAT GGC GTT AGT - 3'

5

Reverse Primer Sequence (SEQ ID NO. 2): 5' - TCC CGG GGC ACT CGC AAG CAC CCT ATC AGG - 3'

10 Note: Those primers amplify a region of 256-nt present within the 5' untranslated region of HCV.

PUTR Probe Sequence (SEQ ID NO. 3): **6FAM** - TGG TCT GCG GAA CCG GTG AGT ACA CC - **TAMRA**

15

No Template Controls (NTC): On each plate, 4 wells are used as "NTC". For these controls, 5µl of water are added to the well in place of RNA.

Thermal Cycling Conditions:

- 5

50°C 2 min

60°C 30 min

95°C 5 min

95°C 15 sec

60°C 1 min

} for 2 cycles
- 10

90°C 15 sec

60°C 1 min

} for 40 cycles

Following the termination of the RT-PCR reaction the data analysis requires setting of threshold fluorescence signal for the PCR plate and a standard curve was constructed by plotting the Ct value versus RNA copy number used in each reference reaction. The Ct values obtained for the assay samples are used to interpolate an RNA copy number based on the standard curve.

Finally, the RNA copy number was normalized (based on the RiboGreen RNA quantification of the total RNA extracted from the cell culture well) and expressed as genome equivalents / µg of total RNA [ge/µg].

The RNA copy number [g.e./µg] from each well of the cell culture plate was a measure of the amount of replicating HCV RNA in the presence of various concentrations of inhibitor. The % inhibition was calculated with the following equation:

$$100 - [(g.e./\mu g \text{ inh})/(g.e./\mu g \text{ ctrl}) \times 100].$$

A non-linear curve fit with the Hill model was applied to the Inhibition-concentration data, and the 50% effective concentration (EC₅₀) was calculated by the use of SAS software (Statistical Software System; SAS Institute, Inc. Cary, N.C.).

When the compounds of this invention were evaluated in the preceding enzymatic and cell based assays, the compounds were found to be highly active. More

55

specifically, the compounds had IC_{50} 's below 0.1 μ M in the NS3-NS4A protease assay, and EC_{50} 's below 0.5 μ M in the cell based HCV RNA replication assay.

EXAMPLE 12

5 Specificity assays

The specificity assays used to evaluate the selectivity of this compound are described in WO 00/09543.

When the compounds were evaluated in the specificity assays, the compounds of
10 formula 1 were found to be selective in that they do not show significant inhibition in the Human Leukocyte Elastase and Cathepsin B assays.

EXAMPLE 13

Pharmacokinetic properties

15 The present compounds also show good pharmacokinetic properties such as significant plasma levels in the rat at 1 hour and 2h after an oral dose of 5mg/kg.

More explicitly, the following assay, an *in vivo* oral absorption screen, was used to determine plasma levels of test compounds in a rat after oral administration:

20

Materials and Methods:

1. Method used to pool compounds ("cassette selection"):

The selection of compounds to be pooled into a "cassette" was based on their structural similarity and physicochemical properties. A solid phase extraction
25 method applicable to all the selected compounds was established. Based on the initial testing where each compound was spiked into rat plasma and run through HPLC or HPLC/MS at a concentration of 0.5 μ M, the retention time, ionic mass, and the possible separation among compounds by HPLC and/or HPLC/MS were used as basis for pooling 3-4 compounds into one "cassette".

30 2. Oral vehicle and compound preparation:

Each "cassette" contains 3-4 compounds at 5 or 4mg/kg for each compound. The cassettes were prepared as an oral suspension in 0.5% aqueous methylcellulose and 0.3% of polyoxyethylene (20) sorbiton monooleate (Tween-80). The dosing volume was 10ml/kg via oral gavage.

3. Dosing and plasma sampling:

Male Sprague Dawley rats were fasted overnight in individual cages, with access to aqueous 10% dextrose. Two rats were dosed with each "cassette". Plasma samples (~1 ml) were collected at 1 and 2h post-dosing from the 2 rats and pooled for extraction and analysis.

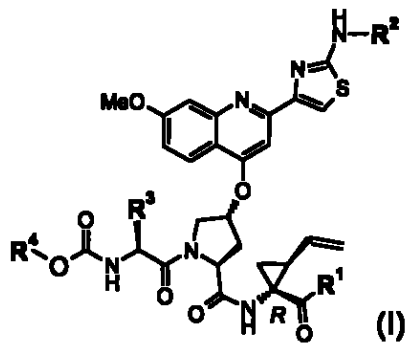
4. Compound extraction and analysis:

From each cassette, plasma samples at 1 and 2h, blank plasma, blank plasma spiked with all the compounds at 0.5 μM of each, are extracted by the solid phase extraction method. Samples were analyzed by HPLC and HPLC/MS for comparison purpose. Plasma concentrations are estimated based on the single concentration of 0.5 μM standard.

When assayed in the preceding screen the compounds of examples 1 to 9 of this invention were found to present in significant levels in the plasma at the 1 hour and 2 hour intervals following oral administration, averaging blood plasma levels of 1.23 μM and 1.16 μM respectively. This demonstration of significant *in vivo* oral absorption for the compounds of this invention is unexpected, in view of the lower oral absorption generally attributed to this class of peptides. The ready oral absorption renders the compounds useful for treating of HCV infection.

The following table lists compounds representative of the invention. Further in keeping with the present disclosure, the compound all had IC_{50} 's below 0.1 μM in the NS3-NS4A protease assay, and EC_{50} 's below 0.5 μM in the cell based HCV RNA replication assay.

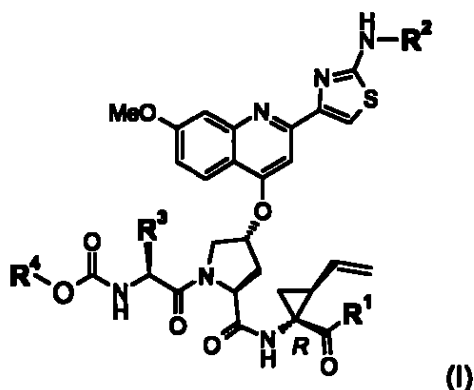
TABLE 1



Cpd No.	R ¹	R ²	R ³	R ⁴	m/z (MH) ⁺
100	OH		tert-Butyl		789.3
101	OH		tert-Butyl		775.4
102	OH		tert-Butyl		803.4
103			tert-Butyl		928.5
104	OH		tert-Butyl		775.4
105	OH		tert-Butyl		803.4
106	OH				815.4
107	OH				801.4

CLAIMS

1. A compound of formula (I):



- wherein R^1 is hydroxy or NHSO_2R^{1A} wherein R^{1A} is (C_{1-6}) alkyl, (C_{3-7}) cycloalkyl or $\{(C_{1-6})$ alkyl- (C_{3-7}) cycloalkyl $\}$, which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, O -($C_{1-6})$ alkyl, amido, amino or phenyl, or R^{1A} is C_6 or C_{10} aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C_{1-6}) alkyl, O -($C_{1-6})$ alkyl, amido, amino or phenyl; R^2 is (C_{4-6}) cycloalkyl; R^3 is *t*-butyl or (C_{5-6}) cycloalkyl and R^4 is (C_{4-6}) cycloalkyl; or a pharmaceutically acceptable salt thereof.
2. The compound of formula I according to claim 1, wherein R^1 is hydroxy, NHSO_2Me , NHSO_2 cyclopropyl or NHSO_2Ph .
 3. The compound of formula I according to claim 2, wherein R^1 is NHSO_2 cyclopropyl or NHSO_2Ph .
 4. The compound of formula I according to claim 2, wherein R^1 is hydroxy.
 5. The compound of formula I according to any one of claims 1 to 4, wherein R^2 is cyclopentyl or cyclohexyl.
 6. The compound of formula I according to claim 5, wherein R^2 is cyclopentyl.
 7. The compound of formula I according to any one of claims 1 to 6, wherein R^3 is *t*-butyl or cyclohexyl.
 8. The compound of formula I according to claim 7, wherein R^3 is *t*-butyl.
 9. The compound of formula I according to any one of claims 1 to 8, wherein R^4 is cyclobutyl or cyclopentyl.
 10. The compound of formula I according to claim 9, wherein R^4 is cyclopentyl.
 11. The compound of formula I according to claim 1, wherein R^1 is hydroxy, R^2 and R^4 each is cyclopentyl and R^3 is *t*-butyl.
 12. The compound of formula I according to claim 1, wherein R^1 is hydroxy, R^2 is cyclobutyl, R^3 is *t*-butyl and R^4 is cyclopentyl.

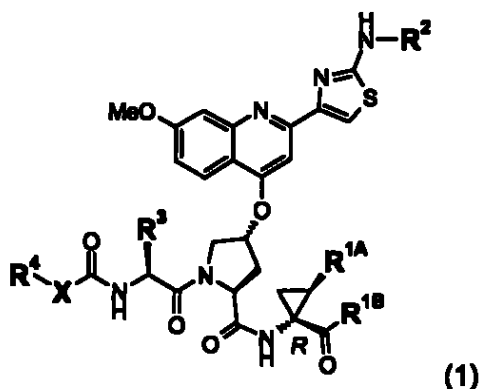
13. The compound of formula I according to claim 1, wherein R¹ is hydroxy, R² is cyclohexyl, R³ is t-butyl and R⁴ is cyclopentyl.
14. The compound of formula I according to claim 1, wherein R¹ is NHSO₂Ph, R² and R⁴ each is cyclopentyl and R³ is t-butyl.
15. The compound of formula I according to claim 1, wherein R¹ is hydroxy, R² is cyclopentyl, R³ is t-butyl and R⁴ is cyclobutyl.
16. The compound of formula I according to claim 1, wherein R¹ is hydroxy, R² is cyclopentyl, R³ is t-butyl and R⁴ is cyclohexyl.
17. The compound of formula I according to claim 1, wherein R¹ is hydroxy, R² and R⁴ each is cyclopentyl and R³ is cyclohexyl.
18. The compound of formula I according to claim 1, wherein R¹ is hydroxy, R², R³ and R⁴ each is cyclopentyl.
19. A pharmaceutical composition comprising an anti-hepatitis C virally effective amount of a compound of formula 1 according to any one of claim 1 to 18 or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.
20. The pharmaceutical composition according to claim 19, further comprising a therapeutically effective amount of one or more other anti-HCV agent.
21. The pharmaceutical composition according to claim 20, wherein said other anti-HCV agent is selected from: α -interferon or pegylated α -interferon.
22. The pharmaceutical composition according to claim 20 or 21, wherein said other anti-HCV agent is ribavirin.
23. The pharmaceutical composition according to claim 20, 21 or 22, wherein said other anti-HCV agent is selected from inhibitors of: helicase, NS2/3 protease and internal ribosome entry site (IRES).
24. A method for the treatment or prevention of a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of a compound of formula I according to any one of claims 1 to 18, or a pharmaceutically acceptable salt thereof.
25. A method for the treatment or prevention of a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of the composition according to any one of claims 19 to 23.
26. A method for the treatment or prevention of a hepatitis C viral infection in a mammal by administering thereto an anti-hepatitis C virally effective amount of a compound

of formula I according to any one of claims 1 to 18, or a therapeutically acceptable salt thereof in combination with one or more other anti-HCV agent.

27. The method according to claim 26, wherein said other anti-HCV agent is selected from: α -Interferon or pegylated α -Interferon.
28. The method according to claim 26 or 27, wherein said other anti-HCV agent is ribavirin.
29. The method according to claim 26, 27 or 28, wherein said other anti-HCV agent is selected from inhibitors of: helicase, NS2/3 protease and internal ribosome entry site (IRES).
30. Use of a compound of formula I according to any one of claims 1 to 18 for the manufacture of a medicament for the treatment or prevention of hepatitis C viral infection.
31. The pharmaceutical composition according to claim 20, 21 or 22, wherein said other anti-HCV agent is an inhibitor of HCV polymerase.
32. The method according to claim 26, 27 or 28, wherein said other anti-HCV agent is an inhibitor of HCV polymerase.

ABSTRACT

Disclosed herein are compounds of formula (1):



5

wherein R¹ is hydroxy or NHSO₂R^{1A} wherein R^{1A} is (C₁₋₈)alkyl, (C₃₋₇)cycloalkyl or {(C₁₋₈)alkyl-(C₃₋₇)cycloalkyl}, which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, O-(C₁₋₈)alkyl, amido, amino or phenyl, or R^{1A} is C₆ or C₁₀ aryl
 10 which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C₁₋₈)alkyl, O-(C₁₋₈) alkyl, amido, amino or phenyl; R² is (C₄₋₈)cycloalkyl; R³ is t-butyl or (C₅₋₈) cycloalkyl and R⁴ is (C₄₋₈)cycloalkyl; or a pharmaceutically acceptable salt thereof.
 The compounds are useful as inhibitors of HCV NS3 protease.

15

SEQUENCE LISTING

<110> BOEHRINGER INGELHEIM INTERNATIONAL GmbH

<120> HEPATITIS C INHIBITOR TRI-PEPTIDES

<130> 13/106

<150> 2,370,396

<151> 2002-08-01

<160> 3

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 30

<212> DNA

<213> Artifical Sequence

<220>

<223> Forward primier

<400> 1

acgcagaaag cgtctagcca tggcgttagt

30

<210> 2

<211> 30

<212> DNA

<213> Artifical Sequence

<220>

<223> Reverse Primer

<400> 2

tcccggggca ctgcgaagca ccctatcagg

30

<210> 3

<211> 26

<212> DNA

<213> Artifical Sequence

<220>

<223> PUTR probe

<400> 3

tgggtctgagg aaccggtgag tacacc

26

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To: BERNIER, Louise G. Boehringer Ingelheim (Canada) Ltd. 2100 Cunard Street Laval, Québec H7S 2G5 Canada		PCT NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE (PCT Rule 20.5(c))	
		Date of mailing (day/month/year)	31 January 2003 (31.01.2003)
Applicant's or agent's file reference 13/106pct		IMPORTANT NOTIFICATION	
International application No. PCT/CA03/00029	International filing date (day/month/year) 14 January 2003 (14.01.2003)	Priority date (day/month/year) 01 February 2002 (01.02.2002)	
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GmbH ET AL			
Title of the invention HEPATITIS C INHIBITOR TRI-PEPTIDES			
1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above. 2. The applicant is further notified that the record copy of the international application: ☒ was transmitted to the International Bureau on <u>31 January 2003 (31.01.2003)</u>. ☐ has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*: ☐ because the necessary national security clearance has not yet been obtained. ☐ because (reason to be specified): * The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).			
Name and mailing address of the Receiving Office Commissioner of Patents Canadian Receiving Office Box PCT, Ottawa/Hull K1A 0C9 Facsimile No. (819) 953-9538		Authorized Officer Carole Millaire Telephone no. (819) 994-6587	

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:
BERNIER, Louise G.
Boehringer Ingelheim (Canada) Ltd.
2100 Cunard Street
Laval, Québec
H7S 2G5 Canada

PCT**NOTIFICATION CONCERNING PAYMENT
OF PRESCRIBED FEES**

(PCT Rules 14, 15 and 16 and Administrative
 Instructions, Sections 102bis(c), 304(a) and (b),
 323(b), 707(b) and 803)

Date of mailing (day/month/year) **31 January 2003 (31.01.2003)**

Applicant's or agent's file reference
13/106pct

PAYMENT DUE

See item 3 for time limits

International Application No.
PCT/CA03/00029

International filing date/Date of receipt
 (day/month/year)
14 January 2003 (14.01.2003)

Priority date (day/month/year)
**01 February 2002
 (01.02.2002)**

Applicant **BOEHRINGER INGELHEIM INTERNATIONAL GmbH ET AL**

1. The applicant is hereby notified that this receiving Office has received:

☐ the payment of all the prescribed fees, and ☒ an overpayment, which will be refunded in due course.

☐ no or insufficient payment of the prescribed fees and the applicant is hereby invited to pay the balance due, as summarized under item 2, within the time limit(s) indicated under item 3.
2. Fees and payment calculation:

<u>\$200.00</u>	-	<u>\$3.067.00</u>	=	<u>\$ 2.867.00</u>
Total fees payable		Amount paid		Balance to be refunded.

☐ The details of the calculation are given in the Annex.
3. Time limit(s) for payment and amount(s) payable (Rules 14.1, 15.4 and 16.1(f)):

☐ within ONE MONTH from the date of receipt of the international application (for the transmittal fee (if any), the search fee, the basic fee and the designation fee). The amount payable for each fee is the amount applicable on the date of receipt of the international application.

☐ within ONE YEAR from the priority date (only for the designation fee and only if this time limit expires later than the above time limit).

 - If the designation fee is paid within one month from the date of receipt of the international application, the amount payable is the amount applicable on that date of receipt.
 - If the designation fee is paid within one year from the priority date but later than one month from the date of receipt of the international application, the amount payable is the amount applicable on the date of payment. The receiving Office should be consulted for the applicable amount.

☐ within 16 MONTHS from the priority date (only for the fee for priority document). The applicant's attention is drawn to the fact the request made by the applicant under Rule 17.1(b) will be considered not to have been made unless the fee is paid within that time limit.
4. Additional observations (if necessary):

☒ The search copy will not be transmitted to the International Searching Authority until the search fee is paid (therefore the start of the international search will be delayed) (Rule 23.1(a) and (b)).

Name and mailing address of the receiving Office
 Commissioner of Patents
 Canadian Receiving Office
 Box PCT, Ottawa/Hull K1A 0C9
 Facsimile No. (819) 953-9538

Authorized Officer

Carole Millaire
 Telephone no. (819) 994-6587

PATENT COOPERATION TREATY

65

From the RECEIVING OFFICE

To: BERNIER, Louise G. Boehringer Ingelheim (Canada) Ltd. 2100 Cunard Street Laval, Québec H7S 2G5 Canada	PCT COMMUNICATION IN CASES FOR WHICH NO OTHER FORM IS APPLICABLE
	Date of mailing 31 January 2003 (31.01.2003) (day/month/year)

Applicant's or agent's file reference 13/106pct	REPLY DUE See paragraph 1 below
International Application No. PCT/CA03/00029	International filing date (day/month/year) 14 January 2003 (14.01.2003)
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GmbH ET AL	

1. ☐ **REPLY DUE** within ____ months/days from the above date of mailing

☐ **NO REPLY DUE**, however, see below _____

☒ **IMPORTANT COMMUNICATION**

☐ **INFORMATION ONLY**

2. **COMMUNICATION:**

RE: PRIORITY DOCUMENT CA (2,370,396) 01-02-2002

PLEASE BE ADVISED THAT THE PRIORITY DOCUMENT IN THE ABOVE INTERNATIONAL APPLICATION HAS NOT YET BEEN RECEIVED.

THE DOCUMENT SHOULD BE SENT TO THE WORLD INTELLECTUAL PROPERTY ORGANIZATION (WIPO), PCT DIVISION, 34, CHEMIN DES COLOMBETTES, 1211 GENEVA 20, SWITZERLAND BEFORE THE END OF THE 16th MONTH FROM THE PRIORITY DATE. WITHIN THE SAME TIME LIMIT, THE DOCUMENT CAN ALSO BE SENT TO THIS OFFICE FOR TRANSMITTAL TO WIPO.

IF FURTHER INFORMATION IS REQUIRED, PLEASE FEEL FREE TO COMMUNICATE WITH THIS OFFICE.

Name and mailing address of the Receiving Office Commissioner of Patents Canadian Receiving Office Box PCT, Ottawa/Hull K1A 0C9 Facsimile No. (819) 953-9538	Authorized Officer Carole Millaire Telephone no. (819) 994-6587
---	---

66

PCT REQUEST

13/106pct

Original (for SUBMISSION) - printed on 19.12.2002 11:44:25 AM

0	For receiving Office use only	
0-1	International Application No.	PCT/CA 03 / 00029
0-2	International Filing Date	14 JANUARY 2003 14.01.03
0-3	Name of receiving Office and "PCT International Application"	EO/CA
0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.92 (updated 01.06.2002)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	Canadian Patent Office (RO/CA)
0-7	Applicant's or agent's file reference	13/106pct
I	Title of invention	HEPATITIS C INHIBITOR TRI-PEPTIDES
II	Applicant	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	BOEHRINGER INGELHEIM INTERNATIONAL GmbH
II-5	Address:	Binger Strasse 173 D- 55216 Ingelheim Germany
II-6	State of nationality	DE
II-7	State of residence	DE
II-8	Telephone No.	011 49 6132 77 2326
II-9	Facsimile No.	011 49 6132 77 3000
III-1	Applicant and/or inventor	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	LLINAS-BRUNET, Montse
III-1-5	Address:	2100 Cunard Street Laval, Québec H7S 2G5 Canada
III-1-6	State of nationality	CA
III-1-7	State of residence	CA

PCT REQUEST

19/108pct

Original (for SUBMISSION) - printed on 19.12.2002 11:44:28 AM

II-2	Applicant and/or inventor	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	GORYS, Vida, J.
III-2-5	Address:	2100 Cunard Street Laval, Québec H7S 2G5 Canada
III-2-6	State of nationality	CA
III-2-7	State of residence	CA
IV-1	Agent or common representative; or address for correspondence The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	BERNIER, Louise, G.
IV-1-2	Address:	Boehringer Ingelheim (Canada) Ltd. 2100 Cunard Street Laval, Québec H7S 2G5 Canada
IV-1-3	Telephone No.	(450) 682-4640
IV-1-4	Facsimile No.	(450) 682-8434
IV-1-5	e-mail	lbernier@lav.boehringer-ingelheim.ca
V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW ME SD SL SZ TE UG ZM ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KE MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE BG CHALI CY CZ DE DK EE ES FI FR GB GR IE IT LU MC NL PT SE SK TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BE CA CHALI 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 KE LC LK LR LS LT LU LV MA MD MG MK MN MW MX ME NO NZ OM PH PL PT RO RU SD SE SG SK SL TJ TM TN TR TT TE UA UG US UZ VC VN YU ZA ZM ZW

AFRICA

CANCELLED
BY RO/CA

PCT REQUEST

13/100pct

Original (for SUBMISSION) - printed on 18.12.2002 11:44:25 AM

V-5	Precautionary Designation Statement In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-8 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 18 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	Exclusion(s) from precautionary designations	NONE	
VI-1	Priority claim of earlier national application		
VI-1-1	Filing date	01 February 2002 (01.02.2002)	
VI-1-2	Number	2,370,396	
VI-1-3	Country	CA	
VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)	
VIII	Declarations	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	
IX	Check list	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	4	-
IX-2	Description (excluding sequence listing part)	46	-
IX-3	Claims	3	-
IX-4	Abstract	1	EZABST00.TXT
IX-5	Drawings	0	-
IX-7a	Sub-total number of sheets	54	
IX-8	Sequence listing part of description	1	-
IX-7	TOTAL	55	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-13	Priority document(s)	Item(s) VI-1, 44	-
IX-16	Sequence listing in computer readable form:		
IX-16 (II)	additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter	-	1 Diskette
IX-17	PCT-EASY diskette	-	Diskette

4 DELETED ROICA

4 ROICA

A

69

4/4

PCT REQUEST

13/106pct

Original (for SUBMISSION) - printed on 19.12.2002 11:44:25 AM

IX-19	Figure of the drawings which should accompany the abstract	_____
IX-20	Language of filing of the international application	English
X-1	Signature of applicant, agent or common representative	<i>Louise G. B.</i>
X-1-1	Name (LAST, First)	BERNIER, Louise, G.

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	14 JANUARY 2003 14.01.03
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	✓
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	✓

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	
------	--	--

70

1/2

PCT (ANNEX - FEE CALCULATION SHEET)

13/106pct

Original (for SUBMISSION) - printed on 19.12.2002 11:44:25 AM

(This sheet is not part of and does not count as a sheet of the international application)

0	For receiving Office use only		
0-1	International Application No.	PCT/CA 03 / 00029	
0-2	Date stamp of the receiving Office	14 JANUARY 2003 14.01.03	
0-4	Form - PCT/RQ/101 (Annex) PCT Fee Calculation Sheet Prepared using	PCT-EASY Version 2.92 (updated 01.06.2002)	
0-9	Applicant's or agent's file reference	13/106pct	
2	Applicant	BOEHRINGER INGELHEIM INTERNATIONAL GmbH, et al.	
12	Calculation of prescribed fees	fee amount/multiplier	Total amounts (CAD)
12-1	Transmittal fee T	⇒	200 4
12-2-1	Search fee S	⇒	1,360 1440
12-2-2	International search to be carried out by	EP	
12-3	International fee		
	Basic fee (first 30 sheets) b1	639	
12-4	Remaining sheets	25	
12-5	Additional amount (X)	15	
12-6	Total additional amount b2	375	
12-7	b1 + b2 = B	1,014	
12-8	Designation fees		
	Number of designations contained in international application	94	
12-9	Number of designation fees payable (maximum 5)	5	
12-10	Amount of designation fee (X)	138	
12-11	Total designation fees D	690	
12-12	PCT-EASY fee reduction R	-197	
12-13	Total international fee (B+D-R) I	⇒	1,507 4
12-17	TOTAL FEES PAYABLE (T+S+I+P)	⇒	2,867 3147
12-18	Mode of payment	authorization to charge deposit account	
12-20	Deposit account instructions The receiving Office:	Canadian Patent Office (RO/CA)	
12-20-1	Authorization to charge the total fees indicated above.	✓	
12-20-2	Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.	✓	
12-20-3	Authorization to charge the fee for priority document.	✓	
12-21	Deposit account No.	cc1127	
12-22	Date	19 December 2002 (19.12.2002)	

RO/CA

AA DELETED RO/CA

PCT (ANNEX - FEE CALCULATION SHEET)

13/108pct

Original (for SUBMISSION) - printed on 19.12.2002 11:44:25 AM

13-23	Name and signature	BERNIER, Louise, G. <i>Louise GB</i>
-------	--------------------	---

VALIDATION LOG AND REMARKS

13-2-3	Validation messages Name	Green? Applicant 1.: Name should preferably be entered in capital letters. Please verify.
		Yellow Applicant 1.:Postal code missing
13-2-7	Validation messages Contents	Yellow! The power of attorney or a copy of the general power of attorney will need to be furnished unless all applicants sign the request form.
		Green? The international application contains no drawings. Please verify.
13-2-8	Validation messages Fees	Green? Please confirm that fee schedule utilized is the latest available
13-2-9	Validation messages Payment	Green? Please ensure that you have a valid deposit account with the receiving Office selected.

