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Industria y Comercio
SUPERINTENDENCIA

PCT

SOLICITUD

PATENTE DE INVENCION

03-50947

21 EXPEDIENTE Nº

54 TÍTULO INHIBIDORES IMIDAZOL Y BENZIMIDAZOL DE CASPASA Y USOS
DE LOS MISMOS

51 CLASIFICACIÓN INTERNACIONAL C07D 233/00, A61K 31/417

71 SOLICITANTE VERTEX PHARMACEUTICALS INCORPORATED

DOMICILIO ESTADOS UNIDOS DE AMERICA

74 APODERADO HELENA CAMARGO WILLIAMS

22 BOGOTÁ, D C 16 de Junio de 2003

202099

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PETITORIO

Industria y Comercio
SUPERINTENDENCIA

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Fecha (HMD): 2003-06-16 14:32:38
Trámite: 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE PCT ENTRADA EN FASE NACIONAL

①

SOLICITUD DE:

☒ Patente de Invención.

☐ Patente de Modelo de Utilidad.

☐ Capítulo I.

☒ Capítulo II.

②

SOLICITANTE
(71)

Nombre: Vertex Pharmaceuticals Incorporated

Dirección: 130 Waverly Street, Cambridge, MA, U.S.A.

Nacionalidad o Domicilio: Estados Unidos de América
Lugar de Constitución: Estados Unidos de América

Teléfono:

Fax:

E-mail:

IDENTIFICACIÓN

C.C. ☐ NIT ☐

C.E. ☐ Otro ☐

Cual _____

Número _____

③

REPRESENTANTE
O APODERADO

Nombre: Helena Camargo Williamson

Dirección: Carrera 9 No. 74-08 OF. 504

Teléfono: 3764200

Fax: 3761707

E-mail: helena.camargo@prc-laws.com

IDENTIFICACIÓN

C.C. ☒ NIT ☐

C.E. ☐ Otro ☐

Cual _____

Número 35.455.268 TP 76985

④

INVENTOR (ES)
(72)

Nombre: Ver hoja anexa (Folio 12)

Dirección:

Nacionalidad o Domicilio

⑤ PCT (86)

Solicitud Internacional No. PCT/US01/47668

Fecha 19 de Noviembre de 2001

Publicación Internacional No. WO 02/42278 A2

Fecha 30 de Mayo de 2002

⑥

Título (54) INHIBIDORES IMIDAZOL Y BENZIMIDAZOL DE CASPASA Y USOS DE LOS MISMOS.

⑦

Clasificación Internacional (51) 0070 233/00 A61K31/417

⑧ Prioridad

(33) País de Origen

(32) Fecha

(31) Número de Solicitud

SI ☒

NO ☐

US

21/11/00

60/252,252

⑨

Comprobante de pago No. 41005 \$ 400.000

Fecha

Jun 11/002

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

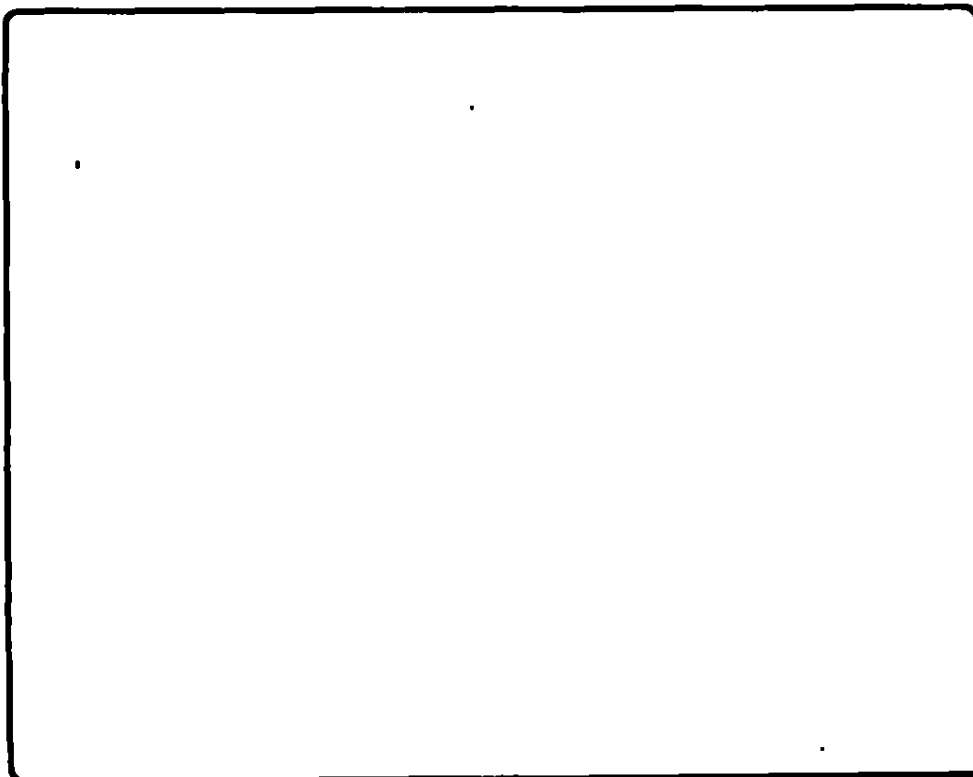
2

10

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud.
- ☒ Documento que acredite 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 internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
- ☐ Traducción del examen preliminar internacional efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☒ 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, relacionados parcial o totalmente con la invención de esta solicitud.
- ☐ De ser el caso, modificaciones efectuadas a la solicitud internacional.
- ☐ Otros

11 FIGURA CARACTERISTICA:



12 Solicito la concesión de la patente,

NOMBRE: Helena Camargo Williamson

FIRMA:

[Handwritten Signature]

C.C. 35.455.268

T.P. 76985

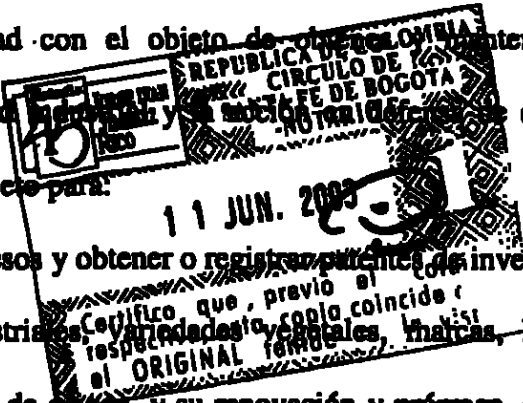
TRADUCCION OFICIAL No. 009/2003 de un documento escrito en Inglés, el cual para su identificación se sella con el sello de la Traductora lo mismo que la presente.—

Traducción de un Poder escrito en Inglés, y sus respectivas autenticaciones.

PODER

La organización VERTEX PHARMACEUTICALS INC., que actualmente cumple con su objeto, actuando por medio de su representante legal, formada y existente de conformidad con las leyes de los Estados Unidos de América, con oficinas registradas en el número 130 Waverly Street, Cambridge, Massachusetts, Estados Unidos de América, por medio del presente nombra a Ernesto Cavelier, con TPA 11.519, Bernardo Rodríguez Ossa, con TPA 41.858, Alvaro Parra Gómez, con TPA 47.452 y Helena Camargo Williamson, con TPA 76.985, todos ellos con oficinas situadas en la Carrera 9 No. 74-08, Oficina 504, Bogotá, D.C., Colombia, en el mismo orden, como sus apoderados reales y verdaderos, para representarla ante cualquier persona, entidades administrativas o judiciales, árbitros, mediadores, tribunal o corte de arbitramento o conciliación, o ante cualquier autoridad con el objeto de obtener, mantener la protección de los derechos de propiedad intelectual y la defensa de dichos derechos, estando facultados para ese objeto para:

1. Solicitar, seguir los respectivos procesos y obtener o registrar patentes de invención, modelos de utilidad, diseños industriales, variedades de plantas, marcas, lemas, nombres y signos y denominaciones de origen, y su renovación y prórroga, cesión de los mismos, registro de cambio de nombre y domicilio, y de contratos y licencias sobre los mismos, y para el registro de la cesión de contratos de tecnología;



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ALICIA GROOT
INTERPRETE OFICIAL
RESOLUCION No. 2183. ABRIL 17. 1988

5

con relación a este poder y ratificará y confirmará todos los actos a que se hayan comprometido los apoderados con relación a este poder, con excepción de los casos de incumplimiento, negligencia o actos que hayan sido llevados a cabo en oposición a las instrucciones expresas del otorgante.

Este poder estará regido e interpretado por las leyes de la República de Colombia, que lo rigen en su totalidad.

Dado y firmado en Cambridge, Massachusetts, el día 3 de abril del 2003.

Por: (firmado) (firma ilegible)

Kenneth S. Boger

Vicepresidente Principal y Asesor Legal General

Vertex Pharmaceuticals, Inc.

Hay un Certificado Notarial, que dice así:

CERTIFICADO DE NOTARIO PUBLICO

Yo, Victoria M. Clifford, suscrita Notaria Pública, debidamente autorizada, habiendo prestado juramento, en ejercicio, por medio del presente certificado: (1) que el día 3 de abril del 2003, compareció personalmente ante mí Kenneth S. Boger, quien me presentó el anterior poder y quien lo identificó bajo su nombre y firma; (2) que he tenido a mi vista los documentos que atestiguan que VERTEX PHARMACEUTICALS INC. existe y ejerce su objeto social de conformidad con las leyes de Massachusetts; (3) que la persona que firma el poder está debidamente autorizada para ello, como representante legal de VERTEX PHARMACEUTICALS INC.



TRADUCCION OFICIAL No. 209/2003
ALICIA GROOT
INTERPRETE OFICIAL
RESOLUCION No. 2188, ABRIL 17, 1988
DEL MINISTERIO DE JUSTICIA

6

Firma y Sello:

(firmado) Victoria M. Clifford

Mi comisión vence el 6 de marzo del 2009.

Hay un sello en relieve que dice: VICTORIA M. CLIFFORD - Notaria Pública -
Massachusetts.

Hay un sello en relieve del ESTADO DE MASSACHUSETTS.

(Este documento deberá ser debidamente legalizado hasta por un Cónsul colombiano o
por Apostilla).

Adjunto se halla un Certificado que dice:

ESTADO DE MASSACHUSETTS

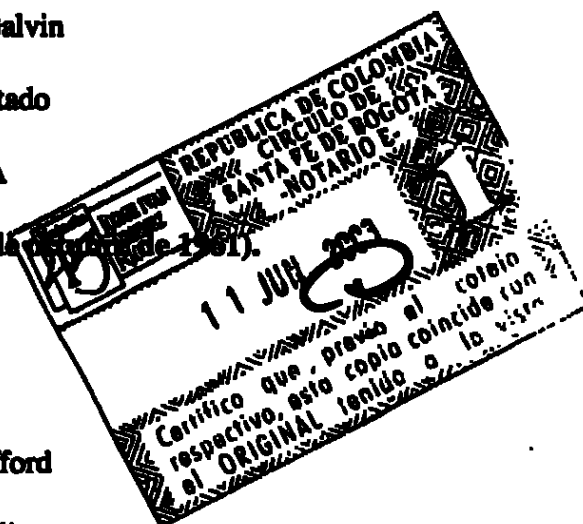
William Francis Galvin

Secretario del Estado

APOSTILLA

(Convención de La Haya del 5 de octubre de 1961).