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:
**WORLD INTELLECTUAL PROPERTY
 ORGANIZATION - PCT DIVISION**
 34, Chemin des Colombettes
 1211 Geneva 20
 SWITZERLAND

PCT

**NOTIFICATION CONCERNING
 REPRESENTATION**
 (PCT Rule 90 and Administrative
 Instructions, Section 328)

Applicant's or agent's file reference 13/106pct	Date of Mailing (day/month/year) 31 January 2003 (31.01.2003)
International application No. PCT/CA03/00029	International filing date (day/month/year) 14 January 2003 (14.01.2003)
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GmbH ET AL	

1. This receiving Office hereby gives notice of the receipt of a document containing:

☒ a power of attorney **BOEHRINGER INGELHEIM INTERNATIONAL GmbH
ET AL**

☐ a revocation of power of attorney

☐ a renunciation of appointment

2. This notification, together with a copy of the document indicated above, is sent to the addressee in its capacity as:

☐ the International Searching Authority.

☐ the International Bureau, which is requested to record a change in the person of the agent or common representative under Rule 92bis.1(a)(ii).

Name and mailing address of the Receiving Office Commissioner of Patents Canadian Receiving Office Box PCT, Ottawa/Hull K1A 0C9 Facsimile No. (819) 953-9538	Authorized Officer Carole Millaire Telephone no. (819) 994-6587
--	--

PATENT COOPERATION TREATY

Corrected Version / Version Corrigée

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

BERNIER, Louise, G.
Boehringer Ingelheim (Canada) Ltd.
2100 Cunard Street
Laval, Québec H7S 2G5
CANADA

PATENT DEPARTMENT

QC: 5-2003

Date of mailing (day/month/year) 07 August 2003 (07.08.03)		~~BOHPO updated~~ Scanned / sent to GmbH ~~Scanned / sent to BIP (with encl.)~~	
Applicant's or agent's file reference 13/106 pct-2		Agent IMPORTANT NOTICE	
International application No. PCT/CA03/00090	International filing date (day/month/year) 24 January 2003 (24.01.03)	Priority date (day/month/year) 01 February 2002 (01.02.02)	
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH			

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

AU, AZ, BY, CH, CO, DE, DZ, GH, HU, KG, KP, KR, MD, MK, MZ, RU, TM, US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE, AG, AL, AM, AP, AT, BA, BB, BG, BR, BZ, CA, CN, CR, CU, CZ, DK, DM, EA, EC, EE, EP, ES, FI, GB, GD, GE, GM, HR, ID, IL, IN, IS, JP, KE, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MG, MN, MW, MX, NO, NZ, OA, OM, PH, PL, PT, RO, SD, SE, SG, SK, SL, TJ, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Inclosed with this notice is a copy of the international application as published by the International Bureau on 07 August 2003 (07.08.03) under No. 03/064456

4. TIME LIMITS for filing a demand for international preliminary examination and for entry into the national phase

The applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be 30 MONTHS from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of 19 months from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see *PCT Gazette* No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the *PCT Newsletter*, October and November 2001 and February 2002 issues.

In practice, time limits other than the 30-month time limit will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For regular updates on the applicable time limits (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/index.html>.

For filing a demand for international preliminary examination, see the *PCT Applicant's Guide*, Volume IA, Chapter IX. Only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

It is the applicant's sole responsibility to monitor all those time limits.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Judith Zahra

PATENT COOPERATION TREATY

7A

From the RECEIVING OFFICE

To:
BERNIER, Louise G.
Boehringer Ingelheim (Canada) Ltd.
2100 Cunard Street
Laval, Quebec
H7S 2G5 Canada

PCT

INVITATION TO CORRECT DEFECTS IN THE INTERNATIONAL APPLICATION

PCT Articles 3(4)(i) and 14(1) and Rule 26)

Deadline March 7/2003

Date of mailing (day/month/year) 07 February 2003 (07.02.2003)

Applicant's or agent's file reference
13/106 pct-2

REPLY DUE within ONE months/days
from the above date of mailing

International application No.
PCT/CA03/00090

International filing date 24 January 2003 (24.01.2003)
(day/month/year)

Applicant BOEHRINGER INGELHEIM INTERNATIONAL GmbH ET AL

1. ☒ The applicant is hereby invited, within the time limit indicated above, to correct, in the international application as filed, the defects specified on the attached

☒ Annex A

☐ Annex B1 (text matter of the international application as filed)

☐ Annex C1 (drawings of the international application as filed)

2. ☐ The applicant is hereby invited, within the time limit indicated above, to correct, in the translation of the international application furnished under Rule 12.3 or 12.4, the defects specified on the attached:

☐ Annex A

☐ Annex B2 (text matter of the translation of the international application)

☐ Annex C2 (drawings of the translation of the international application)

Additional observations (if necessary):

HOW TO CORRECT THE DEFECTS?

Correction must be submitted by filing a replacement sheet embodying the correction and a letter accompanying the replacement sheet, which shall draw attention to the difference between the replaced sheet and the replacement sheet. A correction may be stated in a letter only if it is of such a nature that it can be transferred from the letter to the record copy without adversely affecting the clarity and direct reproducibility of the sheet onto which the correction is to be transferred (Rule 26.4).

ATTENTION

Failure to correct the defects will result in the international application being considered withdrawn by this receiving Office (see Rule 26.5 for further details).

A copy of this invitation and any attachment has been sent to the International Bureau
☒ and the International Searching Authority.

Name and mailing address of the Receiving Office
Commissioner of Patents
Canadian Receiving Office
Box PCT, Ottawa/Hull K1A 0C9
Facsimile No. (819) 953-9538

Authorized Officer

Carole Millaire
Telephone no. (819) 994-6587

The receiving Office has found the following defects in the international application as filed:

1. As to signature* of the international application (Rules 4.15 and 90.4), the request:

- a. ☐ is not signed.
- b. ☐ is not signed by all the applicants.
- c. ☐ is not accompanied by the statement referred to in the check list in Box No. VIII of the request explaining the lack of the signature of an applicant for the designation of the United States of America.
- d. ☒ is signed by what appears to be an agent/common representative but:
 - ☒ the international application is not accompanied by a power of attorney appointing him. *
 - ☐ the power of attorney accompanying the international application was not signed by all the applicants.
- e. ☐ other (specify):

* All applicants must sign, including inventors if they are also applicants (e.g. where the United States of America is designated).

2. As to indications concerning the applicant, the request (Rules 4.4 and 4.5):

- a. ☐ does not properly indicate the applicant's name (specify):
- b. ☐ does not indicate the applicant's address.
- c. ☐ does not properly indicate the applicant's address (specify):
- d. ☐ does not indicate the applicant's nationality.
- e. ☐ does not indicate the applicant's residence.
- f. ☐ other (specify):

3. As to the language of certain elements of the international application, other than the description and claims (Rules 12.1(c) and 26.3(a) and (c)):

- a. ☐ the request is not in a language of publication accepted by this receiving Office; (the) language(s) accepted by this receiving Office is/are:
- b. ☐ the text matter of the drawings is not in the language in which the international application is to be published, which is:
- c. ☐ the abstract is not in the language in which the international application is to be published, which is:

4. The title of the invention:

- a. ☐ is not indicated in Box No. I of the request (Rule 4.1(a)).
- b. ☐ is not indicated at the top of the first sheet of the description (Rule 5.1(a)).
- c. ☐ as appearing in Box No. I of the request is not identical with the title heading the description (Rule 5.1(a)).

5. As to the abstract (Rule 8):

- ☐ the international application does not contain an abstract.



Boehringer
Ingelheim

Via the Regional Office of Montreal

Commissioner of Patents
Canadian Receiving Office
Box PCT
Ottawa/Hull (Ontario)
K1A 0C9

c/o Carole Millaire

Boehringer Ingelheim
(Canada) Ltd.

Research and Development
Patents

February 13, 2003

**Re.: Invitation to correct defects in the International Application
PCT/CA03/00090 filed January 24, 2003 entitled:
"Hepatitis C Inhibitor Tri-Peptides"
Our case: 13/106 pct2**

Louise G. Bernier
Telephone 450-682-4640
Telefax 450-682-8434
E-Mail lbernier@
lv.boehringer-ingelheim.com

Dear Ms. Millaire:

This is in response to the "*Invitation to Correct Defects in the International Application*" for the above mentioned application for which reply is due by March 7, 2003.

2100 Cunard Street
Laval (Québec)
Canada H7S 2G5
Telephone 450-682-4640
Telefax 450-682-8434
www.boehringer-ingelheim.ca

To correct defect 1(d) mentioned in Annex A to form PCT/RO/106 please find enclosed a duly signed Power of Attorney appointing the agent.

It is believed that the Office is now in a position to process this application and request an acknowledgement of receipt of these documents.

Respectfully submitted,

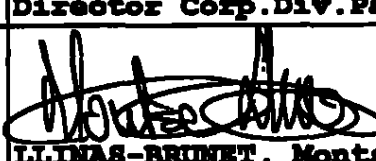
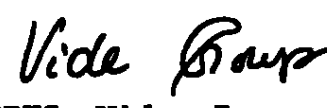
A handwritten signature in cursive script, reading "Louise G. Bernier".

Louise G. Bernier, Ph.D.
Agent for Applicant

Enclosures: Duly signed Power of Attorney

/ra

77

0-1	PCT Power of Attorney (for an international application filed under the Patent Cooperation Treaty) (PCT Rule 90.4)	
0-1-1	Prepared using	PCT-EASY Version 2.92 (updated 01.06.2002)
1	The undersigned applicant(s)	BOEHRINGER INGELHEIM INTERNATIONAL GmbH; LLINAS-BRUNET, Montse; GORYS, Vida, J.
1-1-1	hereby appoints (appoint) the following person	BERNIER, Louise, G. Boehringer Ingelheim (Canada) Ltd. 2100 Cunard Street Laval, Québec H7S 2G5 Canada
1-2	as	agent
1-3	to represent the undersigned before	all the competent International Authorities
1-4	In connection with the international application identified below:	
1-4-1	Title of the invention	HEPATITIS C INHIBITOR TRI-PEPTIDES
1-4-2	Applicant's or agent's file reference	13/106 pct-2
1-4-3	International application number (if already available)	
1-4-4	filed with the following Office as receiving Office	Canadian Patent Office (RO/CA)
1-5	and to make or receive payments on behalf of the undersigned.	
2-1	Signature of applicant	 
2-1-1	Name	BOEHRINGER INGELHEIM INTERNATIONAL GmbH
2-1-2	Name of signatory	Heinz Hammann / Heinz-Gerd Klaes
2-1-3	Capacity	Director Corp.Div.Patents/Head of Patent
2-2	Signature of applicant	
2-2-1	Name	LLINAS-BRUNET, Montse
2-3	Signature of applicant	
2-3-1	Name	GORYS, Vida, J.
3	Date	22 January 2003 (22.01.2003)

78

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20
Switzerland

PCT

NOTIFICATION OF DATE OF RECEIPT
OF PRIORITY DOCUMENT OR OF
PRIORITY APPLICATION NUMBER

(PCT Administrative Instructions,
Section 323(a), (b) and (c))

Applicant's or agent's file reference 88/106 pct-2	Date of mailing (day/month/year) 07 February 2003 (07.02.2003)
International application No. PCT/CA03/00090	International filing date (day/month/year) 24 January 2003 (24.01.2003)
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GmbH ET AL	

1. ☒ This receiving Office hereby gives notice of the receipt of the priority document(s) identified below on:

24 JANUARY 2003 (24.01.2003)

2. ☐ This receiving Office hereby gives notice of the receipt of a request (made under Rule 17.1(b)) to prepare and transmit to the International Bureau the priority document(s) identified below on:

Identification of the priority document(s):

Priority date	Priority application No.	Country or regional Office or PCT receiving Office
01 February 2002 (01.02.2002)	2,370,396	CA

Name and mailing address of the receiving Office Commissioner of Patents Canadian Receiving Office Box PCT, Ottawa/Hull K1A 0C9 Facsimile No. (819) 953-9538	Authorized Officer Carole Millaire Telephone no. (819) 994-6587
--	---

PATENT COOPERATION TREATY

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

From the INTERNATIONAL BUREAU

To:

BERNIER, Louise, G.
Boehringer Ingelheim (Canada) Ltd.
2100 Cunard Street
Laval, Québec H7S 2G5
Canada

Date of mailing (day/month/year) 26 February 2003 (26.02.03)	
Applicant's or agent's file reference 13/106 pct-2	IMPORTANT NOTIFICATION
International application No. PCT/CA03/00090	International filing date (day/month/year) 24 January 2003 (24.01.03)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 01 February 2002 (01.02.02)
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
01 Febr 2002 (01.02.02)	2,370,396	CA	18 Febr 2003 (18.02.03)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338 88 95	Authorized officer MIKI KOBAYASHI (Fax 338-8995) Telephone No. (41-22) 338 8401
--	---

PATENT COOPERATION TREATY

80

PATENT DEPARTMENT

From the INTERNATIONAL BUREAU

PCT

To:

MAR 18 2003

Agent

NOTIFICATION OF RECEIPT OF
RECORD COPY

(PCT Rule 24.2(e))

BERNIER, Louise, G.
Boehringer Ingelheim (Canada) Ltd.
2100 Cunard Street
Laval, Québec H7S 2G5
Canada

Date of mailing (day/month/year) 26 February 2003 (26.02.03)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 13/106 pct-2	International application No. PCT/CA03/00090

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

BOEHRINGER INGELHEIM INTERNATIONAL GMBH (for all designated States except US)
LLINAS-BRUNET, Montse et al (for US)

International filing date : 24 January 2003 (24.01.03)

Priority date(s) claimed : 01 February 2002 (01.02.02)

Date of receipt of the record copy
by the International Bureau : 18 February 2003 (18.02.03)

List of designated Offices :

AP : GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW

EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

EP : AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR

OA : BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

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, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT,
TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

☒ time limits for entry into the national phase - see updated important information (as of April 2002)

☒ confirmation of precautionary designations (if applicable)

☐ requirements regarding priority documents (if applicable)

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer: Miki KOBAYASHI (Fax 338-8996)
Facsimile No. (41-22) 338.89.96	Telephone No. (41-22) 338 8401

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated on the cover sheet of this Notification by paying national fees and furnishing translations, as prescribed by Articles 22 and 39 and the applicable national laws. In addition, the applicant may also have to comply with other special requirements applicable in certain Offices. It is the applicant's responsibility to ensure the necessary steps to enter the national phase are taken in a timely fashion. Most Offices do not issue reminders to applicants in connection with the entry into the national phase.

The applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be 30 MONTHS from the priority date, not only in respect of any elected Office where a demand for international preliminary examination is filed before the expiration of 18 months from the priority date (see Article 38(1)), but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see PCT Gazette No. 44/2001 of 1 November 2001, pages 19928, 19932 and 19934, as well as the PCT Newsletter, October and November 2001 and February 2002 issues.

In practice, time limits other than the 30-month time limit will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For regular updates on the applicable time limits (20, 21, 30 or 31 months, or other time limits), Office by Office, refer to the PCT Gazette ("Section IV" part published on a weekly basis), to the PCT Newsletter (on a monthly basis) and to the relevant National Chapters in Volume II of the PCT Applicant's Guide (the paper version of which is updated usually twice a year and the Internet version of which is updated usually on a weekly basis). Finally, a cumulative table of all applicable time limits for entering the national phase is available from WIPO's Internet site, via links from various pages the site including those of the Gazette, Newsletter and Guide, at <http://www.wipo.int/pct/en/index.html>.

Information about the requirements for filing a demand for international preliminary examination is set out in the PCT Applicant's Guide, Volume IA, Chapter IX. Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.8(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.8(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.8(c) before the expiration of 15 months from the priority date (this time limit may not be extended). If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fee. The Notice of confirmation and payment must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 18 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 18-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 18-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within the time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 18-month time limit is the filing date of the earliest application whose priority is claimed.

82

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL SEARCHING AUTHORITY

To:

BOEHRINGER INGELHEIM (CANADA) LTD.
Attn. Bernier, Louise G.
2100 Cunard Street
Laval Quebec H7S 2G5
CANADA

COMMUNICATION IN CASES FOR WHICH
NO OTHER FORM IS APPLICABLE

Date of mailing
(day/month/year)

04/03/2003

Applicant's or agent's file reference

13/106 pct-2

REPLY DUE

See paragraph 1 below

International application No.

PCT/CA 03/ 00090

International filing date
(day/month/year)

24/01/2003

Applicant

BOEHRINGER INGELHEIM INTERNATIONAL GMBH

1. ☐ REPLY DUE within _____ WORKDAYS from the above date of mailing

☒ NO REPLY DUE

2. COMMUNICATION:

The applicant is informed that establishment of the international search report (ISR) for non first-filings may be delayed due to a current search backlog.

Although the time limit for entering the national phase before designated offices under Article 22(1) PCT and elected offices under Article 39(1) PCT has, with effect from 1 April 2002 (see PCT Gazette 44/2001 Section IV) been set at 30 months from the priority date (before the EPO the time limit is 31 months from the priority date - see Rule 107 EPC as amended with effect from 2 January 2002 - OJ EPO 8-9/2001, 373) not all PCT contracting states have yet made the necessary changes to their national laws and will for the time being continue to require entry to the national phase at 20/21 months from the priority date if a demand has not been filed before the end of 18 months from the priority date - see PCT Gazette/PCT Newsletter available on the WIPO Internet site at <http://www.wipo.int/pct/en/index.html> for an up to date list of the applicable time limits.

In these circumstances, the EPO acting as IPEA will accept, without any late payment fee under Rule 58bis PCT, the handling fee and the preliminary examination fee due in respect of the demand relating to the present application, even if they are not paid within the time limit prescribed in Rules 57.3 and 58.1(b) PCT, provided that they are paid within one month from the date of transmittal of the ISR; i.e., the EPO will only send an invitation pursuant to Rule 58bis.1(a) PCT after expiry of this one-month period. In all cases where the EPO has sent an invitation to pay and the applicant has not paid in full the amount due, the demand shall be considered as if it had not been submitted (Rule 58bis.1(b)-(d) PCT). A loss of rights may well be the consequence in designated states where the time limit for entry into the national phase under Article 22 PCT has already expired (see also Article 37(4) PCT).

Note that if the competent IPEA chosen by the applicant is not the EPO and if the fees mentioned above are not paid within the time limit prescribed in Rules 57.3 and 58.1(b) PCT, the competent IPEA is entitled to apply Rule 58bis.1(a) PCT immediately thereafter.

If your application is affected, we apologise for any inconvenience caused.

Finally, applicants are reminded that as of 3 January 2002 a rationalised PCT II procedure may apply, see OJ EPO 11/2001, 539 and that the EPO as ISA will not carry out international search on an application which relates to no more than a method of doing business, see OJ EPO 10/2001, 482. Applicants should also bear in mind the restriction of the EPO's competence as ISA and IPEA in certain technical fields in respect of certain international applications, see OJ EPO 1/2002, 52 and PCT Newsletter 1/2002 for further details.

Name and mailing address of the International Searching Authority



European Patent Office, P.B. 5018 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 851 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

ISA/EP

83
The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA		Date of receipt of DEMAND	
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 13/106 pct-2	
International application No. PCT/CA03/00090	International filing date (day/month/year) 24/01/2003	(Earliest) Priority date (day/month/year) 01/02/2002	
Title of invention HEPATITIC C INHIBITOR TRI-PEPTIDES			
Box No. II APPLICANT(S)			
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) BOEHRINGER INGELHEIM INTERNATIONAL GMBH Binger Strasse 173 D-55216 Ingelheim GERMANY		Telephone No. 011 49 6132 77 2326	
		Facsimile No. 011 49 6132 77 3000	
		Teleprinter No.	
		Applicant's registration No. with the Office	
State (that is, country) of nationality: DE		State (that is, country) of residence: DE	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) LLINAS-BRUNET, Montse 2100 Cunard Street Laval, Québec H7S 2G5 CANADA			
State (that is, country) of nationality: CA		State (that is, country) of residence: CA	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) GORYS, Vida, J. 2100 Cunard Street Laval, Québec H7S 2G5 CANADA			
State (that is, country) of nationality: CA		State (that is, country) of residence: CA	
☐ Further applicants are indicated on a continuation sheet.			

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BERNIER, Louise, G.
c/o BOEHRINGER INGELHEIM (CANADA) LTD.
2100 Cunard Street
Laval, Québec H7S 2G5
CANADA

Telephone No.

(450) 682-4640

Facsimile No.

(450) 682-8434

Teleprinter No.

Agent's registration No. with the Office

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION
Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filed

the description ☒ as originally filed
☐ as amended under Article 34

the claims ☒ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34

the drawings ☒ as originally filed
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

☒ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☐ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | |
|--|---|--------|
| 1. translation of international application | : | sheets |
| 2. amendments under Article 34 | : | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | sheets |
| 5. letter | : | sheets |
| 6. other (<i>specify</i>) | : | sheets |

For International Preliminary
Examining Authority use only

received	not received
----------	--------------

- | | |
|--------------------------|--------------------------|
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |

The demand is also accompanied by the item(s) marked below:

- | | |
|--|---|
| 1. ☒ fee calculation sheet | 5. ☐ statement explaining lack of signature |
| 2. ☐ original separate power of attorney | 6. ☐ sequence listings in computer readable form |
| 3. ☐ original general power of attorney | 7. ☐ tables in computer readable form related to sequence listings |
| 4. ☐ copy of general power of attorney; reference number, if any: | 8. ☐ other (<i>specify</i>): |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Louise G. BERNIER
Agent for Applicant

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.

☐ The applicant has been informed accordingly.

4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.

5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

86

PCT

FEE CALCULATION SHEET

Annex to the Demand

International application No. PCT/CA03/00090		For International Preliminary Examining Authority use only	
Applicant's or agent's file reference 13/106 pct-2		Date stamp of the IPEA	
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH			
CALCULATION OF PRESCRIBED FEES			
1. Preliminary examination fee		1530.00 EURO	P
2. Handling fee (<i>Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.</i>)		159.00 EURO	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box		1689.00 EURO	
		TOTAL	
MODE OF PAYMENT			
☐ authorization to charge deposit account with the IPEA (see below)	☐ cash		
☐ cheque	☐ revenue stamps		
☐ postal money order	☐ coupons		
☒ bank draft	☐ other (<i>specify</i>):		
AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT (<i>This mode of payment may not be available at all IPEAs</i>)			
☐ Authorization to charge the total fees indicated above.		IPEA/	
☐ (<i>This check-box may be marked only if the conditions for deposit accounts of the IPEA so permit</i>) Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.		Deposit Account No.:	
		Date:	
		Name:	
		Signature:	

PATENT COOPERATION TREATY

PATENT DEPARTMENT

AUG 28 2003

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

BERNIER, Louise, G.
Boehringer Ingelheim (Canada) Ltd.
2100 Cunard Street
Laval, Québec H7S 2G5

SCH 100 updated

Scanned / sent to GmbH

Scanned / sent to BIPI (with encl.) AUG 28 2003

ENP due:

Agent:

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY(PCT Rules 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))Date of mailing
(day/month/year)

22-08-2003

Applicant's or agent's file reference

13/106 pct-2

IMPORTANT NOTIFICATION

International application No.

PCT/CA 03/00090

International filing date (day/month/year)

24/01/2003

Priority date (day/month/year)

01/02/2002

Applicant

BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

19/08/2003

2. This date of receipt is:

- ☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **ATTENTION:** That date of receipt is AFTER the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

- ☐ (If applicable) This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer

MORENO R A

Tel. ext: 2658



38

PATENT DEPARTMENT

PATENT COOPERATION TREATY

SEP 19 2003

From the INTERNATIONAL BUREAU

Agent: _____ PCT

INFORMATION CONCERNING ELECTED
OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:

BERNIER, Louise, G.
Boehringer Ingelheim (Canada) Ltd.
2100 Cunard Street
Laval, Québec H7S 2G5
Canada

F.

Date of mailing (day/month/year)

08 September 2003 (08.09.03)

Applicant's or agent's file reference

13/108 pct-2

IMPORTANT INFORMATION

International application No.

PCT/CA03/00090

International filing date (day/month/year)

24 January 2003 (24.01.03)

Priority date (day/month/year)

01 February 2002 (01.02.02)

Applicant

BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

EP : AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,
SK, TR

National : BG, CA, CN, DE, GB, IL, JP, KP, KR, MN, NO, PL, RO, RU, SK, US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

AP : GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW

EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

OA : BF, BJ, CF, CG, CI, CM, GA, GN, GO, GW, ML, MR, NE, SN, TD, TG

National : AE, AG, AL, AM, AT, AU, AZ, BA, BB, BR, BY, BZ, CH, CO, CR, CU, CZ, DK, DM, DZ, EC,
EE, ES, FI, GD, GE, GH, GM, HR, HU, ID, IN, IS, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MW, MX, MZ, NZ, OM, PH, PT, SD, SE, SG, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN,
YU, ZA, ZM, ZW

3. The applicant is reminded that he must enter the "national phase" before the expiration of 30 months from the priority date before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 38(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 38(3)(b) and Rule 74.1).

Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed until 31 months from the priority date for all States designated for the purposes of obtaining a European patent.

2003-09-19 updated

Scanned / sent to GmbH

Scanned / sent to BIP (with email)

ENP due: Aug. 1, 2004

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 338.89.95

Authorized officer:

Catherine TOLU (Fax 338-8995)

Telephone No. (41-22) 338 9858

694324E



P.B.5618 - Patentkan 2
2280 HV Rijkswijk (Z1)
TE (070) 3 40 20 40
FAX (070) 3 40 30 16

Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

Generaldirektion 1

Directorate General 1

Direction générale 1

BERNIER, Louise, G.
Boehringer Ingelheim (Canada) Ltd.
2100 Cunard Street
Laval, Québec H7S 2G5

PATENT DEPARTMENT

~~Canada~~
~~sent to EPO updated~~

~~sent to GmbH~~

~~sent to BIRL (with encl.)~~

~~Agent~~

ENP due: August 1/2004

JUN 15 2004

epoline® Customer Services

Tel.: +31 (0)70 340 48 00

Date

14.06.04

Reference	Application No./Patent No. 03700744.0 - 2404 PCT/CA0300090 13/106
Applicant/Proprietor Boehringer Ingelheim International GmbH	

Entry into the European phase before the European Patent Office

These notes describe the procedural steps required for entry into the European phase before the European Patent Office (EPO). You are advised to read them carefully: failure to take the necessary action in time can lead to your application being deemed withdrawn.

1. The above-mentioned international patent application has been given European application No. 03700744.0.
2. Applicants without a residence or their principal place of business in an EPC contracting state may themselves initiate European processing of their international applications, provided they do so before expiry of the 31st month from the priority date (see also point 6 below).

During the European phase before the EPO as designated or elected Office, however, such applicants must be represented by a professional representative (Arts. 133(2) and 134(1), (7), EPC).

Procedural acts performed after expiry of the 31st month by a professional representative who acted during the international phase but is not authorised to act before the EPO have no legal effect and therefore lead to loss of rights.

Please note that a professional representative authorised to act before the EPO and who acted for the applicant during the international phase does not automatically become the representative for the European phase. Applicants are therefore strongly advised to appoint in good time any representative they wish to initiate the European phase for them; otherwise, the EPO has to send all communications direct to the applicant.

3. Applicants with a residence or their principal place of business in an EPC contracting state are not obliged to appoint, for the European phase before the EPO as designated or elected Office, a professional representative authorised to act before the EPO. However, in view of the complexity of the procedure it is recommended that they do so.
4. Applicants and professional representatives are also strongly advised to initiate the European phase using EPO Form 1200 (available free of charge from the EPO). This however is not compulsory.

1
90

5. To enter the European phase before the EPO, the following acts must be performed.
(N.B.: Failure validly to do so will entail loss of rights or other adverse legal consequences.)

5.1 If the EPO is acting as designated or elected Office (Arts. 22(1)(3) and 39(1) PCT respectively), applicants must, within 31 months from the date of filing or (where applicable) the earliest priority date:

- a) Supply a translation of the international application into an EPO official language, if the International Bureau did not publish the application in such a language (Art. 22(1) PCT and Rule 107(1)(a) EPC).**
If the translation is not filed in time, the international application is deemed withdrawn before the EPO (Rule 108(1) EPC).
This loss of rights is deemed not to have occurred if the translation is then filed within a two-month grace period as from notification of an EPO communication, provided a surcharge is paid at the same time (Rule 108(3) EPC).
- b) Pay the national basic fee (EUR 125,00) and, where a supplementary European search report has to be drawn up, the search fee (EUR 690,00 ; Rule 107(1)(c) and (e) EPC).**
- c) If the time limit under Article 79(2) EPC expires before the 31-month time limit, pay the designation fee (EUR 75,00) for each contracting state designated (Rule 107(1)(d) EPC).**
- d) If the time limit under Article 94(2) EPC expires before the 31-month time limit, file the written request for examination and pay the examination fee (EUR 1430,00 ; Rule 107(1)(f) EPC).**
- e) Pay the third-year renewal fee (EUR 380,00) if it falls due before expiry of the 31-month time limit (Rule 107(1)(g) EPC).**

If the fees under (b) to (d) above are not paid in time, or the written request for examination is not filed in time, the international application is deemed withdrawn before the EP, or the contracting-state designation(s) in question is (are) deemed withdrawn (Rule 108(1) and (2) EPC). However, the fees may still be validly paid within a two-month grace period as from notification of an EPO communication, provided the necessary surcharges are paid at the same time (Rule 108(3) EPC). For the renewal fee under (e) above, the grace period is six months from the fee's due date (Article 86(2) EPC).

5.2 If the application documents on which the European grant procedure is to be based comprise more than ten claims, a claims fee is payable within the 31-month time limit under Rule 107(1) EPC for the eleventh and each subsequent claim (Rule 110(1) EPC). The fee can however still be paid within a one-month grace period as from notification of an EPO communication pointing out the failure to pay (Rule 110(2) EPC).

6. If the applicant had a representative during the application's international phase, the present notes will be sent to the representative, asking him to inform the applicant accordingly.

All subsequent communications will be sent to the applicant, or - if the EPO is informed of his appointment in time - to the applicant's European representative.



Date

Sheet 3

Application No. 03700744.0

7. For more details about time limits and procedural acts before the EPO as designated and elected Office, see the EPO brochure

How to get a European patent
Guide for applicants - Part 2
PCT procedure before the EPO - "Euro-PCT"

This brochure, the list of professional representatives before the EPO, Form 1200 and details of the latest fees are now all available on the internet under

<http://www.european-patent-office.org>

RECEIVING SECTION



PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 13/106 pct-2	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/CA 03/ 00090	International filing date (day/month/year) 24/01/2003	(Earliest) Priority Date (day/month/year) 01/02/2002
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

TRIPEPTIDES HAVING A HYDROXYPROLINE ETHER OF A SUBSTITUTED QUINOLINE FOR THE INHIBITION OF NS3 (HEPATITIS C)

5. With regard to the abstract,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

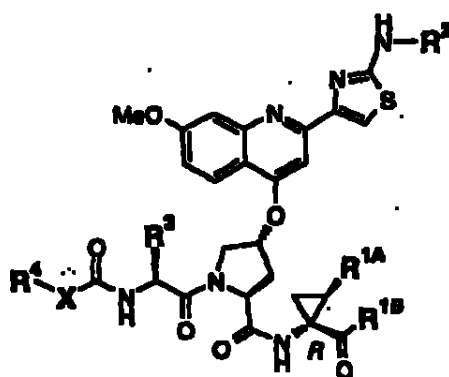
☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

ABSTRACT

Disclosed herein are compounds of formula (1):



(1)

wherein R^1 is hydroxy or $NHSO_2R^{1A}$ wherein R^{1A} is (C_{1-6}) alkyl, (C_{2-7}) cycloalkyl or $((C_{1-6})$ alkyl- (C_{2-7}) cycloalkyl), which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, $O-(C_{1-6})$ alkyl, amido, amino or phenyl, or R^{1A} is C_6 or C_{10} aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C_{1-6}) alkyl, $O-(C_{1-6})$ alkyl, amido, amino or phenyl; R^2 is (C_{4-8}) cycloalkyl; R^3 is t-butyl or (C_{2-6}) cycloalkyl and R^4 is (C_{4-8}) cycloalkyl; or a pharmaceutically acceptable salt thereof.

The compounds are useful as inhibitors of HCV NS3 protease for the treatment of Hepatitis C.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/00090

94

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K5/08 A61K38/06 A61P31/14

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 C07K

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, WPI Data, PAJ, CHEM ABS 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 09543 A (BOEHRINGER INGELHEIM CA LTD ;GOUDREAU NATHALIE (CA); GHIRO ELISE ()) 24 February 2000 (2000-02-24) cited in the application claims 1,11,17,18,29,33,35,60; table 7	1-32
---	---	------

☐ 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.

"Z" document member of the same patent family

Date of the actual completion of the international search

15 April 2003

Date of mailing of the international search report

02/05/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentien 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Fausti, S

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.1

Although claims 24-29 are 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/composition.

Continuation of Box I.1

Claims Nos.: 24-29

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 03/00090

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1. ☒ Claims Nos.: 24-29
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
- 2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
- 3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

- 1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
- 2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
- 3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
- 4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims No.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REP RT

Information on patent family members

International Application No

PCT/CA 03/00090

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0009543	A	24-02-2000	AU 5273199 A	06-03-2000
			BG 105232 A	30-11-2001
			BR 9913646 A	05-06-2001
			CA 2338946 A1	24-02-2000
			WO 0009543 A2	24-02-2000
			CN 1323316 T	21-11-2001
			CZ 20010516 A3	15-08-2001
			EE 200100081 A	15-08-2002
			EP 1105413 A2	13-06-2001
			HR 20010102 A1	28-02-2002
			HU 0105144 A2	29-04-2002
			JP 2002522554 T	23-07-2002
			NO 20010683 A	02-04-2001
			PL 346626 A1	25-02-2002
			SK 2062001 A3	08-10-2001
			TR 200100432 T2	21-09-2001
			TR 200200129 T2	21-06-2002
			US 6534523 B1	18-03-2003
			US 6323180 B1	27-11-2001
			US 6268207 B1	31-07-2001
			US 6329379 B1	11-12-2001
			US 6329417 B1	11-12-2001
			US 2002016442 A1	07-02-2002
			US 2002037998 A1	28-03-2002

PATENT COOPERATION TREATY

PATENT DEPARTMENT

SEP 08 2003

Agent: _____

PCT

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

BERNIER, Louise, G.
Boehringer Ingelheim (Canada) Ltd.
2100 Cunard Street
Laval, Québec H7S 2G5
CANADA

Scanned / sent to GmbH
Scanned / sent to GmbH (with email)

ENP due: _____

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

01/09/2003

Applicant's or agent's file reference

03/106 pct-2

IMPORTANT NOTIFICATION

International application No.

PCT/CA 03/00090

International filing date (day/month/year)

24/01/2003

Priority date (day/month/year)

01/02/2002

Applicant

BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions are patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

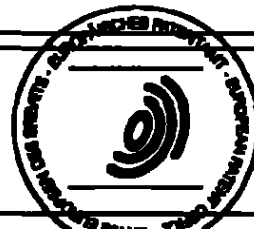
Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523636 epon d
Fax: (+49-89) 2399-4465

Authorized officer

MORENO R A
Tel. (+49-89) 2399 2828



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

(Rationalized Report according to the Notice of the President of the EPO published in the OJ11/2001)

Applicant's or agent's file reference 13/106 pct-2	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/CA 03/ 00090	International filing date (day/month/year) 24/01/2003	Priority date (day/month/year) 01/02/2002
International Patent Classification (IPC) or national classification and IPC C07K5/08		
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH 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 13/08/2003	Date of completion of this report 27/08/2003
Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer WEAVER M R Tel. (+49-89) 2399 2828



100

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No PCT/CA 03/00090

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.

Traducción

101

TRATADO DE COOPERACIÓN DE PATENTES
PCT
INFORME DE EXAMEN PRELIMINAR INTERNACIONAL
(Artículo 36 y Regla 70 del PCT)

Referencia del expediente del agente o solicitante 13/108 PCT-2	PARA ACTUACIONES ADICIONALES	Ver notificación de Transmisión del Reporte de Examen Preliminar (Formato PCT/IPEA416)
Solicitud Internacional No. PCT/CA03/00080	Fecha de presentación Internacional (día/mes/año) 24/01/2003	Fecha de prioridad (día/mes/año) 01/02/2002
Clasificación Internacional de Patentes (IPC) o clasificación nacional a IPC C07K 5/08		
Solicitante BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al.		

1. Este informe de examen preliminar internacional ha sido preparado por esta Administración encargada del Examen Preliminar Internacional y se envía al solicitante en concordancia con el artículo 36.
2. Este INFORME consta de una total de 2 hojas, incluyendo la portada
- ☐ Este informe también se acompaña de ANEXOS, esto es documentos de la descripción, reivindicaciones y/o dibujos que han sido corregidos y que son la base de este informe y/o documentos que contienen rectificaciones realizadas ante esta Administración (Ver Regla 70.16 y Sección 607 de las Instrucciones Administrativas según el PCT)

Estos anexos constan de un total de ____ hojas.

3. Este informe contiene indicaciones relativas a los siguientes aspectos:

- I ☒ Fundamentos del Informe
- II ☐ Prioridad
- III ☒ No se establece concepto relativo a la novedad, nivel inventivo y aplicación industrial
- IV ☐ Carencia de unidad de la invención
- V ☒ Declaración razonada según el artículo 35(2), en relación con la novedad, nivel inventivo o aplicación industrial; citaciones y explicaciones que soportan dicha declaración
- VI ☐ Ciertos documentos citados
- VII ☐ Ciertos defectos de la solicitud internacional
- VIII ☐ Ciertas observaciones respecto a la solicitud internacional

Fecha de presentación de la demanda 13/08/2003	Fecha de terminación de este reporte 27/08/2003
Nombre y dirección de correo de la autoridad encargada del examen preliminar  Oficina Europea de Patentes D-80298 Munich Tel. +49 89 2399 - 0 Tlx 523656 epmu d Fax: +49 89 2399 - 4485	Funcionario Autorizado WEAVER M R Teléfono No. (+49-89) 2399 2828

Formato PCT/IPEA408 (portada) (julio 1998)

I. Base del Informe

La base del presente examen preliminar internacional es la solicitud como originalmente fue presentada.

III. No-establecimiento de opinión con respecto a la novedad, nivel inventivo y aplicabilidad industrial.

La pregunta de si la invención reivindicada parece tener novedad, nivel inventivo, o que sea aplicable industrialmente no ha sido sujeto del examen preliminar internacional con respecto a las reivindicaciones que no han sido sujeto de búsqueda (Artículo 17(2)(a) o (3) y Regla 66.1 (e) PCT); ver también el reporte de búsqueda internacional.

V. Declaración motivada según el Artículo 35(2) en cuanto a la novedad, el nivel inventivo y la aplicación industrial; citas y explicaciones que apoyan esta declaración.

En la medida que este examen preliminar internacional se ha realizado (ver punto III arriba), lo siguiente puede señalarse:

A la luz de los documentos citados en el reporte de búsqueda internacional, se considera que la invención como se encuentra definida en las reivindicaciones independientes, que han sido sujeto de un reporte de búsqueda internacional, cumplen con los criterios mencionados en el Artículo 33(1) PCT, es decir que parece ser novedosa, alcanzar el nivel inventivo y ser industrialmente aplicable.

TRIPÉPTIDOS INHIBIDORES DE LA HEPATITIS C

CAMPO DE LA INVENCION

La presente invención se refiere a compuestos, procedimiento para su síntesis, composiciones y métodos para el
5 tratamiento de una infección del virus de la hepatitis C (HCV). En particular, la presente invención proporciona nuevos análogos de péptidos, composiciones farmacéuticas que contienen tales análogos y métodos para usar estos análogos en el tratamiento de una infección de HCV.

10 ANTECEDENTES DE LA INVENCION

El virus de la hepatitis C (HCV) es el agente etiológico principal de hepatitis no A y no B posterior a transfusiones y adquirido en comunidades a nivel mundial. Se estima que por encima de 200 millones de personas en el mundo
15 están infectadas por el virus. Un elevado porcentaje de portadores resulta crónicamente infectado y muchos progresan a enfermedades crónicas del hígado, la denominada hepatitis C crónica. Este grupo a su vez tiene un riesgo elevado de enfermedad grave del hígado como cirrosis hepática, cáncer hepatocelular y enfermedad terminal del hígado que
20 conduce a la muerte.

El mecanismo mediante el que el HCV establece la persistencia viral y provoca una tasa elevada de enfermedad hepática crónica no ha sido dilucidado a fondo. Es conocido
25 el modo en que el HCV interacciona con el sistema inmune del hospedante y lo evade. Además, las funciones de las respuestas inmunes celulares y tumorales en protección contra la infección por HCV y la enfermedad no han sido todavía establecidas. Las inmunoglobulinas han sido descritas
30 para la profilaxis de la hepatitis viral asociada a transfusiones, sin embargo, la institución "Center for Disease Control" no recomienda actualmente el tratamiento con inmunoglobulinas para estos fines. La ausencia de una respuesta inmune protectora eficaz es un obstáculo en el desarrollo
35 de una vacuna o medidas adecuadas de profilaxis posteriores

a la exposición, por lo que a corto plazo, las esperanzas se asientan firmemente en intervenciones antivirales.

Se han realizado diversos estudios clínicos con el objetivo de identificar agentes farmacéuticos capaces de tratar eficazmente la infección por HCV en pacientes afectados con hepatitis C crónica. Estos estudios han incluido el uso de interferón-alfa, solo o en combinación con otros agentes antivirales. Estos estudios han mostrado que un número sustancial de los participantes no respondieron a estas terapias, y de los que respondieron favorablemente, una elevada proporción se encontró que recaía tras terminar el tratamiento.

Hasta recientemente, el interferón (IFN) era la única terapia disponible de provecho demostrado aprobada en el campo clínico para pacientes con hepatitis C crónica. Sin embargo, la velocidad de respuesta sostenida es baja, y el tratamiento con interferón induce también efectos secundarios graves (es decir, retinopatía, tiroiditis, pancreatitis aguda o depresión) que disminuyen la calidad de vida de los pacientes tratados. Recientemente, el interferón en combinación con ribavirina ha sido aprobado para pacientes que no responden a IFN solo. Sin embargo, los efectos secundarios provocados por el IFN no son aliviados con esta terapia de combinación. Las formas pegiladas (tratadas con polietilenglicol) de interferones como PEG-Intron® y Pegasys® pueden abordar de forma aparentemente parcial estos efectos secundarios perjudiciales, pero los fármacos antivirales siguen siendo todavía la vía de elección para el tratamiento oral de HCV.

Por lo tanto, existe una necesidad de desarrollo de agentes antivirales eficaces para el tratamiento de una infección por HCV que superen las limitaciones de las terapias farmacéuticas existentes.

El HCV es un virus de RNA de cadena positiva envuelta

en la familia Flaviviridae. El genoma de RNA de HCV de cadena única tiene una longitud de aproximadamente 9.500 nucleótidos y tiene un marco de lectura abierta único (ORF) que codifica una única poliproteína grande de aproximadamente 3.000 aminoácidos. En las células infectadas, esta poliproteína es escindida en múltiples sitios por proteasas celulares y virales para producir las proteínas estructurales y no estructurales (NS). En el caso del HCV, la generación de proteínas no estructurales maduras (NS2, NS3, NS4A, NS4B, NS5A y NS5B) se efectúa por dos proteasas virales. La primera, como todavía está escasamente caracterizada, se escinde en la unión NS2-NS3 (denominada en lo sucesivo proteasa NS2/3); la segunda es una proteasa-serina combinada con la región N-terminal de NS3 (proteasa NS3) y media todas las escisiones posteriores aguas abajo de NS3, tanto en *cis*, en el sitio de escisión NS3-NS4A, como en el *trans*, para los restantes sitios NS4A-NS4B, NS4B-NS5A y NS5A-NS5B. La proteína NS4A parece servir para múltiples funciones, actuando como un co-factor para la proteasa NS3 y ayudando posiblemente a la localización de membranas de NS3 y otros componentes de replicasas virales. La formación de complejos de proteasa de NS3 con NS4A parece necesaria para los acontecimientos de los tratamientos, aumentando la eficacia proteolítica en todos los sitios. La proteína de NS3 exhibe actividades de nucleósido-trifosfatasa y RNA-helicasa. La NS5B es una RNA-polimerasa dependiente de RNA que está involucrada en la replicación de HCV.

Una estrategia general para el desarrollo de agentes antivirales es inactivar viralmente enzimas codificadas que son esenciales para la replicación del virus.

Lo que sigue es una lista de solicitudes de patentes publicadas en los últimos años que describen análogos de péptidos inhibidores de NS3-proteasa de HCV que son estructuralmente diferentes de los compuestos de la presente invención:

106

GB 2,337,262; JP10298151; JP 11126861; JP 11292840; JP
2001-103993; US 6,159,938; US 6,187,905; WO 97/43310; WO
98/17679; WO 98/22496; WO 98/46597; WO 98/46630; WO
99/38888; WO 99/50230; WO 99/64442; WO 99/07733; WO
5 99/07734; WO 00/09543; WO 00/09558; WO 00/20400; WO
00/59929; WO 00/31129; WO 01/02424; WO 01/07407; WO
01/16357; WO 01/32691; WO 01/40262; WO 01/58929; WO
01/64678; WO 01/74768; WO 01/77113; WO 01/81325; WO
02/08187; WO 02/08198; WO 02/08244; WO 02/08251; WO
10 02/08256; WO 02/18369; WO 02/60926 y WO 02/79234.

Una ventaja de la presente invención es que proporci-
na compuestos de tripéptidos que son inhibidores de la NS3-
proteasa, una enzima esencial para la replicación del virus
de la hepatitis C. Además de ello, los compuestos son capa-
15 ces de inhibir la replicación de RNA de HCV en el modelo de
célula de replicón.

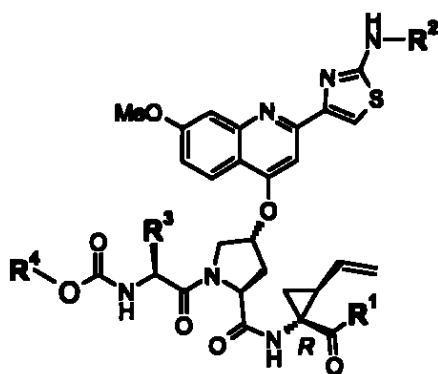
Una ventaja adicional de un aspecto de la presente in-
vención consiste en el hecho de que los compuestos inhiben
específicamente la NS3-proteasa y no muestran una actividad
20 inhibidora significativa frente a las demás serina-
proteasas como elastasa de leucocitos humanos (HLE), elas-
tasa pancreática porcina (PPE) o quimotripsina pancreática
bovina, o cisteína-proteasas como catepsina B hepática
humana (CatB).

25

SUMARIO DE LA INVENCION

Está incluido en el alcance de la invención un com-
puesto de fórmula (I):

30



(1)

en la que R¹ es hidroxí o NHSO₂R^{1A} en donde R^{1A} es al-
quilo (C₁₋₈), cicloalquilo (C₃₋₇) o {alquilo(C₁₋₈)-
5 cicloalquilo (C₃₋₇)}, que están todos opcionalmente susti-
tuidos de 1 a 3 veces con halo, ciano, nitro, O-alquilo
(C₁₋₈), amido, amino o fenilo, o R^{1A} es arilo de C₆ o C₁₀ que
está opcionalmente sustituido de 1 a 3 veces con halo, cia-
no, nitro, alquilo (C₁₋₈), O-alquilo (C₁₋₈), amido, amino o
10 fenilo; R² es cicloalquilo (C₄₋₆); R³ es t-butilo o cicloal-
quilo (C₃₋₆) y R⁴ es cicloalquilo (C₄₋₆), o una sal farmacéu-
ticamente aceptable del mismo.

Está incluida en el alcance de esta invención una com-
posición farmacéutica que comprende una cantidad viralmente
15 eficaz anti-hepatitis C de un compuesto de fórmula I, o una
sal terapéuticamente aceptable del mismo, mezclada con un
medio de vehículo farmacéuticamente aceptable o agente
auxiliar.

Según una realización, la composición farmacéutica de
20 esta invención incluye adicionalmente interferón (pegilado
o no), o ribavirina, o uno o más de otro agente anti-HCV, o
cualquier combinación de los anteriores.

Otro aspecto importante de la invención incluye un mé-
todo para tratar una infección viral de hepatitis C en un
25 mamífero administrando al mamífero una cantidad viralmente
eficaz anti-hepatitis C de un compuesto de fórmula I, una
sal terapéuticamente aceptable del mismo, o una composición

como se describió anteriormente, solos o en combinación con uno o más de: interferón (pegilado o no) o ribavirina, o uno o más de otros agentes anti-HCV, administrados conjunta o separadamente.

5 Otro aspecto importante de la invención incluye un método para prevenir una infección viral de hepatitis C en un mamífero administrando al mamífero una cantidad viralmente eficaz anti-hepatitis C de un compuesto de fórmula I, una
10 sal farmacéuticamente aceptable del mismo, o una composición como se describió anteriormente, solos o en combinación con uno o más de: interferón (pegilado o no) o ribavirina, o uno o más de otros agentes anti-HCV, todos los cuales administrados conjunta o separadamente.

También está dentro del alcance de esta invención el
15 uso de un compuesto de fórmula I, como se describe en la presente memoria descriptiva, para la elaboración de un medicamento para el tratamiento o prevención de una infección viral de hepatitis C.

20 DESCRIPCIÓN DETALLADA DE REALIZACIONES PREFERIDAS

Definiciones

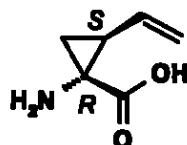
Tal como se usan en la presente memoria descriptiva, las siguientes definiciones se aplican, salvo que se indi-
25 que otra cosa:

Con referencia a los casos en que (R) o (S) se usen para indicar la configuración absoluta de un sustituyente o centro asimétrico de un compuesto de fórmula I, la indicación se hace en el contexto del compuesto completo y no en
30 el contexto del sustituyente o centro asimétrico solo.

La indicación "P1, P2 y P3", tal como se usa en la presente memoria descriptiva, se refiere a la posición de los residuos de aminoácidos partiendo del extremo del C terminal de los análogos de péptidos y extendiéndose hacia
35 el N terminal (es decir, P1 se refiere a la posición 1 del

C terminal, P2: segunda posición desde el C terminal, etc.) (véase Berger A. & Schechter I., Transactions of the Royal Society London series B257, 249-264 (1970).

Tal como se usa en la presente memoria descriptiva, el término "vinil-ACCA" se refiere a un compuesto de fórmula:



a saber, ácido (1R,2S)-1-amino-2-etenilciclopropilcarboxílico.

El término "alquilo (C₁₋₈)", como se usa en la presente memoria descriptiva, solo o en combinación con otro sustituyente, significa sustituyentes alquílicos lineales o ramificados acíclicos que contienen de 1 a 8 átomos de carbono e incluyen, por ejemplo, metilo, etilo, 2-metilhexilo, 1,1-dimetilhexilo (o t-butilo) y octilo.

El término "cicloalquilo (C₃₋₇)", como se usa en la presente memoria descriptiva, solo o en combinación con otro sustituyente, significa un sustituyente cicloalquilo que contiene de 3 a 7 átomos de carbono e incluye ciclopropilo, ciclobutilo, ciclopentilo, ciclohexilo y cicloheptilo.

El término {(alquilo (C₁₋₆)-cicloalquilo (C₃₋₆))}, como se usa en la presente memoria descriptiva, significa un radical cicloalquilo que contiene de 3 a 6 átomos de carbono directamente unidos a un radical alquilenos que contiene 1 a 6 átomos de carbono; por ejemplo, ciclopropilmetilo, ciclopentiletilo, ciclohexilmetilo y ciclohexiletilo. En el caso en que R^{1A} sea un {alquilo (C₁₋₆)-cicloalquilo (C₃₋₆)}, este grupo está unido al grupo SO₂ a través del alquilo (C₁₋₆) (es decir, la parte de alquilenos).

El término "arilo de C₆ o C₁₀", tal como se usa en la presente memoria descriptiva, solo o en combinación con otro radical, significa un grupo monocíclico aromático que

10

contiene 6 átomos de carbono o un grupo bicíclico aromático que contiene 10 átomos de carbono. Por ejemplo, arilo incluye fenilo, 1-naftilo o 2-naftilo.

El término "O-alquilo (C_{1-6})", como se usa en la presente memoria descriptiva, solo o en combinación con otro radical, significa el radical -O-alquilo (C_{1-6}) en el que alquilo es como se definió anteriormente que contiene hasta seis átomos de carbono, e incluye metoxi, etoxi, propoxi, 1-metiletoxi, butoxi y 1,1-dimetiletoxi. Este último radical es conocido comúnmente como terc-butoxi.

El término "halo", como se usa en la presente memoria descriptiva, significa un sustituyente halógeno seleccionado entre bromo, cloro, flúor o yodo.

La expresión "sal farmacéuticamente aceptable" significa una sal de un compuesto de fórmula (1) que, dentro del alcance de un criterio médico prudencial, sea adecuada para ser usado en contacto con los tejidos de seres humanos y animales inferiores sin una toxicidad excesiva, irritación, respuesta alérgica y similares, compatible con una relación ventaja/riesgo razonable, generalmente soluble o dispersable en agua o aceite, y eficaz para su uso previsto. El término incluye sales por adición de ácidos farmacéuticamente aceptables y sales por adición de bases farmacéuticamente aceptables. Las listas de sales adecuadas se encuentran, por ejemplo, en la publicación de S.M. Birge et al., J. Pharm. Sci., 1977, 66, pag. 1-19, que se incorpora como referencia a la presente memoria descriptiva en su totalidad.

La expresión "sal por adición de ácidos farmacéuticamente aceptable" significa las sales que retienen la eficacia y las propiedades biológicas de las bases libres y que no son indeseables biológicamente o en lo demás, formadas con ácidos inorgánicos como ácido clorhídrico, ácido bromhídrico, ácido yodhídrico, ácido sulfúrico, ácido sulfámico, ácido nítrico, ácido fosfórico y similares, y ácidos

orgánicos como ácido acético, ácido tricloroacético, ácido trifluoroacético, ácido adípico, ácido algínico, ácido ascórbico, ácido aspártico, ácido bencenosulfónico, ácido benzoico, ácido 2-acetoxibenzoico, ácido butírico, ácido canfórico, ácido canforsulfónico, ácido cinámico, ácido cítrico, ácido diglucónico, ácido etanosulfónico, ácido glutámico, ácido glicólico, ácido glicerofosfórico, ácido hemisulfico, ácido heptanoico, ácido hexanoico, ácido fórmico, ácido fumárico, ácido 2-hidroxietanosulfónico (ácido isetiónico), ácido láctico, ácido maleico, ácido hidroximaléico, ácido málico, ácido malónico, ácido mandélico, ácido mesitilenosulfónico, ácido metanosulfónico, ácido naftalenosulfónico, ácido nicotínico, ácido 2-naftalenosulfónico, ácido oxálico, ácido pimoico, ácido pectínico, ácido fenilacético, ácido 3-fenilpropiónico, ácido pítrico, ácido piválico, ácido propiónico, ácido pirúvico, ácido salicílico, ácido esteárico, ácido succínico, ácido sulfanílico, ácido tartárico, ácido p-toluenosulfónico, ácido undecanoico y similares.

La expresión "sal por adición de bases farmacéuticamente aceptable" significa las sales que retienen la eficacia y propiedades biológicas de los ácidos libres y que no son indeseables biológicamente o en lo demás, formadas con bases inorgánicas como amoníaco o hidróxido, carbonato o bicarbonato de amonio o un catión metálico como sodio, potasio, litio, calcio, magnesio, hierro, cinc, cobre, manganeso, aluminio y similares. Son particularmente preferidas las sales de amonio, potasio, sodio, calcio y magnesio. Las sales derivadas de bases no tóxicas orgánicas farmacéuticamente aceptables incluyen sales de compuestos de aminas primarias, secundarias y terciarias, compuestos de amina cuaternaria, aminas sustituidas incluidas las aminas sustituidas que se producen de forma natural, aminas cíclicas y resinas básicas de intercambio iónico, como metilamina, dimetilamina, trimetilamina, etilamina, dietilamina, trieti-

lamina, isopropilamina, tripropilamina, tributilamina, etanolamina, dietanolamina, 2-dimetilaminoetanol, 2-dietilaminoetanol, diciclohexilamina, lisina, arginina, histidina, cafeína, hidrabramina, colina, betaína, etilendiamina, glucosamina, metilglucamina, teobromina, purinas, piperazina, piperidina, N-etilpiperidina, compuestos de tetrametilamonio, compuestos de tetraetilamonio, piridina, N,N-dimetilanilina, N-metilpiperidina, N-metilmorfolina, diciclohexilamina, dibencilamina, N,N-dibencilfenetilamina, 10 1-efenamina, N,N'-dibenciletilendiamina, resinas de poliaminas y similares. Las bases no tóxicas orgánicas particularmente preferidas son isopropilamina, dietilamina, etanolamina, trimetilamina, diciclohexilamina, colina y cafeína.

La expresión "agente antiviral", como se usa en la presente memoria descriptiva, significa un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación de un virus en un mamífero. Esto incluye agentes que interfieren con los mecanismos del hospedante o virales necesarios para la formación y/o replicación de un virus en un mamífero. Los agentes antivirales incluyen, por ejemplo, ribavirina, amantadina, VX-497 (merimepodib, Vertex Pharmaceuticals), VX-498 (Vertex Pharmaceuticals), Lecocirin, Viramidina, Ceplene (maxamina), XTL-001 y XTL-002 (XTL Biopharmaceuticals).

La expresión "otro agente anti-HCV", como se usa en la presente memoria descriptiva, significa los agentes que son eficaces para disminuir o prevenir la progresión de síntomas de la enfermedad relacionados con la hepatitis C. Estos agentes se pueden seleccionar entre: agentes anti-virales, 30 agentes inmunomoduladores, inhibidores de polimerasa de HCV, inhibidores de NS3-proteasa de HCV o inhibidores de otra diana en el ciclo vital del HCV.

La expresión "agente inmunomodulador", como se usa en la presente memoria descriptiva, significa los agentes 35 (compuestos o biológicos) que son eficaces para aumentar o

potenciar la respuesta del sistema inmune en un mamífero. Los agentes inmunomoduladores incluyen, por ejemplo, interferones de clase I (como α -, β - y omega-interferones, tau-interferones, interferones de consenso y asialo-

5 interferones), interferones de clase II (como γ -interferones) e interferones pegilados.

La expresión "inhibidor de NS3-proteasa de HCV", como se usa en la presente memoria descriptiva, significa un agente (compuesto o biológico) que es eficaz para inhibir

10 la función de NS3-proteasa de HCV en un mamífero. Los inhibidores de NS3-proteasa de HCV incluyen, por ejemplo, los compuestos descritos en los documentos WP 99/07733, WO 99/07734, WO 00/09558, WO 00/09543, WO 00/59929 o WO 02/060926, el candidato clínico de Boehringer Ingelheim

15 identificado como BILN 2061 y el candidato de pre-desarrollo de Vertex/Eli Lilly identificado como VX-950 o LY-570310. En particular, los compuestos n°s 2, 3, 5, 6, 8, 10, 11, 18, 19, 29, 30, 31, 32, 33, 37, 38, 55, 59, 71, 91, 103, 104, 105, 112, 113, 114, 115, 116, 120, 122, 123, 124,

20 125, 126 y 127, descritos en la tabla de las páginas 224-226 en el documento WO 02/060926, se pueden utilizar en combinación con los compuestos de la presente invención.

La expresión "inhibidor de polimerasa de HCV", tal como se utiliza en esta memoria, significa un agente (com-

25 puesto o biológico) que es eficaz para inhibir la función de una polimerasa de HCV en un mamífero. Esto incluye, por ejemplo, inhibidores de la polimerasa NS5B de HCV. Inhibidores de polimerasa de HCV incluyen no nucleósidos, por ejemplo los compuestos descritos en:

- 30 - solicitud de EE.UU. n° 10/198.680, que corresponde al documento PCT/CA02/01127, ambos presentados el 18 de julio de 2002 (Boehringer Ingelheim),
- solicitud de EE.UU. n° 10/198.384, que corresponde al documento PCT/CA02/01128, ambos presentados el 18 de ju-

lio de 2002 (Boehringer Ingelheim),

- solicitud de EE.UU. n° 10/198.259, que corresponde al documento PCT/CA02/01129, ambos presentados el 18 de julio de 2002 (Boehringer Ingelheim),
- 5 - documento WO 02/100846 A1 y documento WO 02/100851 A2 (ambos de Shire),
- documento WO 01/85172 A1 y documento WO 02/098424 A1 (ambos de GSK),
- documento WO 00/06529 y documento WO 02/06246 A1 (am-
- 10 bos de Merck),
- documento WO 01/47883 y documento WO 03/000254 (ambos de Japan Tobacco) y
- documento EP 1 256 628 A2 (Agouron).

Además, otros inhibidores de polimerasa de HCV incluyen análogos de nucleósidos, por ejemplo los compuestos descritos en:

- documento WO 01/90121 A2 (Idenix),
- documento WO 02/069903 A2 (Biocryst Pharmaceuticals Inc.), y
- 20 - documento WO 02/057287 A2 y documento WO 02/057425 A2 (ambos de Merck/Isis).

Ejemplos específicos de inhibidores de una polimerasa de HCV incluyen JTK-002, JTK-003 y JTK-109 (Japan Tobacco).

La expresión "inhibidor de otra diana en el ciclo vital de HCV", como se usa en la presente memoria descriptiva, significa un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación del HCV en un mamífero de forma distinta que inhibiendo la función de la NS3-proteasa del HCV. Esto incluye agentes que interfieren con los mecanismos virales del hospedante o el HCV necesarios para la formación y/o replicación del HCV en un mamífero. Los inhibidores de otra diana en el ciclo vital del HCV incluyen, por ejemplo, agentes que inhiben una diana seleccionada entre una helicasa, una NS2/3-proteasa del

35 HCV y un inhibidor del sitio de entrada ribosomal interno

(IRES). Ejemplos específicos de inhibidores distintos de la diana en el ciclo vital del HCV incluyen ISIS-14803 (ISIS Pharmaceuticals).

La expresión "inhibidor de HIV", como se usa en la presente memoria descriptiva, significa un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación del HIV en un mamífero. Esto incluye agentes que interfieren con los mecanismos del hospedante o virales necesarios para la formación y/o replicación del HIV en un mamífero. Los inhibidores del HIV incluyen, por ejemplo, inhibidores nucleosídicos, inhibidores no nucleosídicos, inhibidores de proteasa, inhibidores de fusión e inhibidores de integrasa.

La expresión "inhibidor de HAV", como se usa en la presente memoria descriptiva, significa un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación del HAV en un mamífero. Esto incluye agentes que interfieren con los mecanismos del hospedante o virales necesarios para la formación y/o replicación del HAV en un mamífero. Los inhibidores del HAV incluyen vacunas de la hepatitis A, por ejemplo, Havrix® (ClaxoSmithKline), VAQ-TA® (Merck) y Avaxim® (Aventis Pasteur).

La expresión "inhibidor de HBV", como se usa en la presente memoria descriptiva, significa un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación del HBV en un mamífero. Esto incluye agentes que interfieren con los mecanismos del hospedante o virales necesarios para la formación y/o replicación del HBV en un mamífero. Los inhibidores del HBV incluyen, por ejemplo, agentes que inhiben la DNA-polimerasa viral del HBV o vacunas de HBV. ejemplos específicos de inhibidores del HBV incluyen Lamivudina (Epivir-HBV®), Adefovir Dipivoxil, Entecavir, FTC (Coviracil®), DAPD (DXG), L-FMAU (Clevudine), AM365 (Amrad), Ldt (Telbivudine), monoval-LdC (Valtorcita-

116

bine), ACH-126,443 (L-Fd4C) (Achillion), MCC478 (Eli Lilly), Racivir (RCV), Fluoro-L y D nucleósidos, Robustaflovona, ICN 2001-3 (ICN), Bam 205 (Novelos), XTL-001 (XTL), Imino-azúcares (Nonyl-DNJ) (Synergy), HpeBzyme; y productos
5 inmunomoduladores como: interferón alfa 2b, HE2000 (Hollis-Eden), Theradigm (Epimmune), EHT899 (Enzo Biochem), Thymosin alfa-1 (Zadaxin®), vacuna de DNA de HBV (PowderJect), vacuna de DNA de HVB (Jefferon Center), antígeno de HBV (OraGen), BayHpeo B® (Bayer), Nabi-HB® (Nabi) y Anti-
10 hepatitis B (Cangene); y productos de vacunas de HBV como los siguientes: Engerix B, Recombivax HB, GenHevac B, Hepacare, Bio-Hep B, TwinRix, Comvax, Hexavac.

La expresión "interferón de clase I", como se usa en la presente memoria descriptiva, significa un interferón
15 seleccionado entre un grupo de interferones que se unen todos al tipo I de receptores. Esto incluye los interferones de clase I producidos tanto natural como sintéticamente. Ejemplos de interferones de clase I incluyen α -, β - y omega-interferones, tau-interferones, interferones de consenso
20 y asialo-interferones.

La expresión "interferón de clase II", como se usa en la presente memoria descriptiva, significa un interferón
seleccionado entre un grupo de interferones que se unen todos al tipo II de receptores. Ejemplos de interferones de
25 clase II incluyen γ -interferones.

Las composiciones farmacéuticas de la invención pueden contener uno o más agentes activos adicionales seleccionados, por ejemplo, entre agentes antivirales, agentes inmunomoduladores, otros inhibidores de NS3-proteasa de HCV,
30 inhibidores de polimerasa de HCV, inhibidores de otra diana en el ciclo vital del HCV, inhibidores de HIV, inhibidores de HAV e inhibidores de HBV. Ejemplos de estos agentes se proporcionan en la sección de Definiciones anterior.

Ejemplos preferidos y específicos de algunos de estos

agentes se citan a continuación:

- agentes antivirales: ribavirina y amantadina;
- agentes inmunomoduladores: interferones de clase I, interferones de clase II e interferones pegilados;
- 5 ▪ inhibidor de otra diana en el ciclo vital del HCV que inhibe una diana seleccionada entre: NS3-helicasa, NS2/3-proteasa de HCV o un sitio de entrada de ribosoma interno (IRES);
- inhibidores de HIV: inhibidores nucleosídicos, inhibidores no nucleosídicos, inhibidores de proteasa, inhibidores de fusión e inhibidores de integrasa; o
- 10 ▪ inhibidores de HBV: agentes que inhiben DNA-polimerasa viral de HBV o es una vacuna de HBV.

Como se expuso anteriormente, la terapia de combinación está contemplada cuando un compuesto de fórmula (1), o una sal farmacéuticamente aceptable del mismo, es administrado conjuntamente con al menos un agente adicional seleccionado entre: un agente antiviral, un agente inmunomodulador, otro inhibidor de NS3-proteasa de HCV, un inhibidor de polimerasa de HCV, un inhibidor de otra diana en el ciclo vital del HCV, un inhibidor de HIV, un inhibidor de HAV y un inhibidor de HBV. Ejemplos de estos agentes se proporcionan en la sección de Definiciones anterior. Estos agentes adicionales pueden ser combinados con los compuestos de esta invención para crear una forma de dosificación farmacéutica única. Alternativamente, estos agentes adicionales pueden ser separadamente administrados al paciente como parte de una forma de dosificación múltiple, por ejemplo, usando un estuche de ensayo. Estos agentes adicionales pueden ser administrados al paciente antes, simultáneamente o a continuación de la administración de un compuesto de fórmula (1), o una sal farmacéuticamente aceptable del mismo.

Como se usa en la presente memoria descriptiva, el término "tratamiento" significa la administración de un

compuesto o composición según la presente invención para aliviar o eliminar los síntomas de la enfermedad de la hepatitis C y/o para reducir el contenido viral en un paciente.

5 Como se usa en la presente memoria descriptiva, el término "prevención" significa la administración de un compuesto o composición según la presente invención con posterioridad a la exposición del individuo al virus, pero antes de la aparición de los síntomas de la enfermedad y/o antes
10 de la detección del virus en la sangre.

Realizaciones preferidas

Preferentemente, en los compuestos de fórmula 1 como se definió anteriormente, R¹ es hidroxil, NHSO₂Me, NHSO₂-
15 ciclopropilo o NHSO₂Ph. Más preferentemente, R¹ es NHSO₂-ciclopropilo o NHSO₂Ph. Alternativamente, y lo más preferentemente, R¹ es hidroxil.

Preferentemente, en los compuestos de fórmula 1 como se definió anteriormente, R² es ciclopentilo o ciclohexilo.
20 Lo más preferentemente, R² es ciclopentilo.

Preferentemente, R³ es t-butilo o ciclohexilo. Lo más preferentemente, R³ es t-butilo.

Preferentemente, en los compuestos de fórmula 1 como se definió anteriormente, R⁴ es ciclobutilo o ciclopentilo.
25 Lo más preferentemente, R⁴ es ciclopentilo.

Más preferentemente, en un compuesto de fórmula 1 como se definió anteriormente, R¹ es hidroxil, R² y R⁴ son cada un ociclopentilo y R³ es t-butilo.

Más preferentemente, en un compuesto de fórmula 1, R¹
30 es hidróxil, R² es ciclobutilo, R³ es t-butilo y R⁴ es ciclopentilo.

Más preferentemente, en un compuesto de fórmula 1, R¹ es hidroxil, R² es ciclohexilo, R³ es t-butilo y R⁴ es ciclopentilo.

35 Más preferentemente, R¹ es NHSO₂Ph, R² y R⁴ son cada un

119

ociclopentilo y R³ es t-butilo.

Más preferentemente, en un compuesto de fórmula 1, R¹ es hidroxilo, R² es ciclopentilo, R³ es t-butilo y R⁴ es clobutilo.

5 Más preferentemente, en un compuesto de fórmula 1, R¹ es hidroxilo, R² es ciclopentilo, R³ es t-butilo y R⁴ es ciclohexilo.

Más preferentemente, en un compuesto de fórmula 1, R¹ es hidroxilo, R² y R⁴ son cada uno ciclopentilo y R³ es ciclohexilo.

10 Más preferentemente, en un compuesto de fórmula 1, R¹ es hidroxilo, R², R³ y R⁴ son cada uno ciclopentilo.

Según una realización alternativa, la composición farmacéutica de esta invención puede comprender adicionalmente otro agente anti-HCV. Ejemplos de agentes anti-HCV incluyen

15 α -(alfa), β -(beta), δ -(delta), γ -(gamma) o ω -(omega)-interferón, ribavirina y amantadina.

Según otra realización alternativa, la composición farmacéutica de esta invención puede comprender adicional-

20 mente otro inhibidor de NS3-proteasa de HCV.

Según otra realización alternativa, la composición farmacéutica de esta invención puede comprender adicional-

25 mente un inhibidor de polimerasa de HCV.

Según todavía otra realización alternativa, la composición farmacéutica de esta invención, puede comprender adicionalmente un inhibidor de otras dianas en el ciclo vital del HCV, que incluyen, pero sin limitación, helicasa, NS2/3-proteasa o sitio de entrada de ribosoma interno (IRES).

30 La composición farmacéutica de esta invención puede ser administrada por vía oral, parenteral o por medio de un depósito implantado. Se prefiere la administración oral o administración por inyección. La composición farmacéutica de esta invención puede contener cualquiera de los exci-

pientes, adyuvantes o vehículos farmacéuticamente aceptables, no tóxicos y convencionales. En algunos casos, el pH de la formulación puede ser ajustado con ácidos, bases o tampones farmacéuticamente aceptables para aumentar la estabilidad del compuesto formulado o su forma de suministro. El término "parenteral", como se usa en la presente memoria descriptiva, incluye las técnicas subcutánea, intracutánea, intravenosa, intramuscular, intra-articular, intrasinovial, intraesternal, intratecal y de inyección o infusión intralesional.

La composición farmacéutica puede estar en la forma de una preparación inyectable esterilizada, por ejemplo, en forma de una suspensión acuosa u oleaginosa inyectable esterilizada. Esta suspensión puede ser formulada según las técnicas conocidas en el estado de la técnica usando agentes dispersantes o humectantes adecuados (como, por ejemplo, Tween 80) y agentes suspensores.

La composición farmacéutica de esta invención puede ser administrada por vía oral en cualquier forma de dosificación aceptable por vía oral incluidos, pero sin limitación, cápsulas, comprimidos y suspensiones y soluciones acuosas. En el caso de comprimidos para uso oral, los vehículos que son comúnmente usados incluyen lactosa y almidón de maíz. Se añaden también normalmente agentes lubricantes, como estearato de magnesio. Para una administración oral en una forma de cápsula, los diluyentes útiles incluyen lactosa y almidón de maíz seco. Cuando las suspensiones acuosas se administran por vía oral, el ingrediente activo se combina con agentes emulsionantes y suspensores. Si se desea, se pueden añadir ciertos agentes edulcorantes y/o aromáticos y/o colorantes.

Otros vehículos o excipientes adecuados para las formulaciones y composiciones anteriormente indicadas se pueden encontrar en los textos farmacéuticos estándar, por ejemplo, en "Remington's Pharmaceuticals Sciences", "The

Science and Practice of Pharmacy", 19° Ed. Mack Publishing Company, Easton, Penn, (1995).

Los niveles de dosificación entre aproximadamente 0,01 y aproximadamente 100 mg/kg de peso corporal por día, preferentemente entre aproximadamente 0,1 y aproximadamente 50 mg/kg de peso corporal por día del compuesto inhibidor de proteasa descrito en la presente memoria descriptiva son útiles en una monoterapia para la prevención y tratamiento de una enfermedad mediada por el HCV. Normalmente, la composición farmacéutica de esta invención se administrará de aproximadamente 1 a aproximadamente 5 veces por día o, alternativamente, en forma de una infusión continua. Esta administración puede ser usada como una terapia crónica o aguda. La cantidad de ingrediente activo que puede ser combinado con los materiales excipientes para producir una forma de dosificación única variará dependiendo del hospedante tratado y del modo particular de administración. Una preparación típica contendrá de aproximadamente 5% a aproximadamente 95% de compuesto activo (p/p). Preferentemente, estas preparaciones contienen de aproximadamente 20% a aproximadamente 80% de compuesto activo.

Como el experto en la técnica apreciará, se pueden requerir dosis inferiores o superiores a las anteriormente citadas. La dosificación específica y los regímenes de tratamiento para cualquier paciente particular dependerán de una diversidad de factores, incluida la actividad del compuesto específico empleado, la edad, peso corporal, estado general de salud, sexo, dieta, tiempo de administración, velocidad de excreción, combinación de fármacos, la gravedad y el curso de la infección, la disposición del paciente para la infección y el criterio del facultativo encargado. Generalmente, el tratamiento se inicia con dosificaciones pequeñas sustancialmente menores que la dosis óptima del péptido. Posteriormente, la dosificación se aumenta en pequeños incrementos hasta que se alcanza el efecto óptimo

122

bajo las circunstancias. En general, el compuesto se administra lo más deseablemente a un nivel de concentración que suministrará generalmente resultados antivirales eficaces sin provocar efectos secundarios peligrosos o perjudiciales.

Cuando la composición de esta invención comprende una combinación de un compuesto de fórmula 1 y uno o más agentes terapéuticos o profilácticos adicionales, tanto el compuesto como el agente adicional deben estar presentes a niveles de dosificación entre aproximadamente 10 y 100% y, más preferentemente, entre aproximadamente 10 y 80% de la dosificación normalmente administrada en un régimen de monoterapia.

Cuando estos compuestos o sus sales farmacéuticamente aceptables se formulan junto con un vehículo farmacéuticamente aceptable, la composición resultante puede ser administrada *in vivo* a mamíferos, como el hombre, para inhibir la NS3-proteasa de HCV o para tratar o prevenir una infección por virus HCV. Este tratamiento se puede conseguir también usando un compuesto de esta invención en combinación con agentes que incluyen, pero sin limitación: α -, β -, δ -, ω -, tau- o γ -interferón, ribavirina, amantadina; otros inhibidores de NS3-proteasa de HCV; inhibidores de polimerasa de HCV, inhibidores de otras dianas en el ciclo vital del HCV, que incluye, pero sin limitación helicasa, NS2/3-proteasa o sitio de entrada de ribosoma interno (IRES); o sus combinaciones. Los agentes adicionales pueden ser combinados con compuestos de esta invención para crear una forma de dosificación única. Alternativamente, estos agentes adicionales pueden ser administrados separadamente a un mamífero como parte de una forma de dosificación múltiple.

Consecuentemente, otra realización de esta invención proporciona un método para inhibir la actividad de NS3-proteasa de HCV en un mamífero administrando un compuesto

de fórmula 1.

En una realización preferida, este método es útil para disminuir la actividad de NS3-proteasa del virus de la hepatitis C que infecta a un mamífero.

5 Si la composición farmacéutica comprende solamente un compuesto de esta invención como el componente activo, este método puede comprender adicionalmente la etapa de administrar a dicho mamífero un agente seleccionado entre un agente inmunomodulador, un agente antiviral, un inhibidor de
10 NS3-proteasa de HCV, un inhibidor de polimerasa de HCV o un inhibidor de otros agentes en el ciclo vital del HCV como helicasa, NS2/3-proteasa o IRES. Este agente adicional puede ser administrado al mamífero antes, simultáneamente o a continuación de la administración de la composición de esta
15 invención.

Un compuesto de fórmula 1 expuesto en la presente memoria descriptiva puede ser usado también como un reactivo de laboratorio. Un compuesto de esta invención puede ser usado también para tratar o prevenir la contaminación viral
20 de materiales y, por lo tanto, reducir el riesgo de infección viral del personal de laboratorio o médico o de pacientes que entran en contacto con tales materiales (por ejemplo, sangre, tejido, instrumentos y vestimentas quirúrgicas, instrumentos y vestimentas de laboratorio y aparatos
25 y materiales de recogida de sangre).

Un compuesto de fórmula 1 expuesto en la presente memoria descriptiva puede ser usado también como un reactivo de investigación.

Un compuesto de fórmula 1 puede ser usado también como
30 un testigo positivo para validar ensayos basados en células sustitutivas o en ensayos de replicación viral *in vitro* o *in vivo*.

Detalles adicionales de la invención se ilustran en los siguientes ejemplos, que debe entenderse que son no limitativos con respecto a las reivindicaciones anejas.
35

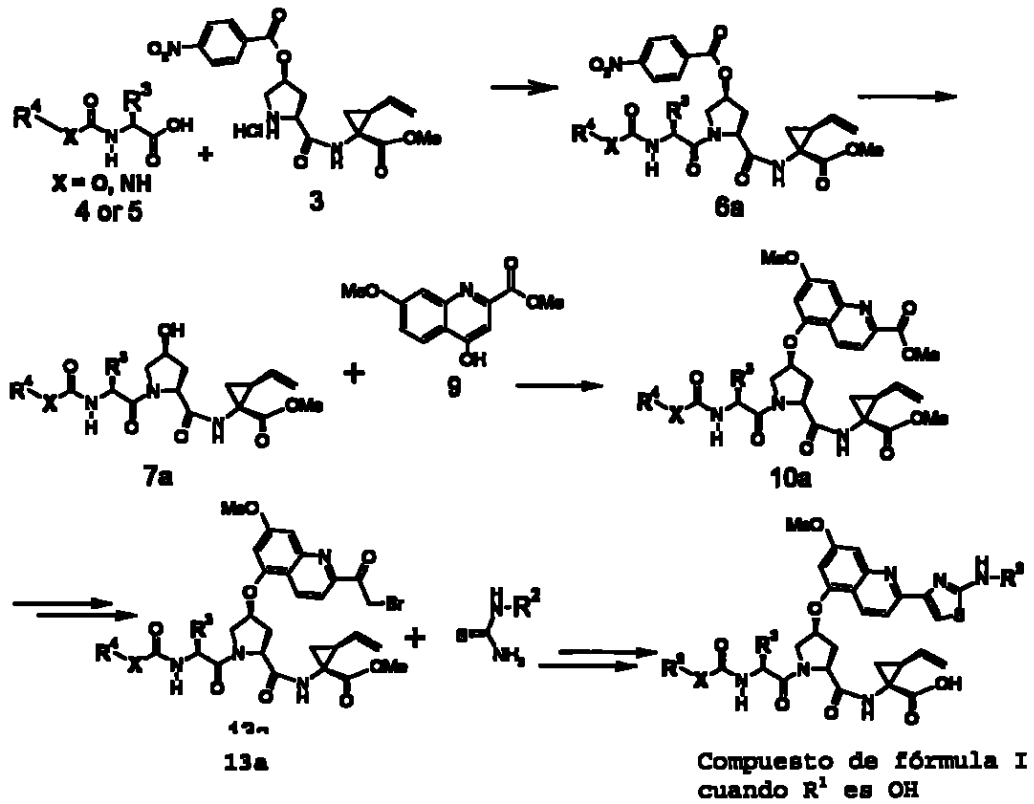
Metodología

En general, los compuestos de fórmula 1, y los intermedios para el mismo, se preparan por métodos conocidos usando condiciones de reacción que se conoce que son adecuadas para los reactantes. Varios de estos métodos se describen en los documentos WO 00/09543, WO 00/09558 y en la patente de EE.UU. n° 6.323.180, incorporados en esta memoria como referencia.

Los compuestos de fórmula I en la que R^1 es NHSO_2R^{1A} , como se definen en la presente memoria descriptiva, se preparan acoplando el correspondiente ácido de fórmula I (es decir, R^1 es hidroxilo) con una sulfonamida apropiada de fórmula $R^{1A}\text{SO}_2\text{NH}_2$ en presencia de un agente de acoplamiento bajo condiciones estándar. Aunque se pueden emplear diversos agentes de acoplamiento comúnmente usados, se ha encontrado que son prácticos TBTU y HATU. Las sulfonamidas están disponibles en el comercio o se pueden preparar por métodos conocidos.

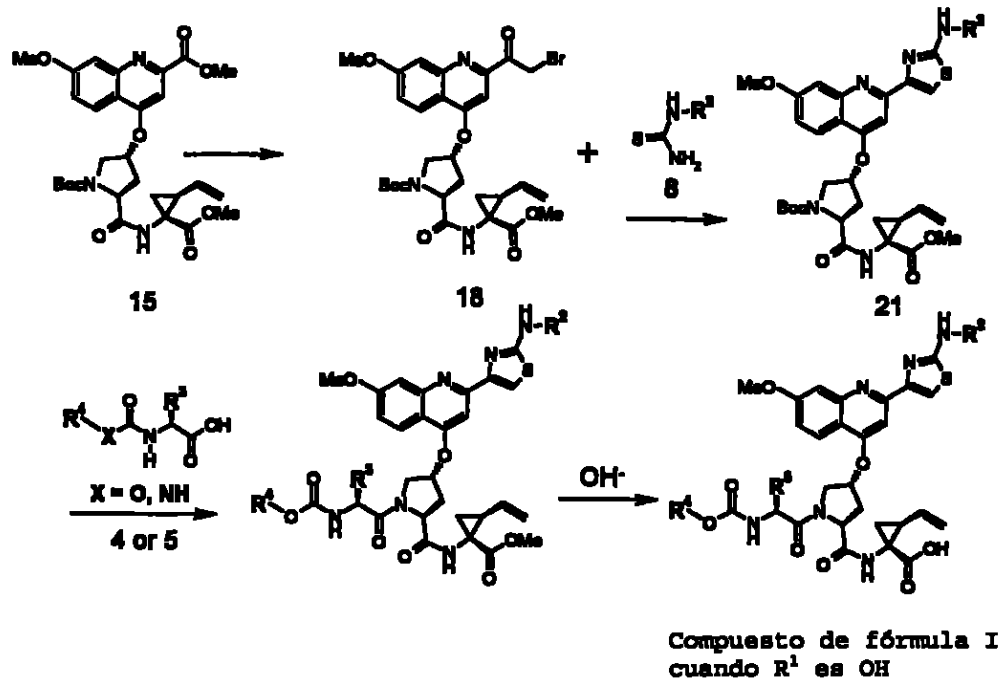
Los siguientes esquemas ilustran dos procedimientos convenientes que usan métodos conocidos para preparar los compuestos de fórmula 1 cuando R^1 es OH.

Esquema 1:



5

Esquema 2:



10

126

EJEMPLOS

Las temperaturas se proporcionan en grados Celsius. Los porcentajes de las soluciones expresan una relación de peso a volumen, y las relaciones de las soluciones expresan una relación de volumen a volumen, salvo que se establezca otra cosa. Los espectros de resonancia magnética nuclear (RMN) se registraron en un espectrómetro de 400 MHz Bruker; los desplazamientos químicos (δ) se expresan en partes por millón. La cromatografía rápida se llevó a cabo sobre gel de sílice (SiO_2) según la técnica de cromatografía rápida de Still et al., J. Org. Chem., (1978), 43, 2923).

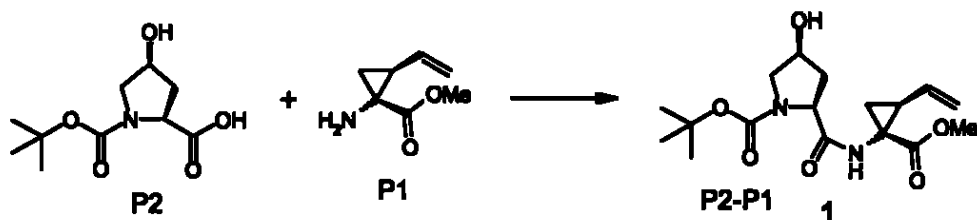
Las abreviaturas usadas en los ejemplos incluyen:

DBU: 1,8-diazabicyclo[5.4.0]undec-7-eno; DCM: diclorometano; DIAD: diisopropilazodicarboxilato; DIEA: diisopropiletilamina; DIPEA: diisopropiletilamina; DMF: N,N-dimetilformamida; DMAP: 4-(dimetilamino)piridina; EtOAc: acetato de etilo; HATU: [hexafluorofosfato de (O-7-azabenzotriazol-1-il)-1,1,3,3-tetrametiluronio]; HPLC: cromatografía líquida de alta resolución; MS: espectrometría de masas (MALDI-TOF: ionización por disorción láser asistida en matriz - tiempo de vuelo, FAB: bombardeo con átomos rápidos); Me: metilo; MeOH: metanol; Ph: fenilo; T.A.: temperatura ambiente (18 a 22°); terc.butilo o t-butilo: 1,1-dimetiletilo; TBG: terc-butil-glicina: terc-leucina; TBTU: tetrafluoroborato de 2-(1H-benzotriazol-1-il)-1,1,3,3-tetrametil-uronio; TFA: ácido trifluoroacético y THF: tetrahidrofurano.

Síntesis de compuestos de formula (I):

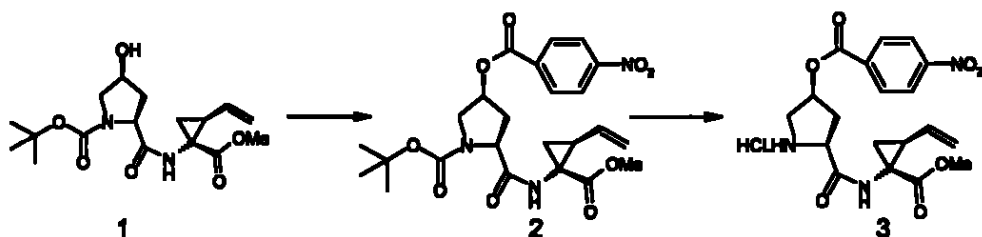
El intermedio de dipéptido 15 (Esquema 2) y 2-carbometoxi-4-hidroxi-7-metoxiquinolina 9 (Esquema 1) se sintetizaron según los métodos descritos en el documento WO 00/09543.

Síntesis de dipéptido 1



Una mezcla de Boc-hidroxiprolina (50,0 g, 216 mmol),
éster metílico de vinyl-ACCA (42,25 g, 238 mmol, 1,1
equiv.), TBTU (76,36 g, 238 mmol) 1,1 equiv.) y DIPEA (113
5 ml, 649 mmol, 3 equiv.) en DMF (800 ml) se agitó a T.A. ba-
jo una atmósfera de nitrógeno. Después de 3,5 h, el disol-
vente se evaporó y el residuo se extrajo con EtOAc. El ex-
tracto se lavó con ácido clorhídrico (10%), bicarbonato de
sodio saturado y salmuera. La fase orgánica se secó segui-
10 damente sobre sulfato de magnesio, se filtró y se evaporó
para suministrar un aceite. Después de secar durante una
noche bajo alto vacío, se obtuvo el dipéptido 1 en forma de
una espuma amarilla (72,0 g, 94%, pureza > 95% por HPLC).

15 Preparación de dipéptido 3

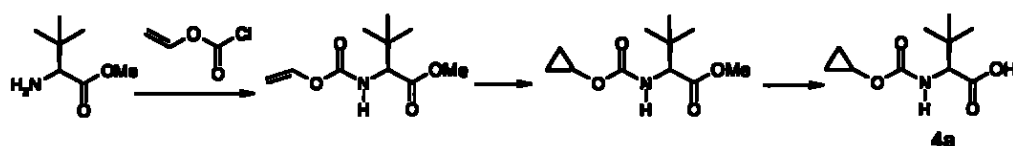


El dipéptido 1 (72,0 g, 203 mmol), trifenilfosfina
(63,9 g, 243,8 mmol, 1,2 equiv.) y ácido 4-nitrobenzoico
(41,08 g, 245,8 mmol, 1,2 equiv.) se disolvieron en THF se-
20 co (1,4 l). La solución agitada se enfrió a 0°C bajo una
atmósfera de nitrógeno. Seguidamente se añadió gota a gota
azodicarboxilato de dietilo (38,4 ml, 244 mmol, 1,2 equiv.)
durante 45 minutos y la reacción se dejó calentar a T.A.
Después de 4 h, el disolvente se evaporó. El residuo se di-
25 vidió en cuatro partes. Cada una de estas se purificó por
cromatografía sobre gel de sílice fina (malla 10-40 µm,

diámetro de la columna 12 cm, longitud de la columna 16 cm) usando un gradiente de 2:1 hexano/EtOAc a 1:1 de hexano/EtOAc hasta EtOAc puro. De esta manera, se obtuvo éster de Boc-dipéptido 2 en forma de un sólido blanco amorfo después de evaporar los disolventes y secar los residuos bajo alto vacío a 70°C durante 1 h (108,1 g, cuantitativo-105%). Una solución de cloruro de hidrógeno 4 N en dioxano se añadió al éster de Boc-dipéptido 2 (108 g, 243 mmol) dando lugar a una solución incolora. La solución se agitó a T.A. durante 1 h. El disolvente se evaporó y el residuo se colocó bajo alto vacío durante 3 h suministrando la sal de hidrocloreuro del compuesto 3 en forma de un sólido amorfo. El sólido se usó como tal.

15 Síntesis de carbamatos 4

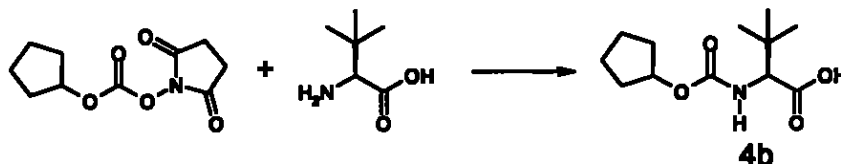
Preparación de carbamato 4a:



A 0°C, se añadieron a una solución de éster metílico de terc-butyl-glicina (590 mg, 3,25 mmol) en THF (8 ml): cloroformiato de vinilo (0,55 ml, 6,47 mmol) y trietilamina (1,5 ml, 8,25 mmol). La temperatura se dejó elevar a T.A. La solución se agitó durante 16 h. La solución se concentró y el residuo se disolvió en EtOAc. La solución de EtOAc se lavó con solución acuosa al 10% de ácido cítrico (2x), una solución acuosa saturada de NaHCO₃ (2x) y salmuera, se secó y se concentró para producir el correspondiente carbamato de vinilo (608 mg) en forma de un aceite incoloro. El aceite se disolvió en DCM (4 ml), se enfrió a 0°C y se añadieron diyodometano (0,15 ml, 1,86 mmol) y dietil-cinc (95 µl, 0,93 mmol). Apareció un sólido blanco en primer lugar pero se disolvió con el tiempo (~ 1 h). La suspensión/solución se agitó a T.A. durante 5 h. Se añadió una solución satura-

da de cloruro de amonio y la solución se extrajo con EtOAc (2x). El extracto orgánico se secó (MgSO_4) y se concentró. El residuo se purificó por cromatografía de columna rápida. Una elución con hexano:EtOAc 95:5 proporcionó el correspondiente éster metílico en forma de un aceite incoloro (96 mg, 90% de rendimiento). Una solución del éster metílico (93 mg; 0,45 mmol) en THF (5 ml), MeOH (1 ml) y una solución acuosa de LiOH (45 mg; 1,81 mmol) en agua (2 ml) se agitó durante 4 h. La solución se diluyó con agua y se extrajo con EtOAc (2x). La solución acuosa se acidificó mediante la adición de HCl 1 N, y la solución ácida se extrajo con EtOAc (2x). Los extractos orgánicos combinados se secaron (MgSO_4), se filtraron y se evaporaron para obtener el carbamato deseado 4a en forma de un sólido blanco (53 mg; 60% de rendimiento).

Preparación de carbamato 4b



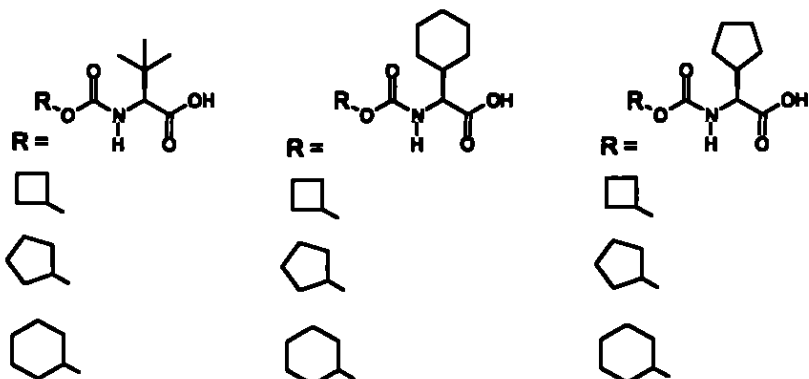
Se añadió THF (350 ml) a un matraz que contenía éster ciclopentílico y éster 2,5-dioxo-pirrolidin-1-ílico de ácido carbónico (9,00 g; 39,6 mmol) y terc-butil-glicina (6,24 g; 47,5 mmol) dando lugar a una suspensión. Se añadió agua destilada (100 ml) con agitación vigorosa. Una pequeña cantidad de sólido permanecía sin disolver. Seguidamente se añadió trietilamina (16,6 ml; 119 mmol) dando lugar a una solución homogénea que se agitó a T.A. Después de 2,5 h, el THF se evaporó y el residuo acuoso se diluyó con agua (100 ml). La reacción se hizo básica mediante la adición de NaOH 1 N (25 ml - pH final > 10). La solución se lavó con EtOAc (2 x 200 ml) y la fase acuosa se acidificó con HCl 1 N (aprox. 70 ml - pH final < 2). La solución turbia se extrajo con EtOAc (200 + 150 ml). El extracto se secó (MgSO_4) y

130

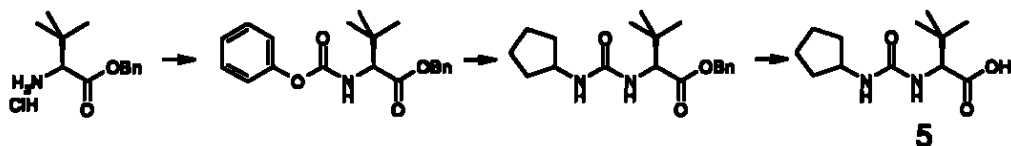
se evaporó para proporcionar carbamato 4b en forma de un sólido blanco (8,68 g).

Preparación de otros carbamatos

5 Usando el procedimiento descrito anteriormente y usando combinaciones apropiadas de terc-butil-glicina, ciclo-
pentil-glicina o ciclohexil-glicina y éster ciclobutílico,
ciclopentílico, ciclohexílico o 2,5-dioxo-pirrolidin-1-
fílico de ácido carbónico, se prepararon los carbamatos de
10 las fórmulas siguientes:



Preparación de ureas 5



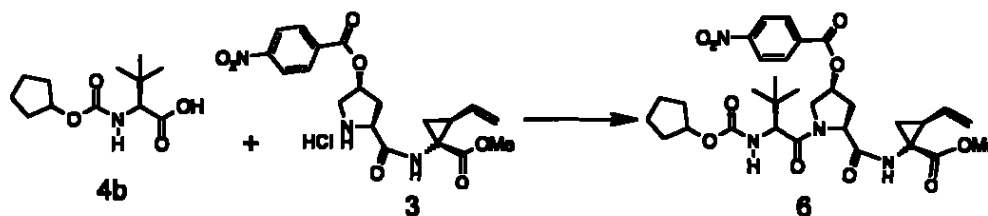
15 Una solución de sal de hidrocloreuro de éster bencílico
de terc-butil-glicina (2,55 g; 9,89 mmol) en THF (20 ml) y
piridina (2,0 ml; 24,73 mmol) se enfrió a 0°C. Se añadió
gota a gota cloroformiato de fenilo (1,30 ml; 10,19 mmol) a
la solución enfriada. La suspensión resultante se agitó du-
20 rante 5 minutos a 0°C y seguidamente a T.A. durante 1,5 h.
La mezcla de reacción se diluyó con EtOAc, se lavó con áci-
do cítrico al 10% (2x), agua (2x), NaHCO₃ saturado (2x),
agua (2x) y salmuera (1x), se secó (MgSO₄), se filtró y se
evaporó para obtener el compuesto en bruto en forma de un
25 aceite casi incoloro (3,73 g; > 100%; supuestamente 9,89

mmol). El producto en bruto (1,01 g; 2,97 mmol) se disolvió en DMSO (6,5 ml) y se añadió gota a gota ciclopentilamina. La mezcla de reacción se agitó a T.A. durante 45 minutos. La mezcla de reacción se diluyó con EtOAc. La fase orgánica se lavó con ácido cítrico al 10% (2x), agua (2x), NaHCO₃ saturado (2x), agua (2x) y salmuera (1x), se secó (MgSO₄), se filtró y se evaporó para proporcionar el producto en bruto de ciclopentilurea-Tbg-OBn en forma de un aceite casi incoloro. El material en bruto se purificó por cromatografía de columna rápida con sílice usando hexano:EtOAc 9:1 para separar las impurezas menos polares y 7:3 para eluir el producto purificado en forma de un aceite incoloro espeso (936 mg; 95%). El producto de éster bencílico (936 mg, 2,82 mmol) se desprotegió bajo un balón relleno de hidrógeno a T.A. en solución de etanol absoluto (15 ml) agitando la solución con 10% de Pd/C (93,6 mg) durante 5,5 h. La mezcla de reacción se filtró a través de un filtro de 0,45 micrómetros y se evaporó hasta sequedad para proporcionar la urea 5 en forma de un sólido blanco (668,8 mg; 98%).

¹H RMN (400 MHz, DMSO-d₆): δ 12,39 (s, 1H), 6,09 (d, J = 7,4 Hz, 1H), 5,93 (d, J = 9,4 Hz, 1H), 3,90 (d, J = 9,4 Hz, 1H), 3,87-3,77 (m, 1H), 1,84-1,72 (m, 2H), 1,63-1,42 (m, 4H), 1,30-1,19 (m, 2H), 0,89 (s, 9H).

M.S. (electropulverización): 241,0 (M-H) - 243,0 (M+H)+. Homogeneidad de HPLC de fase inversa (0.06% TFA; CH₃CN : H₂O) : 99%.

Síntesis de tripéptido 6

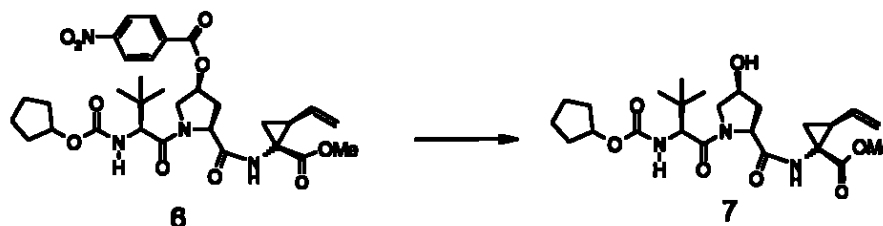


El carbamato 4b (6,15 g, 22,5 mmol) y TBTU (7,72 g,

132

24,7 mmol) se pusieron en suspensión en DCM y la suspensión se agitó rápidamente. Se añadió DIPEA (3,92 ml, 22,5 mmol) a T.A. y después de 10 minutos la reacción era casi homogénea. Seguidamente se vertió en la reacción una solución de
 5 dipéptido 3 (10,39 g, 23,6 mmol) en DCM anhidro (100 ml) que contenía DIPEA (4,11 ml, 23,62 mmol). La solución amarilla resultante se dejó agitar durante 14 h. El disolvente se evaporó seguidamente produciendo un jarabe amarillo que se extrajo con EtOAc (300 + 150 ml) y se lavó con HCl 0,05
 10 N (2 x 200 ml), Na₂CO₃ saturado (300 ml) y salmuera (150 ml). Los extractos combinados se secaron sobre MgSO₄ y se evaporaron para producir el tripéptido 6 en forma de una espuma amarilla pálida (15,68 g, cuantitativo).

15 Síntesis de tripéptido 7:

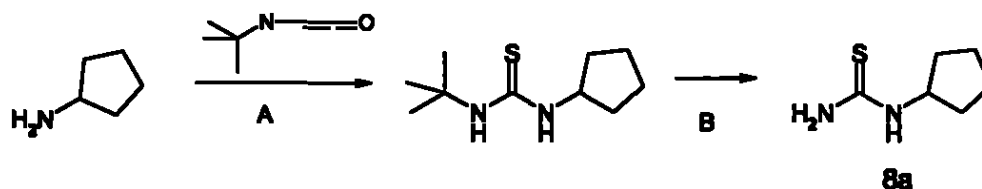


El tripéptido 6 (15,68 g) se disolvió en THF (200 ml) y se añadió agua (30 ml). La solución resultante se enfrió a 0°C y se añadió una solución de monohidrato de hidróxido
 20 de litio (1,18 g, 28,12 mmol) durante 3 minutos con agitación vigorosa. Después de 3 h a 0°C, la base en exceso se neutralizó con HCl 1 N (pH final de aprox. 6) y el THF se evaporó, dando lugar a una suspensión acuosa (goma amarilla). La mezcla se extrajo con EtOAc (2 x 200 ml) y se lavó
 25 con NaHCO₃ saturado (2 x 300 ml). Los extractos combinados se secaron sobre MgSO₄ y se evaporaron para producir una espuma amarilla pálida. Una cromatografía rápida de la espuma sobre gel de sílice usando EtOAc como eluyente proporcionó 7 en forma de un sólido amorfo blanco (9,77 g, 91%).

30

Preparación de Tioureas 8

Síntesis de tiourea 8a:



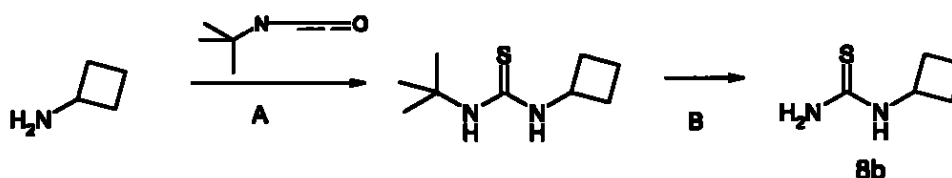
A una solución de isotiocianato de ter-butilo (5,0 ml; 39,4 mmol) en DCM (200 ml) se añadió ciclopentilamina (4,67 ml; 47,3 mmol) y seguidamente DIEA, y la mezcla de reacción se agitó a T.A. durante 2 h. La mezcla se diluyó con EtOAc, se lavó con una solución acuosa al 10% de ácido cítrico (2x), NaHCO₃ saturado (2x), H₂O (2x) y salmuera (1x). La capa orgánica se secó sobre MgSO₄ anhidro, se filtró y se evaporó para producir N-terc-butil-N'-ciclopentil-tiourea en forma de un sólido blanco (3,70 g; rendimiento de 47%). La N-terc-butil-N'-ciclopentil-tiourea (3,70 g) se disolvió en HCl concentrado (46 ml). La solución amarilla oscura se calentó a reflujo suave. Después de 40 minutos, la mezcla de reacción se dejó enfriar a T.A. y posteriormente se enfrió en hielo y se hizo básica a pH 9,5 con sólido y una solución acuosa saturada de NaHCO₃. El producto se extrajo en EtOAc (3x). Los extractos de EtOAc combinados se lavaron con H₂O (2x) y salmuera (1x). La capa orgánica se secó (MgSO₄), se filtró y se concentró para producir un sólido color beige (2,46 g en bruto). La tritución del material en bruto en hexano/EtOAc 95/5 proporcionó, después de filtrar, la N-ciclopentilurea 8a en forma de un sólido blanco (2,38 g, 90% de rendimiento).

¹H RMN (400 MHz, DMSO-d₆): δ 7,58 (bs, 1H), 7,19 (bs, 1H), 6,76 (bs, 1H), 4,34 (bs, 1H), 1,92-1,79 (m, 2H), 1,66-1,55 (m, 2H), 1,55-1,30 (m, 4H). MS; es⁺ 144,9 (M + H)⁺, es⁻: 142,8 (M - H)⁻.

Preparación de tiourea 8b

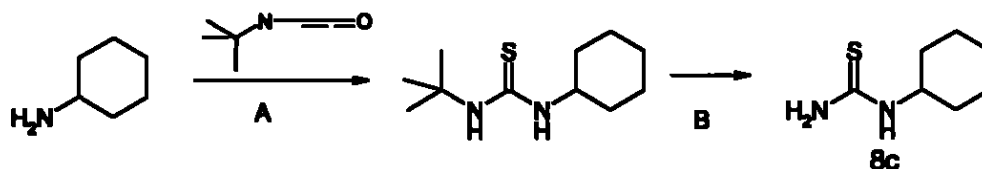
Usando el procedimiento anteriormente descrito y usan-

do ciclobutilamina disponible en el comercio en lugar de ciclopentilamina, se produjo la tiourea 8b:



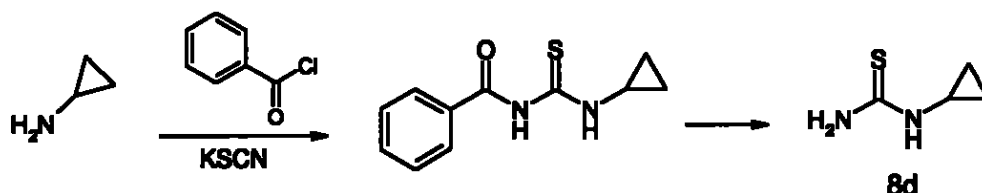
5 Preparación de tiourea 8c

Usando el procedimiento anteriormente descrito y usando ciclohexilamina disponible en el comercio en lugar de diclopentilamina, se produjo la tiourea 8c:



10

Preparación de tiourea 8d



A una solución de KSCN (4,60 g; 47,33 mmol) en acetona
 15 (35 ml) a 0°C, se añadió gota a gota cloruro de benzoilo
 (5,0 ml; 43,03 mmol). La solución lechosa se agitó en un
 baño con hielo durante 1,5 h y seguidamente se añadió gota
 a gota ciclopropilamina (3,2 ml; 46,0 mmol). La mezcla de
 reacción se agitó durante 1,5 h a 0°C y seguidamente se
 20 añadió más ciclopropilamina (0,50 ml, 7,22 mmol) y la mez-
 cla de reacción se agitó a T.A. durante 30 minutos adicio-
 nales. La mezcla de reacción se vertió en hielo/H₂O (300
 ml), se agitó durante 5 minutos y el sólido amarillo lumi-
 noso se filtró y se lavó varias veces con H₂O y se secó pa-
 25 ra proporcionar N-benciloxi-N'-ciclopropil-tiourea (6,62
 g). La tiourea se puso en suspensión en una solución de

NaOH 2 N (50 ml) y se calentó a reflujo durante 15 minutos. La solución se enfrió a T.A., se saturó con NaCl sólido y se extrajo con EtOAc (3x). Los extractos combinados de EtOAc se lavaron con H₂O (2x) y salmuera (1x), se secaron
5 (MgSO₄), se filtraron y se evaporaron para proporcionar el producto en bruto en forma de un sólido blanco apagado. El sólido se trituró en hexano/EtOAc 5/5 para proporcionar la N-ciclopropil-tiourea 8d en forma de un sólido cristalino blanco (2,5 g; 50% de rendimiento).

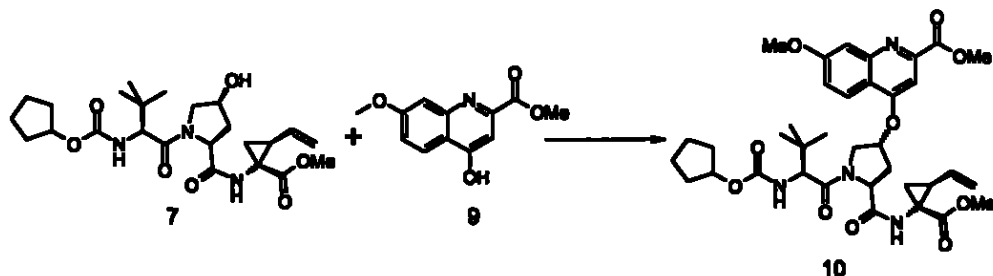
10 ¹H RMN (400 MHz, DMSO-d₆): δ 7,92 (bs, 1H), 7,61 (bs, 1H), 7,13 (bs, 1H), 2,39 (bs, 1H), 0,67-0,63 (m, 2H), 0,51-0,44 (m, 2H).

MS; es⁺ 116,9 (M + H)⁺, es⁻: 114,8 (M - H)⁻

15 **EJEMPLO 1**

Preparación de Compuesto 100

Etapas 1: Síntesis de tripéptido 10:



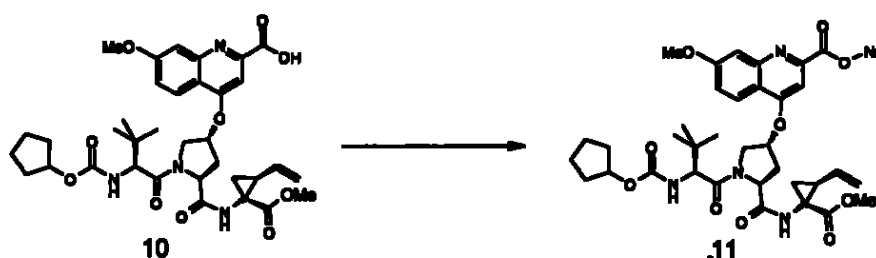
Al tripéptido 1 (1,0 g; 2,09 mmol) disuelto en THF (35
20 ml) se añadieron hidroquinolina 9 (729 mg; 3,13 mmol) y
trifenilfosfina (1,1 g; 4,2 mmol). La suspensión amarilla
se enfrió en un baño con hielo y se añadió gota a gota DIAD
(821 µl, 4,2 mmol). La solución se agitó a la temperatura
de un baño con hielo durante 30 minutos y a T.A. durante 16
25 h. La solución se evaporó hasta sequedad y el residuo se
disolvió en EtOAc, se lavó con solución saturada de bicar-
bonato de sodio (2x), agua (2x) y salmuera (1x), se secó
(MgSO₄), se filtró y se evaporó para obtener un aceite ama-
rillo que precipitó en reposo. El sólido en bruto se puso

en suspensión en DCM y el material insoluble se separó por filtración. La solución se concentró y el residuo se purificó por cromatografía rápida en hexano:EtOAc; 5:5, para separar todas las impurezas menos polares y en CHCl₃:EtOAc; 80:20 hasta que se había eluido todo el Ph₃P=O. El compuesto deseado se eluyó con CHCl₃:EtOAc; 65:35 en forma de un sólido blanco (1c; 70% de rendimiento).

M.S.(electropulverización) : 693.3 (M-H)- 695.4
(M+H)+ 717.4 (M+Na)+ .

Homogeneidad de HPLC de fase inversa (0,06% de TFA; CH₃CN:H₂O):99%.

Etapas 2: Monohidrólisis selectiva de éster 10:

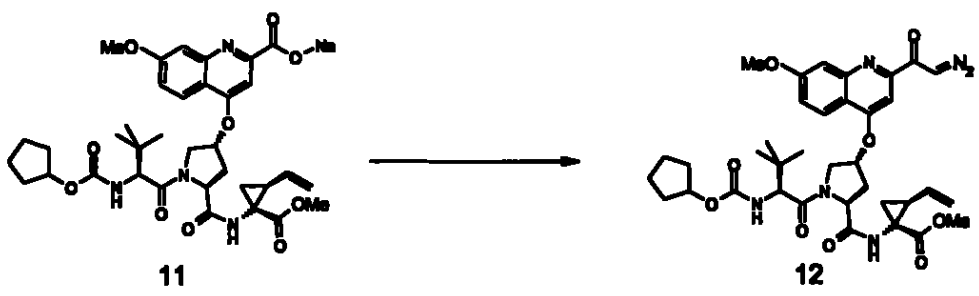


El tripéptido 10 (1 g; 1,44 mmol) se disolvió en THF (10 ml) y se añadieron MeOH (5 ml), agua (5 ml) y una solución acuosa 1 N de NaOH (1,5 ml) y la solución se agitó a T.A. durante 2 h. La mezcla se evaporó hasta sequedad y seguidamente se evaporó conjuntamente con MeOH:tolueno (1:1; 4x), tolueno (2x) y dietil-éter (2x) para obtener el producto (exento de agua) en forma de un sólido escamoso blanco (1,04 g; 100% de rendimiento).

M.S.(electropulverización): 679.3 (M-H)- 681.3
(M+H)+ 703.3 (M+Na)+ .

Homogeneidad de HPLC de fase inversa (0,06% de TFA; CH₃CN:H₂O):95%.

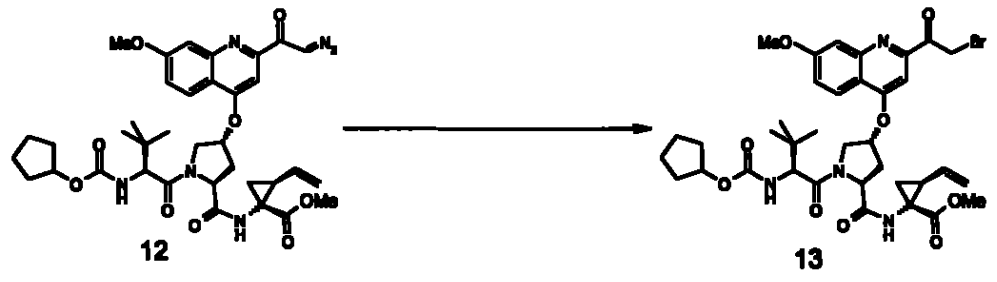
Etapas 3: Síntesis de diazocetona 12:



Se disolvió sal de sodio 11 (supuestamente 1,44 mmol) en THF (16 ml), se añadió trietilamina (301 μ l; 2,16 mmol) y la solución se enfrió a 0°C. Se añadió gota a gota cloroformiato de isobutilo (280 μ l; 2,16 mmol) y la suspensión blanca se agitó a 0°C durante 75 minutos y seguidamente se añadió una solución de diazometano (0,67 M en dietil-éter; 13 ml; 8,64 mmol). La mezcla de reacción se agitó 1 h a 0°C, 45 minutos a T.A. y se evaporó para proporcionar una suspensión espesa. Esta suspensión se disolvió en EtOAc y agua. La solución orgánica se lavó con NaHCO₃ saturado (2x), agua (2x) y salmuera (1x), se secó (MgSO₄), se filtró y se evaporó para proporcionar el producto de diazocetona en forma de un sólido color marfil (el material en bruto se usó para la siguiente etapa; supuestamente 1,44 mmol).

M.S.(electropulverización) : 703.3 (M-H) - 705.3 (M+H)+. Homogeneidad de HPLC de fase inversa (0.06% TFA; CH₃CN: H₂O) : 91%.

20 Etapas 4: Síntesis de bromocetona 13:



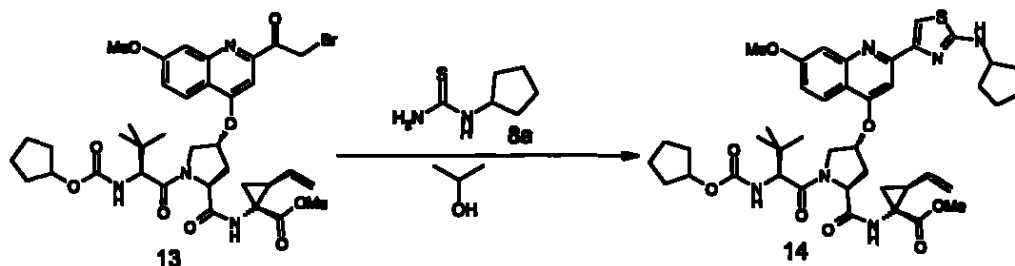
A 0°C, a una solución de diazocetona 12 (1,44 mmol) en THF (24 ml) se añadió gota a gota una solución de HBr (1,0 ml) y la mezcla se agitó durante 1 h. La solución se diluyó con EtOAc, se lavó con una solución saturada de NaHCO₃,

25

(2x), agua (2x) y salmuera (1x), se secó (MgSO_4), se filtró y se evaporó para proporcionar la bromocetona deseada en forma de un sólido color marfil-beige (1,1 g; supuestamente 1,44 mmol).

5 M.S. (electropulverización): 757,3 (M), 759,3 (M+2).

Etapas 5: Síntesis de triazolil-tripeptido 14:



Se disolvieron α -bromocetona 13 (0,40 mmol) y N-ciclopentiltiourea (68,5 mg; 0,48 mmol) en isopropanol (15 ml) y la solución amarilla se calentó a 70°C durante 75 minutos. La solución se dejó enfriar a T.A. y se evaporó hasta sequedad. El residuo se disolvió en EtOAc. La solución se lavó con NaHCO_3 saturado (2x), agua (2x) y salmuera (1x), se secó (MgSO_4), se filtró y se concentró para proporcionar el producto en forma de una espuma naranja-marrón. Una cromatografía de columna rápida en hexano:EtOAc 7:3 separó las impurezas menos polares y una con 6:4 recuperó el compuesto deseado en forma de una espuma amarilla luminosa (218 mg; 69%).

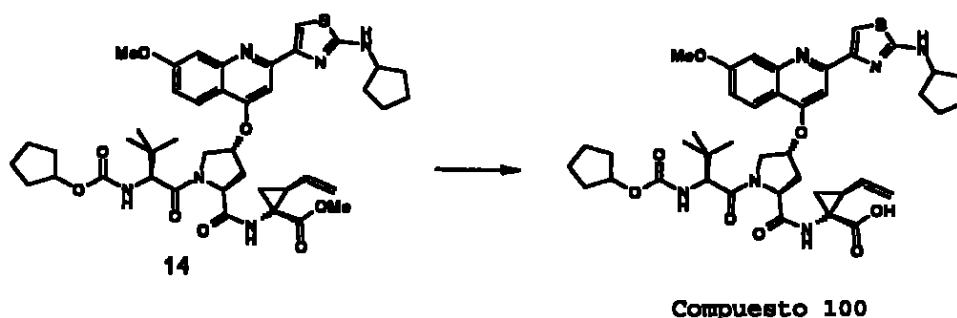
10
15
20

M.S.(electropulverización) : 801.4 (M-H)- 803.4 (M+H)+ 825 (M+Na)+ .

Homogeneidad de HPLC de fase inversa (0.06% TFA; CH_3CN : H_2O): 99%.

25

Etapas 6: hidrólisis de éster 14:



Una solución de éster metílico 14 (145 mg; 0,181 mmol) en THF (3 ml), MeOH (1,5 ml) y una solución acuosa de LiOH (75,8 mg; 1,81 mmol) en agua (1,5 ml) se agitó durante 18 h. La solución orgánica se concentró para proporcionar una suspensión blanca apagada que se diluyó con EtOAc y salmuer

10 ra para obtener una solución total. El pH se ajustó a 6 mediante la adición de HCl 1 N y la capa orgánica se extrajo adicionalmente con EtOAc (2x). Los extractores orgánicos combinados se lavaron con agua (2x), salmuera (1x), se secaron (MgSO₄), se filtraron y se evaporaron para proporcionar el compuesto deseado en forma de un sólido amarillo (138,2 mg,

15 97% de rendimiento).

Conversión a sal de Na:

El compuesto 100 (138,2 mg; 0,175 mmol) se disolvió en MeOH (30ml) y se añadió 1 equivalente de NaOH 0,01 N (17,5 ml). La solución amarilla transparente se concentró para separar MeOH y se diluyó con agua, se congeló y se liofilizó para obtener el producto (sal de Na) en forma de un sólido amorfo amarillo (139 mg; rendimiento teórico: 142 mg; P.M. de la sal de Na: 810,95).

25 M.S.(electropulverización): 787.2 (M-H)- 789.3 (M+H)+ 811.3 (M+Na)+. Homogeneidad de HPLC de fase inversa (0.06% TFA; CH₃CN : H₂O): 98%.

¹H RMN (400 MHz,DMSO-d₆): mezcla de rotámeros aprox. 5:1; δ 8,14 (bs, 1H) , 8,02 (d, J = 9,2 Hz, 1H) , 7,89 (d, J = 6,7 Hz, 1H), 7,49-7,36 (m, 2H), 7,27 (bs, 1H),

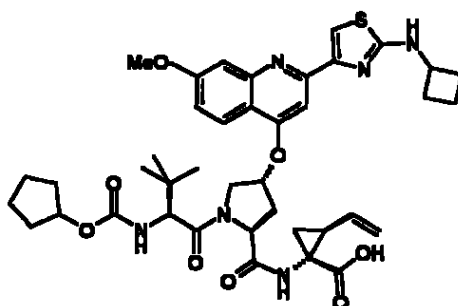
30

7,06-6,96 (m, 2H), 6,10-5,90 (m, 1H), 5,33 (s, 1H), 5,01 (d, J = 16,8 Hz, 1H), 4,84 (d, J = 10,6 Hz, 1H), 4,79-4,65 (m, 1H), 4,47-4,40 (m, 1H), 4,30 (d, J = 11,5 Hz, 1H),), 4,15 (d, J = 8,8 Hz, 1H), 4,00-3,85 (m, 2H), 3,90 (s, 3H), 2,37-2,26 (m, 1H), 2,15-1,91 (m, 2H), 1,80-1,23 (m, 18H), 0,96 y 0,86 (2x s, 9H).

EJEMPLO 2

Preparación de compuesto 101

Usando el mismo procedimiento descrito en el Ejemplo 1 y usando N-ciclobutil-tiourea 8b en lugar de usar N-ciclopentil-tiourea 8a en la etapa 5 se dio lugar al compuesto 101 en forma de la sal de TFA:



Compuesto 101

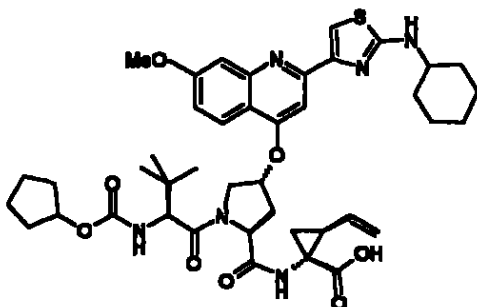
^1H RMN (400 MHz, DMSO- d_6): mezcla de rotámeros aprox. 90:10, descripción de isómeros principales; δ 8,59 (s, 1H), 8,45-8,39 (m, 1H), 8,25 (bs, 1H), 8,20 (d, J = 9,2 Hz, 1H), 7,84 (bs, 1H), 7,74 (s, 1H), 7,32-7,26 (m, 1H), 7,01 (d, J = 8,3 Hz, 1H), 5,78-5,66 (m, 2H), 5,20 (dd, J = 17,0, 1,6 Hz, 1H), 5,09-5,04 (m, 1H), 4,53-4,36 (m, 4H), 4,05-3,92 (m, 2H), 3,97 (s, 3H), 2,63-2,55 (m, 1H), 2,44-2,29 (m, 3H), 2,07-1,95 (m, 3H), 1,79-1,23 (m, 12H), 0,96 (s, 9H).

M.S.(electropulverización): 773.4 (M-H) - 775.4 (M+H)+. Homogeneidad de HPLC de fase inversa (0.06% TFA; CH_3CN : H_2O): 98%

EJEMPLO 3

Preparación de Compuesto 102

Usando el mismo procedimiento descrito en el Ejemplo 1 y usando N-ciclohexil-tiourea 8c en lugar de usar N-ciclopentil-tiourea 8a en la etapa 5 se dio lugar al Compuesto 102 en forma de la sal de TFA:



Compuesto 102

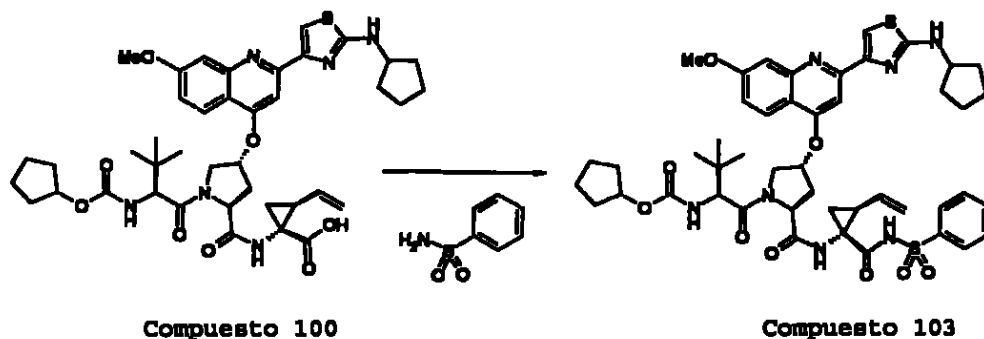
H^1 RMN (400 MHz, DMSO- d_6): mezcla de rotámeros aprox. 90:10, descripción del isómero principal; δ 8,61 (s, 1H), 8,26-8,17 (m, 2H), 8,13-8,04 (m, 1H), 7,81 (bs, 1H), 7,73 (bs, 1H), 7,32-7,24 (m, 1H), 7,07 (d, $J = 8,2$ Hz, 1H), 5,78-5,65 (m, 2H), 5,23-5,15 (m, 1H), 5,09-5,03 (m, 1H), 4,51-4,43 (m, 3H), 4,05-3,77 (m, 3H), 3,97 (s, 3H), 2,64-2,55 (m, 1H), 2,38-2,27 (m, 1H), 2,05-1,95 (m, 3H), 1,79-1,71 (m, 2H), 1,66-1,19 (m, 16H), 0,96 (s, 9H).

M.S. (electropulverización): 801,4 (M-H) - 803,4 (M+H)+. Homogeneidad de HPLC de fase inversa (0.06% TFA; CH_3CN : H_2O): 99%

20

EJEMPLO 4

Preparación de Compuesto 103



El Compuesto 100 (30 mg, 0,038 mmol) se combinó con HATU (17 mg, 0,045 mmol) y se disolvió en DMF anhidra (4 ml). La solución se agitó a T.A. antes de que se añadiera gota a gota DIPEA (26 μ l, 0,15 mmol) durante aprox. 1 minuto. La mezcla se agitó durante 60 minutos a T.A. y se analizó por HPLC analítica en cuanto a la formación del éster activado. A continuación de esto, se añadió una solución de bencenosulfonamida (23 mg, 0,15 mmol), DMAP (17 mg, 0,14 mmol) y TBU (22 μ l, 0,15 mmol) en DMF (1 ml). La mezcla de reacción se agitó 24 h a T.A. antes de ser vertida en EtOAc (50 ml) y se lavó con soluciones saturada de NaHCO_3 y saturada de salmuera. La fase orgánica se secó sobre MgSO_4 , se filtró y se concentró. El residuo se reconstituyó en DMSO y se purificó por HPLC preparativa. Una liofilización proporcionó el producto final (17 mg, 48%) en forma de un sólido amorfo amarillo pálido.

^1H RMN (400MHz, DMSO-d_6), δ 10,89 (s, 1H), 8,84 (s, 1H), 8,20 (d, $J = 8,8$ Hz, 1H), 8,18-8,08 (m, 2H), 7,89 (d, $J = 7,6$ Hz, 2H), 7,70 (dd, $J = 7,6, 7,6$ Hz, 2H), 7,58 (dd, $J = 7,7, 7,7$ Hz, 2H), 7,28 (bs, 1H), 7,11 (d, $J = 6,6$ Hz, 1H), 5,75 (bs, 1H), 5,37-5,25 (m, 1H), 5,12 (d, $J = 17$ Hz, 1H), 4,92 (d, $J = 12$ Hz, 1H), 4,58-4,42 (m, 3H), 4,24 (bs, 1H), 4,05 (d, $J = 7,8$ Hz, 1H), 3,97 (s, 3H), 3,93 (d, $J = 7,8$ Hz, 1H), 2,69-2,61 (m, 1H), 2,35-2,21 (m, 1H), 2,13-1,98 (m, 3H), 1,78-1,68 (m, 4H), 1,68-1,51 (m, 8H), 1,50-1,41 (m, 4H), 1,29-1,22 (m, 1H), 0,99 (s, 9H).

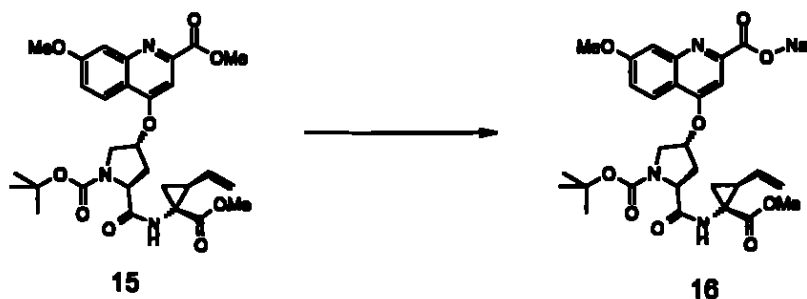
MS (electropulverización): 928,5 (M + H)+, y 926,5 (M-H) -.

RP-HPLC: Rt = 7,3 minutos (homogeneidad = 99%).

Usando la secuencia sintética mostrada en el Esquema 5 2, se prepararon los siguientes compuestos:

EJEMPLO 5

Etapla 1: síntesis de dipéptido 16:

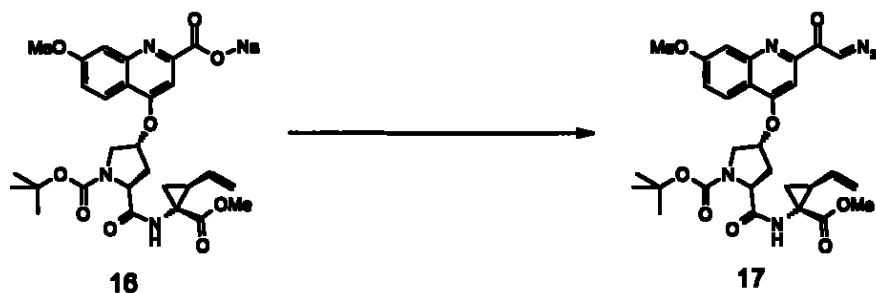


Se disolvió dipéptido 15 (4,0 g; 7,02 mmol) en THF (20 ml), MeOH (10 ml) y agua (10 ml) y se añadió una solución acuosa 1 N de NaOH (1,05 equivalentes; 7,4 ml). La solución se agitó a T.A. durante 2,75 h. La mezcla se evaporó hasta sequedad. El residuo se diluyó con agua, se congeló y se liofilizó para proporcionar la sal de sodio 16 en forma de un sólido amorfo blanco (4,28 mg).

M.S.(electropulverización): 554.2 (M-H)- 556.3 (M+H)+ 578.2 (M+Na)+.

Homogeneidad de HPLC de fase inversa (0.06% TFA; CH₃CN: H₂O): 96%.

Etapla 2: síntesis de dipéptido-diazocetona 17:



La sal de sodio 16 (supuestamente 7,0,2 mmol) se di-

144

solvió en THF (78 ml); se añadió trietilamina (1,37 ml, 9,83 mmol) y la solución se enfrió a 0°C. Se añadió gota a gota cloroformiato de isobutilo (1,28 ml; 9,83 mmol) y la suspensión blanca se agitó a 0°C durante 2 h, y seguidamente se añadió una solución de diazometano (0,67 M en dietil-éter; 63 ml; 42,13 mmol). La mezcla de reacción se agitó 1 h a 0°C, 1,25 h a T.A. y se evaporó para proporcionar una suspensión espesa. Esta suspensión se disolvió en EtOAc y agua. La solución orgánica se lavó con NaHCO₃ saturado (2x), agua (2x) y salmuera (1x), se secó (MgSO₄), se filtró y se evaporó para proporcionar la diazocetona 17 en forma de un sólido color beige (material en bruto usado para la siguiente etapa; supuestamente 7,02 mmol).

M.S. (electropulverización): 578.2 (M-H) - 580.3 (M+H)+. Homogeneidad de HPLC de fase inversa (0,06% de TFA; CH₃CN:H₂O): 90%.

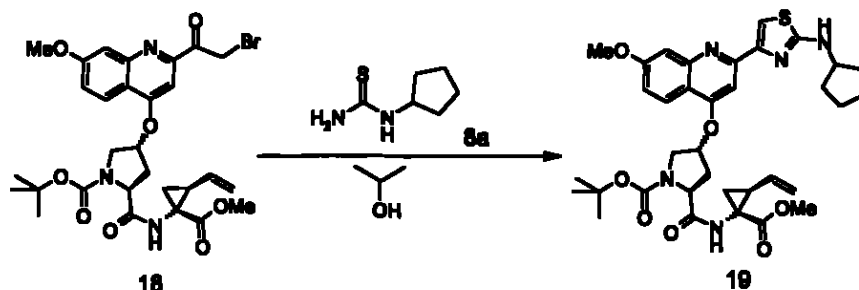
Etapa 3: Síntesis de dipéptido-bromocetona 18:



A 0°C, a una solución de diazocetona (supuestamente 1,44 mmol) en THF (116 ml) se añadió gota a gota una solución acuosa al 48% de HBr (5,1 ml) y la mezcla se agitó durante 2 h. La solución se diluyó con EtOAc, se lavó con una solución saturada de NaHCO₃ (2x), agua (2x) y salmuera (1x), se secó (MgSO₄), se filtró y se evaporó para proporcionar la bromocetona deseada en forma de un sólido color beige (4,25 g; 6,72 mmol).

M.S. (electropulverización): 632 (M) 634,2 (M+2).

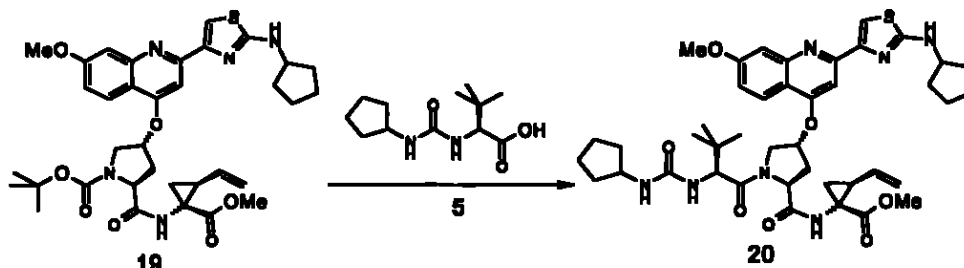
Etapa 4: Síntesis de dipéptido 19:



Se disolvieron α -bromocetona 18 (512 mg; 0,81 mmol) y N-ciclopentiltiurea (128,4 mg; 0,89 mmol) en isopropanol (20 ml). La solución amarilla resultante se calentó a 70°C durante 1,5 h. La solución se dejó enfriar a T.A. y se evaporó hasta sequedad. El residuo se diluyó con EtOAc. La solución de EtOAc se lavó con NaHCO₃ saturado (2x), agua (2x) y salmuera (1x), se secó (MgSO₄), se filtró y se concentró para proporcionar el producto en forma de una espuma color naranja-marrón. Una cromatografía en columna rápida en hexano:EtOAc 7:3 separó las impurezas menos polares y con 6:4 se recuperó el compuesto deseado en forma de un sólido amarillo luminoso (411,5 mg; 75%).

M.S. (electropulverización): 676,3 (M-H) - 678,3 (M+H)⁺
Homogeneidad HPLC de fase inversa (0,06% TFA; CH₃CN:H₂O): 99%.

Etapa 5: Síntesis de tripéptido 20



Se disolvió el Boc-dipéptido (32,6 g; 0,048 mmol) en HCl/dioxano 4 N (3 ml) y se agitó a T.A. Después de 2 h, la mezcla de reacción fue tratada evaporando hasta sequedad. La sal de HCl se obtuvo así en forma de un sólido color blanco apagado. La sal de HCl se sometió a alto vacío du-

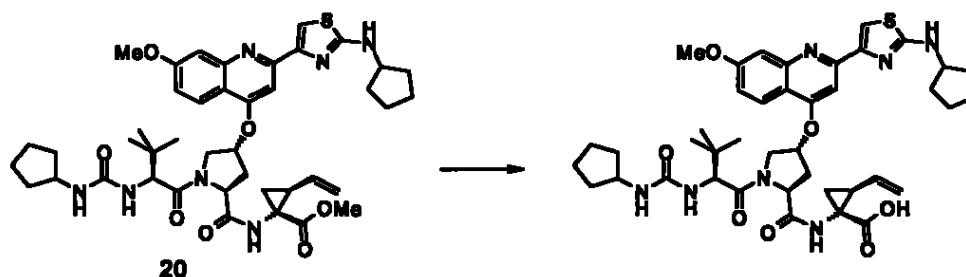
46

rante 30 minutos. A una solución de la sal de HCl en DCM (2 ml) y DIEA (33 μ l; 0,192 mmol) se añadió urea 5 (13,96 mg; 0,058 mmol), y seguidamente un agente de acoplamiento de HATU (21,90 mg; 0,058 mmol). La mezcla de reacción se agitó a T.A. durante 3 h. La mezcla de reacción se diluyó con EtOAc, se lavó con NaHCO₃ saturado (2x), agua (2x) y salmuera (1x), se secó (MgSO₄), se filtró y se evaporó para proporcionar el producto en bruto en forma de un aceite espeso amarillo (supuestamente 0,048 mmol).

M.S. (electropulverización): 800,4 (M-H) - 802,4 (M+H)⁺. Homogeneidad de HPLC de fase inversa (0,06% TFA; CH₃CN:H₂O): 95%.

Etapas 6: Hidrólisis de éster metílico de tripéptido

20:



Una solución de éster metílico 20 (77,80 mg; 0,097 mmol) en THF (2 ml) y MeOH (1 ml) y una solución acuosa de LiOH (40,7 mg; 0,97 mmol) en agua (1 ml) se agitó durante 16 h. La solución orgánica se concentró para proporcionar una suspensión color blanco apagado. El material en bruto se purificó por HPLC preparatoria (YMC Combiscreen ODS-AQ, 50 x 20 mm ID S-5 micrómetros, 120A; λ =220 nm) usando un gradiente lineal y 0,06% de TFA CH₃CN/H₂O. Las fracciones puras se combinaron, se concentraron y se convirtieron en la sal de sodio.

Conversión en la sal de Na:

Las fracciones concentradas se diluyeron con EtOAc y unos pocos ml de salmuera (basificadas a pH ~ 13 con NaOH 5

147

N y seguidamente neutralizadas a pH 5,5-6,0 con HCl 1 N). El producto se extrajo con EtOAc (3x), se lavó con agua (2x) y salmuera (1x), se secó (MgSO₄), se filtró y se evaporó hasta sequedad para proporcionar el producto neutro en forma de un sólido amarillo (52,7 mg; 70%). El producto neutro (49,4 mg; 0,0627 mmol) se disolvió en MeOH (10 ml) y se añadió 1 equivalente de NaOH 0,01 N (6,27 ml). La solución amarilla transparente se concentró para separar MeOH y se diluyó con agua, se congeló y se liofilizó para proporcionar el producto (sal de Na) en forma de un sólido amorfo amarillo (50,8 mg; rendimiento teórico: 50,8 mg; p.m. de la sal de Na: 809,75).

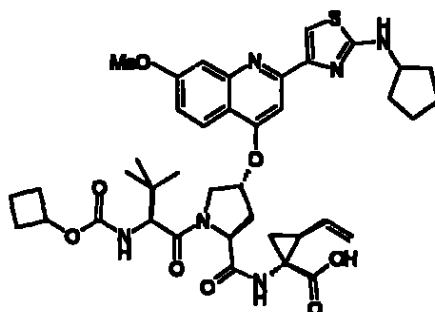
¹H RMN (400 MHz, DMSO-d₆): mezcla de rotámeros aprox. 8:1; δ 8,20 (bs, 1H), 7,96 (d, J = 8,8 Hz, 1H), 7,86 (d, J = 5,7 Hz, 1H), 7,51-7,47 (m, 2H), 7,26 (s, 1H), 6,97 (d, J = 8,6 Hz, 1H), 6,19-6,02 (m, 1H), 5,33 (bs, 1H), 4,99 (d, J = 16,8 Hz, 1H), 4,77 (d, J = 10,0 Hz, 1H), 4,52-4,41 (m, 2H), 4,34 (d, J = 11,0 Hz, 1H), 4,04-3,96 (m, 2H), 3,89 (s, 3H), 3,79-3,68 (m, 1H), 3,68-3,15 (bajo pico de agua, 2H), 2,45-2,36 (m, 1H), 2,05-1,92 (m, 2H), 1,82-1,35 (m, 16H), 1,35-1,12 (m, 2H), 0,91 y 0,84 (2x s, 9H).

M.S. (electropulverización): 786.4 (M-H) - 788.3 (M+H)+ 810 (M+Na)+. Homogeneidad de HPLC de fase inversa (0.06% TFA; CH₃CN: H₂O): 99%.

EJEMPLO 6

Compuesto 104:

Usando el mismo procedimiento descrito en el Ejemplo 6 y usando el ciclobutil-carbamato de terc-butil-glicina en la Etapa 5 en lugar de urea 5 y purificando el ácido carboxílico en bruto después de la Etapa 6 por HPLC, se proporcionó el compuesto del título en forma de una sal de TFA:



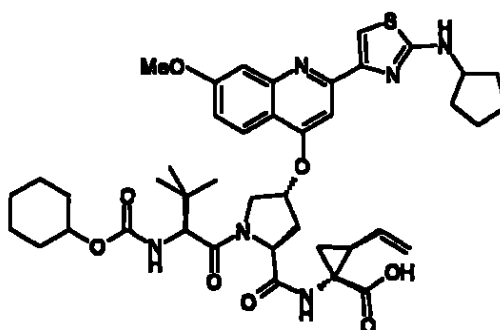
Compuesto 104

H^1 RMN (400 MHz, DMSO- d_6): mezcla de rotámeros aprox.
 5 7:1; δ 8.09 (bs, 1H), 8.02 (d, $J = 9.0$ Hz, 1H), 7.88 (d, $J = 6.5$ Hz, 1H), 7.45 (s, 1H), 7.40 (s, 1H), 7.28 (d, $J = 2.3$ Hz, 1H), 7.16-7.08 (m, 1H), 7.07-7.00 (m, 1H), 6.10-5.95 (m, 1H), 5.32 (bs, 1H), 5.00 (d, $J = 17.2$ Hz, 1H), 4.82 (d, $J = 11.4$ Hz, 1H), 4.64-4.52 (m, 1H), 4.48-
 10 4.41 (m, 1H), 4.29 (d, $J = 11.7$ Hz, 1H),), 4.12 (d, $J = 8.6$ Hz, 1H), 3.97-3.85 (m, 2H), 3.91 (s, 3H), 2.36-2.27 (m, 1H), 2.18-2.04 (m, 2H), 2.03-1.81 (m, 6H), 1.77-1.43 (m, 9H), 1.41-1.34 (m, 1H), 0.96 y 0.85 (2x s, 9H).

M.S. (electropulverización) : 773,3 (M-H) - 775,4
 15 (M+H)+. Homogeneidad de HPLC de fase inversa (0.06% TFA; CH_3CN : H_2O): 99%.

EJEMPLO 7**Compuesto 105**

20 Usando el mismo procedimiento descrito en el Ejemplo 6 y usando el ciclohexil-carbamato de terc-butil-glicina en la Etapa 5 en lugar de urea 5, se suministró el compuesto del título en forma de sal de sodio:



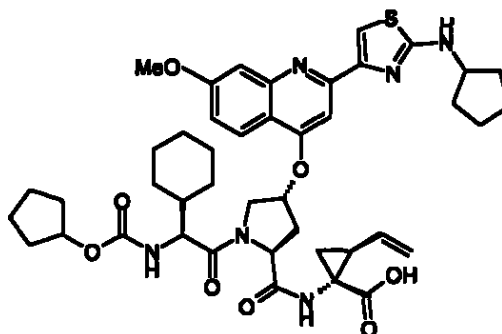
Compuesto 105

^1H RMN (400 MHz, DMSO- d_6): mezcla de rotámeros aprox.
 5 5:1; δ 8,29 (bs, 1H) , 8,02 (d, J = 9,0Hz, 1H) , 7,89 (d,
 J = 6,5 Hz, 1H), 7,45 (s, 1H), 7,40 (s, 1H), 7,27 (d, J
 = 2,2 Hz, 1H), 7,05 (d, J = 8,6 Hz, 1H), 7,00 (dd, J =
 2,0, 9,0 Hz, 1H), 5,89 (bs, 1H), 5,34 (s, 1H), 5,08 (d, J
 = 17,0 Hz, 1H), 4,91 (d, J = 9,4 Hz, 1H), 4,43 (dd, J =
 10 8,4 , 16,8 Hz, 1H), 4,36-4,24 (m, 1H), 4,14 (d, J = 8,6
 Hz, 1H),), 4,00-3,86 (m, 3H), 3,89 (s, 3H), 2,35-2,23
 (m, 1H), 2,04-1,91 (m, 5H), 1,79-1,41 (m, 10H), 1,39-1,08
 (m, 7H), 0,97 y 0,86 (2x s, 9H).

M.S.(electropulverización) : 801,4 (M-H)- 803,4
 15 (M+H)+. Homogeneidad de HPLC de fase inversa (0.06% TFA;
 CH₃CN: H₂O): 98%.

EJEMPLO 8

Compuesto 106



20

Usando el mismo procedimiento descrito en el Ejemplo 6
 y usando el ciclopentil-carbamato de ciclohexil-glicina en

130

la Etapa 5 en lugar de urea 5, se suministró el compuesto del título en forma de la sal de sodio:

^1H RMN (400 MHz, DMSO- d_6): mezcla de rotámeros aprox. 1:4; δ 8,22 y 8,04 (2xs, 1H), 8,00 (d, J = 9,2Hz, 1H), 7,91 (d, J = 6,5 Hz, 1H), 7,46 (s, 1H), 7,41 (s, 1H), 7,27 (d, J = 2,4 Hz, 1H), 7,20 (d, J = 8,4 Hz, 1H), 7,01 (dd, J = 2,4, 9,0 Hz, 1H), 6,09-5,98 (m, 1H), 5,34 (s, 1H), 4,98 (dd, J = 1,6, 17,4 Hz, 1H), 4,80 (d, J = 11,9 Hz, 1H), 4,73-4,67 (m, 1H), 4,43-4,31 (m, 2H), 4,05-3,95 (m, 2H), 3,95-3,84 (m, 1H), 3,90 (s, 3H), 2,38-2,29 (m, 1H), 2,03-1,92 (m, 2H), 1,83-1,21 (m, 24H), 1,21-0,83 (m, 5H)

M.S. (electropulverización): 813,4 (M-H) - 815,4 (M+H)+. Homogeneidad de HPLC de fase inversa (0.06% TFA; $\text{CH}_3\text{CN} : \text{H}_2\text{O}$) : 99%.

EJEMPLO 9

Compuesto 107:

Usando el mismo procedimiento descrito en el Ejemplo 6 y usando el ciclopentil-carbamato de ciclopentil-glicina en lugar de urea 5 en la Etapa 5, se suministró el compuesto del título, que se convirtió en su correspondiente sal de Na.

25 EJEMPLO 10

Ensayo de proteasa NS3-NS4A

El ensayo enzimático usado para evaluar el presente compuesto se describe en los documentos WO 00/09543 y WO 00/59929.

30

EJEMPLO 11

Ensayo de replicación de RNA de HCV basado en células

Cultivo celular

Células Huh7 que mantienen establemente un replicón de

HCV subgenómico fueron establecidas como se describió previamente (Lohman et al., 1999. Science 285: 110-113) y se denominaron la línea celular S22.3. Se mantienen células S22.3 en medio Earle modificado de Dulbecco (DMEM) complementado con 10% de FBS y 1 mg/ml de neomicina (Standard Medium). Durante el ensayo, se usó medio DMEM complementado con 10% de FBS, que contenía 0,5% de DMSO y carecía de neomicina (Medio de Ensayo). antes de 16 horas de la adición del compuesto, células S22.3 fueron tripsinizadas y diluidas hasta 50.000 células/ml en Medio Standard. Se distribuyeron 200 µl (10.000 células) en cada pocillo de una placa de 96 pocillos. La placa se incubó seguidamente a 37°C con 5% de CO₂ hasta el día siguiente.

15 Reactivos y materiales:

Producto	Empresa	Catálogo N°	Almacenamiento
DMEM	Wisent Inc.	10013CV	4°C
DMSO	Sigma	D-2650	TA
PBS de Dublecco	Gibco-BRL	14190-136	TA
Suero Bovino fetal	Bio-Whittaker	14-901F	-20°C/4°C
Neomicina (G418)	Gibco-BRL	10131-027	-20°C/4°C
Tripsina-EDTA	Gibco-BRL	25300-054	-20°C/4°C
Placas de 96 pocillos	Costar	3997	TA
Unidad de filtración de 0,22 µm de PVDF	Millipore	SLGV025LS	TA
Placa de titulación de pocillos profundos de polipropileno	Beckman	267007	TA

Preparación de Compuesto del Ensayo

Se añadieron 10 µl del compuesto del ensayo (en 100% de DMSO) a 2 ml de Medio de Ensayo hasta una concentración final de DMSO de 0,5% y la solución fue sonicada durante 15 minutos y se filtró a través de una unidad de filtración

Millipore de 0,22 μ M. SE transfirieron 900 μ l en la hilera A de una placa de titulación de pocillos profundos de polipropileno. Las hileras B a H contenían 400 μ l de partes alícuotas de Medio de Ensayo (que contenía 0,5% de DMSO) y
5 se usaron para preparar diluciones en serie 1/2) transfiriendo 400 μ l de una hilera a otra (no se incluyó ningún compuesto en la hilera A).

Aplicación de compuesto del ensayo a células

10 El medio de cultivo celular fue aspirado de la placa de 96 pocillos que contenía las células S22.3. Se transfirieron 175 μ l del medio del ensayo con la dilución apropiada de compuesto del ensayo desde cada pocillo de la placa de compuesto al pocillo correspondiente de la placa de cul-
15 tivo celular (la hilera H se usó como el "testigo sin inhibición"). La placa de cultivo celular se incubó a 37° con 5% de CO₂ durante 72 h.

Extracción de RNA celular total

20 a continuación del período de incubación de 72 h, el RNA celular total fue extraído de las células S22.3 de la placa de 96 pocillos usando el estuche de ensayo RNeasy 96 (Qiagen®, RNeasy Handbook. 1999). Brevemente, el medio de ensayo fue completamente separado de las células y se añadieron a cada pocillo 100 μ l de tampón RLT (Qiagen®) que
25 contenía β -mercaptoetanol 143 mM de la placa de cultivo celular de 96 pocillos. La microplaca fue suavemente agitada durante 20 segundos. Seguidamente se añadieron 100 μ l de etanol al 70% a cada pocillo de lamicroplaca y se mezcló
30 con pipeteo. El lisado fue separado y fue aplicado a pocillos de una placa RNeasy 96 (Qiagen®) que se colocó en la parte superior de un bloque de pocillos Qiagen® Square-Well. La placa RNeasy 96 fue sellada con cinta y el bloque Square-Well con la placa RNeasy 96 fue introducida en la

153

sujeción y colocada en un cubo rotor de una centrifugadora 4K15C. La muestra fue centrifugada a 6.000 rpm (~ 5.600 x g) durante 4 minutos a temperatura ambiente. La cinta se retiró de la placa y se añadieron 0,8 ml de tampón Buffer RW1 (estuche de ensayo Qiagen® RNeasy) a cada pocillo de la placa RNeasy 96. La placa RNeasy 96 fue sellada con un nuevo trozo de cinta y se centrifugó a 6.000 rpm durante 4 minutos a temperatura ambiente. La placa RNeasy 96 se colocó en la parte superior de otro bloque Square-Well limpio, la cinta se retiró y se añadieron 0,8 ml de tampón RPE (estuche de ensayo Qiagen® RNeasy) a cada pocillo de la placa RNeasy 96. La placa RNeasy 96 fue sellada con un nuevo trozo de cinta y se centrifugó a 6.000 rpm durante 4 minutos a temperatura ambiente. La cinta se retiró y se añadieron otros 8 ml de tampón Buffer RPE (estuche de ensayo Qiagen RNeasy) a cada pocillo de la placa RNeasy 96. La placa RNeasy fue sellada con un nuevo trozo de cinta y se centrifugó a 6.000 rpm durante 10 minutos a temperatura ambiente. La cinta se retiró, la placa RNeasy 96 se colocó en la parte superior de un estante que contenía microtubos de recogida de 1,2 ml. El RNA fue eluido añadiendo 50 ml de RNasa exento de agua, sellando la placa con un nuevo trozo de cinta e incubando durante 1 minuto a temperatura ambiente. La placa se centrifugó seguidamente a 6.000 rpm durante 4 minutos a temperatura ambiente. La etapa de elución se repitió con un segundo volumen de 50 µl de RNasa exenta de agua. Los microtubos con RNA celular total se almacenaron a -70°.

30 Cuantificación de RNA celular total.

Se cuantificó RNA en el sistema STORM® (Molecular Dynamics®) usando el estuche de ensayo de cuantificación de RNA RiboGreen® (Molecular Probes®). Brevemente, el reactivo RiboGreen se diluyó 200 veces en TE (Tris-HCl 10 mM, pH

= 7,5, EDTA 1 mM). Generalmente, se diluyeron 50 μ l de reactivo en 10 ml de TE. Una curva estándar de RNA ribosomal se diluyó en TE hasta 2 μ g/ml y cantidades predeterminadas (100, 50, 40, 20, 10, 5, 2 y 0 μ l) de la solución de RNA ribosomal fueron seguidamente transferidas a una placa de 96 pocillos nueva (COSTAR n° 3997) y el volumen se completó hasta 100 μ l con TE. Generalmente, la columna 1 de la placa de 96 pocillos se usó para la curva estándar y los demás pocillos se usaron para cuantificar las muestras de RNA. Se transfirieron 10 μ l de cada muestra de RNA que iba a ser cuantificada al correspondiente pocillo de la placa de 96 pocillos y se añadieron 90 μ l de TE. Se añadió un volumen (100 μ l) de reactivo RiboGreen diluido a cada pocillo de la placa de 96 pocillos y se incubó durante 2 a 5 minutos a temperatura ambiente, con protección de la luz (una muestra de RNA de 10 μ l en un volumen final de 200 μ l genera una dilución 20). La intensidad de la fluorescencia de cada pocillo se midió en el sistema STORM® (Molecular Dynamics®). Se creó una curva estándar sobre la base de las cantidades conocidas de RNA ribosomal y las intensidades fluorescentes resultantes. La concentración de RNA en las muestras experimentales se determinó a partir de la curva estándar y se corrigió para la dilución 20X.

25

30

155

Reactivos y materiales:

Producto	Empresa	Catálogo n°	Almacenamiento
DEPC	Sigma	D5758	4°C
EDTA	Sigma	E5134	TA
Trizma-Base	Sigma	T8524	TA
Trizma-HCl	Sigma	T7149	TA
Collection Tube Strips	Qiagen	19562	TA
Estuche de ensayo de cuantificación de RNA Ribogreen	Molecular Probe	R11490	-20°C
Estuche de ensayo Rneasy 96	Qiagen	74183	TA
Bloques Square-Well	Qiagen	19573	TA

RT-PCR en tiempo real.

El RT-PCR en tiempo real se realizó en un sistema de
5 detección de secuencias ABI Prism 7700 usando el estuche de
ensayo TaqMan EZ RT-PCR (de la empresa Perkin-Elmer Ap-
plied Biosystems®). El RT-PCR fue optimizado para la cuan-
tificación del 5'IRES de RNA de HCV usando la tecnología de
TaqMan (Roche Molecular Diagnostics Systems) similar a la
10 técnica previamente descrita (Martell et al., 1999. J.
Clin. Microbiol. 37:327-332). El sistema explota la activi-
dad nucleolítica en 5'-3' de la DNA-polimerasa de AmpliTaq.
Brevemente, el método utiliza una sonda de hibridación
fluorógena de etiqueta dual (sonda PUTR) que se reasocia
15 específicamente a la plantilla entre los cebadores de PCR
(cebadores 8125 y 7024). El extremo 5' de la sonda contiene
un reportero fluorescente (6-carboxifluoresceína [FAM]) y
el extremo 3' contiene un inactivador fluorescente (6-
carboxitetrametil-rodamina [TAMRA]). El espectro de emisión
20 del reportero FAM fue suprimido por el inactivador en la

sonda de hibridación intacta. La degradación de nucleasa de la sonda de hibridación libera el reportero, dando lugar a un aumento de la emisión de fluorescencia. El detector de secuencias ABI Prism 7700 mide el aumento de la emisión de fluorescencia continuamente durante la amplificación por PCR de forma que el producto amplificado fue directamente proporcional a la señal. La representación de la amplificación fue analizada tempranamente en la reacción en un punto que representa la fase logarítmica de la acumulación de producto. Un punto que representa un umbral de detección definida del aumento en la señal fluorescente asociada con el crecimiento exponencial del producto de PCR para el detector de secuencias se definió como el umbral del ciclo (C_T). Los valores de C_T son inversamente proporcionales a la cantidad de RNA de HCV producido; de forma que bajo condiciones idénticas de PCR, cuanto mayor es la concentración de partida de RNA de HCV, más bajo es el valor de C_T . Se creó una curva estándar automáticamente por el sistema de detección ABI Prism 7700 representando gráficamente el valor de C_T frente a cada dilución estándar de concentración conocida de RNA de HCV.

Las muestras de referencia para la curva estándar están incluidas en cada placa de RT-PCR. El RNA de replicón de HCV se sintetizó (por transcripción de T7) *in vitro*, se epurificó y se cuantificó por OD_{260} . Considerando que $1 \mu\text{g}$ de este RNA = $2,15 \times 10^{11}$ copias de RNA, se hicieron diluciones con el fin de tener 10^8 , 10^7 , 10^6 , 10^5 , 10^4 , 10^3 ó 10^2 copias de RNA genómico/ $5 \mu\text{l}$. El RNA de Huh-7 celular total se incorporó también con cada dilución ($50 \text{ ng}/5 \mu\text{l}$). Se combinaron $5 \mu\text{l}$ de cada patrón de referencia (replicón de HCV + RNA de Huh-7) con $45 \mu\text{l}$ de reactivo Mix, y se usaron en la reacción de RT-PCR en tiempo real.

La reacción de RT-PCR en tiempo real se ajustó para las muestras experimentales que se purificaron en placas de 96 pocillos RNeasy combinando $5 \mu\text{l}$ de cada muestra de RNA

157

celular total con 45 µl de reactivo Mix.

Reactivos y materiales:

Producto	EMPRESA	Catalogo n°	Almacenamiento
Estuche de ensayo TaqMan EZ RT-PCR	PE Applied Biosystems	N808-0236	-20°C
MicroAmp Optical Caps	PE Applied Biosystems	N801-0935	TA
Placa de reacción de 96 pocillos MicroAmp Optical	PE Applied Biosystems	N801-0560	TA

5 Preparación de mezcla de reactivos:

Componente	Volumen para una muestra (µl)	Volumen para una placa (µl) (91 muestras + volumen muerto)	Conc. final
Rnase-exento de agua	16,5	1617	
Tampón 5X TaqMan EZ	10	980	1X
Mn(OAc) ₂ (25mM)	6	588	3 mM
dATP (10mM)	1,5	147	300 µM
dCTP (10mM)	1,5	147	300 µM
dGTP (10mM)	1,5	147	300 µM
dUTP (20mM)	1,5	147	600 µM
Cebador directo (10µM)	1	98	200 nM
Cebador inverso (10µM)	1	98	200 nM
Sonda PUTR (5µM)	2	196	200 nM
rTth DNA-polymerase (2.5 U/µL)	2	196	0.1 U/µL
AmpErase UNG (1U/µL)	0,5	49	0.01 U/µL
Volumen total	45	4410	

Secuencia del cebador directo (SEQ ID. 1): 5'-ACG CAG
AAA GCG TCT AGC CAT GGC GTT AGT-3'

Secuencia del cebador inverso (SEQ ID n° 2): 5'-TCC
CGG GGC ACT CGC AAG CAC CCT ATC AGG-3'

Nota: Los cebadores amplifican una región de 256-nt
5 presente en la región sin traducir en 5' de HCV.

Secuencia de la sonda PUTR (SEQ ID n° 3): 6FAM - TGG
TCT GCG GAA CCG GTG AGT ACA CC - TAMRA

10 Testigos sin plantillas (NTC): En cada placa, se usa-
ron 4 pocillos como "NTC". Para estos testigos, se añadie-
ron 5 µl de agua al pocillo en lugar de RNA.

Condiciones de ciclación térmica:

50°C	2 minutos	
60°C	30 minutos	
95°C	5 minutos	
95°C	15 segundos	para 2 ciclos
60°C	1 minutos	
90°C	15 segundos	para 40 ciclos
60°C	1 minutos	

15 A continuación de la terminación se la reacción de RT-
PCR, el análisis de datos requiere el ajuste de la señal de
fluorescencia umbral para la placa de PCR y se construyó
una curva estándar representando gráficamente el valor de
Ct frente al número de copias de RNA usado en cada reacción
20 de referencia. Los valores de Ct obtenidos para las mues-
tras del ensayo se usan para interpolar un número de copias
de RNA basado en la curva estándar.

Finalmente, el número de copias de RNA fue normalizado
(basándose en la cuantificación de RNA RiboGreen del RNA
25 extraído del pocillo de cultivo de células) y se expresó

como equivalentes de genoma/ μ g de RNA total [ge/ μ g].

El número de copias de RNA (g.e./ μ g) de cada pocillo para la placa de cultivo celular fue una medida de la cantidad de RNA de HCV replicante en presencia de diversas concentraciones de inhibidor. El % de inhibición se calculó con la siguiente ecuación:

$$100 - [(g.e./\mu g \text{ inh}) / (g.e./\mu g \text{ ctl}) \times 100].$$

Se aplicó un ajuste de curva no lineal con el modelo de Hill a los datos de inhibición-concentración, y la concentración eficaz en 50% (EC₅₀) se calculó mediante el uso del software SAS (Statistical Software System; SAS Institute, INC. Cary, N.C.).

Cuando los compuestos de esta invención se valoraron en los ensayos precedentes enzimáticos y basados en la células, se encontró que los compuestos eran altamente activos. Más específicamente, los compuestos tenían una IC₅₀ por debajo de 0,1 μ M en el ensayo de NS3-NS4A-proteasa y una EC₅₀ por debajo de 0,5 μ M en el ensayo de replicación de RNA de HCV basado en células.

EJEMPLO 12

Ensayos de la especificidad

Los ensayos de la especificidad usados para evaluar la selectividad de este compuesto se describen en el documento 00/09543.

Cuando los compuestos se evaluaron en los ensayos de especificidad, los compuestos de Fórmula 1 se encontró que eran selectivos en cuanto que no muestran ninguna inhibición significativa en los ensayos de elastasa y catepsina B de leucocitos humanos.

EJEMPLO 13

Propiedades farmacocinéticas

Los presentes compuestos muestran también buenas propiedades farmacocinéticas como niveles en plasma significa-

tivos en rata al cabo de 1 hora y 2 h de una dosis oral de 5 mg/kg.

Más explícitamente, el siguiente ensayo, una selección de absorción oral *in vivo*, se usó para determinar niveles en plasma de los compuestos del ensayo en una rata después de una administración oral:

Materiales y métodos:

1. Método para reunir compuestos ("selección de cassette"):

La selección de compuestos para ser reunidos en una "cassette" se basó en su analogía estructural y propiedades fisicoquímicas. Se estableció un método de extracción en fase sólida aplicable a todos los compuestos seleccionados. Basándose en el ensayo inicial, en el que cada compuesto fue salpicado en plasma de rata y experimentado por medio de HPLC o HPLC/MS a una concentración de 0,5 μ M, se usaron el tiempo de retención, masa iónica y separación posible entre compuestos por HPLC y/o HPLC/MS como una base para reunir 3-4 compuestos en una "cassette".

2. Vehículo oral y preparación de compuestos:

Cada "cassette" contiene 3-4 compuestos a 5 ó 4 mg/kg para cada compuesto. Las cassette se prepararon en forma de una suspensión oral en 0,5% de metilcelulosa acuosa y 0,3% de monoleato de polioxietileno (20)-sorbitan (Tween-80). El volumen de dosificación fue 10 ml/kg por medio de gavaje oral.

3. Dosificación y toma de muestras de plasma:

Ratas machos Sprague Dawley se mantuvieron en ayunas durante una noche en jaulas individuales, con acceso a dextrosa acuosa al 10%. Dos ratas fueron dosificadas con cada "cassette". Las muestras de plasma (~ 1 ml) se recogieron a las 1 y 2 h después de la dosificación de las 2 ratas y se reunieron para la extracción y análisis.

4. Extracción de compuestos y análisis:

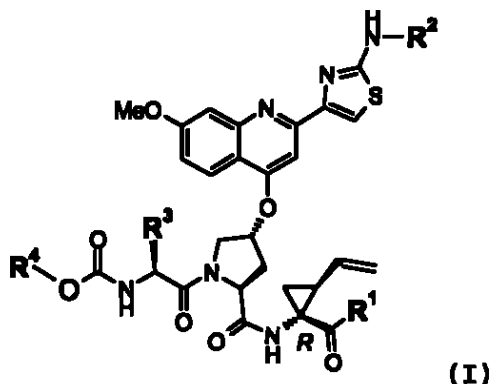
A partir de cada cassette, las muestras de plasma a las 1 y 2 h, plasma en blanco, plasma en blanco salpicado con todos los compuestos a 0,5 μ M cada uno, son extraídos por el método de extracción en fase sólida. Las muestras se
5 analizaron por HPLC y HPLC/MS para fines de comparación. Las concentraciones en plasma se estimaron basándose en la concentración única de patrón 0,5 μ M.

Cuando se ensayan en la selección precedente, los compuestos de los Ejemplos 1 a 9 de esta invención se encontró
10 que estaban presentes en niveles significativos en el plasma a los intervalos de 1 hora y 2 horas a continuación de la administración oral, con una media de niveles en plasma de sangre de 1,23 μ M y 1,16 μ M, respectivamente. Esta demostración de la absorción oral significativa *in vivo* para
15 los compuestos de esta invención es inesperada, teniendo en cuenta la inferior absorción oral generalmente atribuida a esta clase de péptidos. La absorción oral fácil hace que estos compuestos sean útiles para tratar una infección de HCV.

20 La siguiente tabla es una lista de compuestos representativos de la invención. Adicionalmente, en congruencia con la presente descripción, los compuestos tenían todos una IC_{50} por debajo de 0,5 μ M en el ensayo de NS3-NS4A-proteasa, y una EC_{50} por debajo de 0,5 μ M en el ensayo de
25 replicación de RNA de HCV basado en células.

REIVINDICACIONES

1. Un compuesto de fórmula (I):



(I)

- 5 en la que R¹ es hidroxil o N¹HSO₂R^{1A} en donde R^{1A} es alquilo (C₁₋₈), cicloalquilo (C₃₋₇) o {alquilo(C₁₋₆)-cicloalquilo (C₃₋₇)}, que están todos opcionalmente sustituidos de 1 a 3 veces con halo, ciano, nitro, O-alquilo (C₁₋₆), amido, amino o fenilo, o R^{1A} es arilo de C₆ o C₁₀ que está opcionalmente
- 10 sustituido de 1 a 3 veces con halo, ciano, nitro, alquilo (C₁₋₆), O-alquilo (C₁₋₆), amido, amino o fenilo; R² es cicloalquilo (C₄₋₆); R³ es t-butilo o cicloalquilo (C₅₋₆) y R⁴ es cicloalquilo (C₄₋₆), o una sal farmacéuticamente aceptable del mismo.
- 15 2. El compuesto de fórmula I según la reivindicación 1, en el que R¹ es hidroxil, N¹HSO₂Me, N¹HSO₂-ciclopropilo o N¹HSO₂-Ph.
3. El compuesto de fórmula I según la reivindicación 2, en el que R¹ es N¹HSO₂-ciclopropilo o N¹HSO₂-Ph.
- 20 4. El compuesto de fórmula I según la reivindicación 2, en el que R¹ es hidroxil.
5. El compuesto de fórmula I según una cualquiera de las reivindicaciones 1 a 4, en el que R² es ciclopentilo o ciclohexilo.
- 25 6. El compuesto de fórmula I según la reivindicación 5, en el que R² es ciclopentilo.
7. El compuesto de fórmula I según una cualquiera de

las reivindicaciones 1 a 6, en el que R^3 es t-butilo o ciclohexilo.

8. El compuesto de fórmula I según la reivindicación 7, en el que R^3 es t-butilo.

5 9. El compuesto de fórmula I según una cualquiera de las reivindicaciones 1 a 8, en el que R^4 es ciclobutilo o ciclopentilo.

10 10. El compuesto de fórmula I según la reivindicación 9, en el que R^4 es ciclopentilo.

11. El compuesto de fórmula I según la reivindicación 1, en el que R^1 es hidroxil, R^2 y R^4 es cada uno ciclopentilo y R^3 es t-butilo.

12. El compuesto de fórmula I según la reivindicación 1, en el que R^1 es hidroxil, R^2 es ciclobutilo, R^3 es t-butilo y R^4 es ciclopentilo.

13. El compuesto de fórmula I según la reivindicación 1, en el que R^1 es hidroxil, R^2 es ciclohexilo, R^3 es t-butilo y R^4 es ciclopentilo.

14. El compuesto de fórmula I según la reivindicación 1, en el que R^1 es NHSO_2Ph , R^2 y R^4 es cada uno ciclopentilo y R^3 es t-butilo.

15. El compuesto de fórmula I según la reivindicación 1, en el que R^1 es hidroxil, R^2 es ciclopentilo, R^3 es t-butilo y R^4 es ciclobutilo.

16. El compuesto de fórmula I según la reivindicación 1, en el que R^1 es hidroxil, R^2 es ciclopentilo, R^3 es t-butilo y R^4 es ciclohexilo.

17. El compuesto de fórmula I según la reivindicación 1, en el que R^1 es hidroxil, R^2 y R^4 es cada uno ciclopentilo y R^3 es ciclohexilo.

18. El compuesto de fórmula I según la reivindicación 1, en el que R^1 es hidroxil, R^2 , R^3 y R^4 es cada uno ciclopentilo.

19. Una composición farmacéutica, que comprende una cantidad viralmente eficaz anti-hepatitis C de un compuesto

de fórmula 1 según una cualquiera de las reivindicaciones 1 a 18, o una sal farmacéuticamente aceptable del mismo, mezclado con un medio excipiente o agente auxiliar farmacéuticamente aceptable.

5 20. La composición farmacéutica según la reivindicación 19, que comprende adicionalmente una cantidad terapéuticamente eficaz de uno o más de otro agente anti-HCV.

 21. La composición farmacéutica según la reivindicación 20, en el que dicho otro agente anti-HCV se selecciona
10 entre: α -interferón o α -interferón pegilado.

 22. La composición farmacéutica según la reivindicación 20 ó 21, en la que dicho otro agente anti-HCV es ribavirina.

 23. La composición farmacéutica según la reivindicación 20, 21 ó 22, en la que dicho otro agente anti-HCV se
15 selecciona entre inhibidores de: helicasa, NS2/3-proteasa y sitio de entrada de ribosomas internos (IRES).

 24. Un método para el tratamiento o prevención de una infección viral de hepatitis C en un mamífero, administrando
20 al mamífero una cantidad viralmente eficaz anti-hepatitis C de un compuesto de fórmula I según una cualquiera de las reivindicaciones 1 a 18, o una sal farmacéuticamente aceptable del mismo.

 25. Un método para el tratamiento o prevención de una
25 infección viral de hepatitis C en un mamífero, administrando al mamífero una cantidad viralmente eficaz anti-hepatitis C de la composición según una cualquiera de las reivindicaciones 19 a 23.

 26. Un método para el tratamiento o prevención de una
30 infección viral de hepatitis C en un mamífero, administrando al mamífero una cantidad viralmente eficaz anti-hepatitis C de un compuesto de fórmula I según una cualquiera de las reivindicaciones 1 a 18 o una sal terapéuticamente aceptable del mismo en combinación con uno o más de

otros agentes anti-HCV.

27.- El método según la reivindicación 26, en el que dicho otro agente anti-HCV se selecciona entre: α -interferón o α -interferón pegilado.

5 28.- El método según la reivindicación 26 ó 27, en el que dicho otro agente anti-HCV es ribavirina.

29. El método según la reivindicación 26, 27 ó 28, en el que dicho otro agente anti-HCV se selecciona entre inhibidores de: helicasa, NS2/3-proteasa y sitio de entrada
10 de ribosomas internos (IRES).

30. Uso de un compuesto de fórmula I según una cualquiera de las reivindicaciones 1 a 18, para la elaboración de un medicamento para el tratamiento o prevención de una infección viral de hepatitis C.

15 31. La composición farmacéutica según la reivindicación 20, 21 ó 22, en la que dicho otro agente anti-HCV es un inhibidor de polimerasa de HCV.

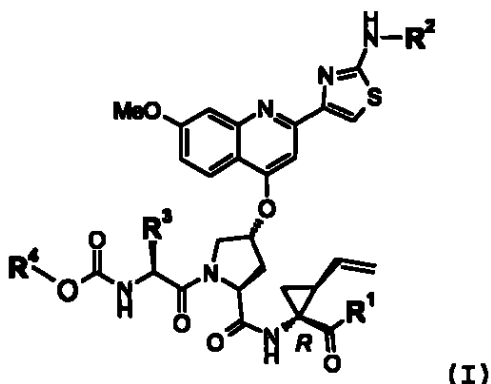
32. El método según la reivindicación 26, 27 ó 28, en el que dicho otro agente anti-HCV es un inhibidor de polimerasa de HCV.
20

25

30

RESUMEN

En la presente memoria descriptiva se describen compuestos de fórmula (1):



5 en la que R^1 es hidroxilo o $NHSO_2R^{1A}$ en donde R^{1A} es alquilo (C_{1-8}), cicloalquilo (C_{3-7}) o {alquilo(C_{1-6})-cicloalquilo (C_{3-7})}, que están todos opcionalmente sustituidos de 1 a 3 veces con halo, ciano, nitro, O-alquilo (C_{1-6}), amido, amino
 10 o fenilo, o R^{1A} es arilo de C_6 o C_{10} que está opcionalmente sustituido de 1 a 3 veces con halo, ciano, nitro, alquilo (C_{1-6}), O-alquilo (C_{1-6}), amido, amino o fenilo; R^2 es cicloalquilo (C_{4-6}); R^3 es t-butilo o cicloalquilo (C_{3-6}) y R^4 es cicloalquilo (C_{4-6}), o una sal farmacéuticamente aceptable de los mismos. Los compuestos son útiles como inhibidores de NS3-proteasa de HCV.

20

25

LISTADO DE SECUENCIAS

	<110> BOEHRINGER INGELHEIM INTERNATIONAL GmbH	
5	<120> TRI-PÉPTIDOS INHIBIDORES DE HEPATITIS C	
	<130> 13/106	
	<150> 2.370.396	
10	<151> 2002-08-01	
	<160> 3	
	<170> FastSEQ for Windows Version 4.0	
15	<210> 1	
	<211> 30	
	<212> DNA	
	<213> Secuencia artificial	
20	<220>	
	<223> Cebador directo	
	<400> 1	
25	acgcagaaag cgtctagcca tggcgtagt	30
	<210> 2	
	<211> 30	
	<212> DNA	
30	<213> Secuencia artificial	
	<220>	
	<223> Cebador inverso	
35	<400> 2	
	tcccggggca ctgcgaagca ccctatcagg	30
	<210> 3	
	<211> 26	
40	<212> DNA	
	<213> Secuencia artificial	
	<220>	
	<223> Sonda PUTR	
45	<400> 3	
	tggctctgcgg aaccggtgag tacacc	26
50		
	COLO 9255-841-007-9	
	PRP/WPCL9255/ID-000241	

Expediente No		04085344	No de Folio
Item		No de unidades	✓
1	CD		
2	DVD		
3	REVISTA		
4	LIBRO		
5	PERIODICO		
6	DISQUETES		
7	SOBRE DE MANILA		
8	SOBRE BLANCO TAMAÑO CARTA ✓	1	
9	SOBRE BLANCO TAMAÑO OFICIO		
10	OTROS:		

Observaciones: Arte final

SUPERINDUSTRIA Y COMERCIO
Radiacion : 04005344 00000000 Folios: 170
Fecha (GRU): 2004-08-31 17:44:25
Tramite : 011 PCT-PATENT 1 REGISTRO/ 411 PRESENTIN
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

SUPERINDUSTRIA Y COMERCIO (COPIA)
Radiacion : 04005344 00000000 Folios: 170
Fecha (GRU): 2004-08-31 17:44:25
Tramite : 011 PCT-PATENT 379 FASE INICI 411 PRESENTIN
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

MIT : 800176089-2

RECIBO OFICIAL DE CAJA : 04 - 68,240
FECHA : AGOSTO 30 DE 2004

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	23490086	30/08/2004	30,125,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	422,000.00
		TOTAL :	\$ 422,000.00

SON: CUATROCIENTOS VEINTIDOS MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

66009255-841-007-9

SUPERINDUSTRIA Y COMERCIO(COPIA)
Radicación : 04005344 00000000 Folios: 170
Fecha (MM): 2004-08-31 17:44:26
Trámite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTA
Dependencia: 2820 DIVISION DE NUEVAS CREACIONES

SUPERINDUSTRIA Y COMERCIO
Radicación : 04005344 00000000 Folios: 170
Fecha (MM): 2004-08-31 17:44:26
Trámite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTA
Dependencia: 2820 DIVISION DE NUEVAS CREACIONES

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCION	()	()
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 ()	()

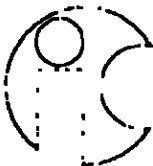
DISEÑO INDUSTRIAL ()		
Indicación de que se solicita el registro de Diseño Industrial	()	()
Datos de identificación del solicitante o de la persona que presenta la solicitud	()	()
Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño	()	()
Comprobante 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

Fecha

31 Ago 04



2020
Bogotá, D.C.,

Doctor:
EMILIO FERRERO
Apoderado
BOEHRINGER INGELHEIM INTERNATIONAL GMBH

Oficio N°
68855

ASUNTO:	Radicación N°	04-85344
	Solicitud internacional	PCT/CA2003/00090
	Fecha inicio fase nacional	01/09/2004
	Trámite	011
	Evento	379
	Actuación	430
	Folios	001

Como resultado del examen de forma, realizado con fundamento en el artículo 38 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 copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486).

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, 24 SET. 2004