1. País: Estados Unidos de América
Este documento público
2. ha sido firmado por la Sra. Victoria M. Clifford
3. quien ha actuado en calidad de Notaria Pública
4. en el Estado de Massachusetts, cuya comisión vence el 3/6/2009



CERTIFICADO

5. en Boston, Massachusetts
6. el 4/23/2003 a las 3:06:14 PM

TRADUCCION OFICIAL No. 009/2003
ALICIA GROOT
INTERPRETE OFICIAL
RESOLUCION No. 2192, ABRIL 17, 1999
DEL MINISTERIO DE JUSTICIA

7. por el Secretario del Estado
8. 44638
9. firmado bajo el Gran Sello del Estado
10. Firma

(firmado) William Francis Galvin

Secretario del Estado

En relieve, Gran Sello del Estado de Massachusetts.

ES TRADUCCION FIEL Y COMPLETA.

Bogotá, Mayo 12 del 2003.

AG.

Alicia Groot

009/2003

TRADUCCION
ALICIA GROOT
INTERPRETE OFICIAL
RESOLUCION No. 2188, ABRIL 17, 1998
DEL MINISTERIO DE JUSTICIA



MINISTERIO DE RELACIONES
EXTERIORES

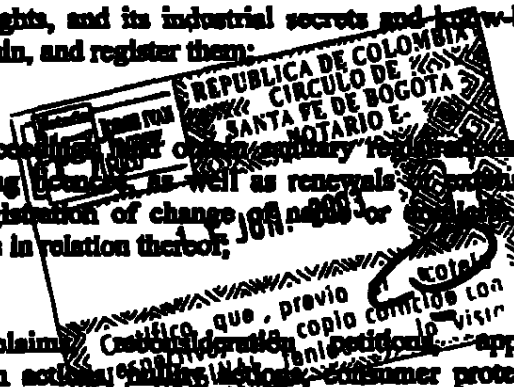
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RESPONSABILIDAD DEL TEXTO
2003 MAY 13 A 11:03
1848
FEE DE LEGALIZACION:
SECRETARÍA DE BOGOTÁ D.C.

B

POWER OF ATTORNEY

The organization **VERTEX PHARMACEUTICALS INC.**, at present carrying out its purpose, acting through its legal representative, formed and existing according to the laws of United States of America, with registered offices at 130 Waverly Street, Cambridge, Massachusetts, United States of America, hereby appoints Ernesto Caveller, with TPA 11.519, Bernardo Rodriguez Ossa, with TPA 41.858, Alvaro Parra Gómez, with TPA 47.452 and Helena Camargo Williamson with TPA 76.985, all of them with offices at Cra. 9 No. 74-08 Of. 504, Bogotá DC, Colombia, in this same order, as its true and lawful attorneys, to represent it before any person, administrative or judicial entities, arbitrator, mediator, court or board of arbitration or conciliation, or before any authority for the purpose of obtaining and maintaining the protection of its industrial property rights and acting in the defense of such rights, being empowered for such purpose to:

1. Apply for, follow the respective proceedings and obtain or register patents of invention, utility models, industrial designs, plant varieties, trademarks, slogans, names and signs and denominations of origin, and their renewal and extension, assignment thereof, registration of change of name and domicile, and of agreements and licenses thereon, and for registration of transfer of technology contracts;
2. Apply for protection of its authors rights, and its industrial secrets and know-how, follow the respective proceedings, obtain, and register them;
3. Apply for, follow the respective proceedings, obtain, register, and renew registrations and permits for its products, and operating licenses, as well as renewals, extensions thereof, assignment of the same, registration of change of name or domicile, and registration of agreements and licenses in relation thereof;
4. File oppositions, observations, claims, appeals, administrative or judicial cancellation actions, appeals, consumer protection actions, protection of industrial secrets and other actions related to rights derived from the above mentioned matters and against unfair competition and restraint of trade, actions related to obtaining or exploiting licenses, as well as intervention in civil, commercial, criminal, administrative contentious and police proceedings, all in relation to actions or proceedings initiated by the grantor or by third parties against or in relation to the grantor;
5. Conciliate, cancel, settle, compromise, desist, receive and take possession of the subject matter of litigation and renounce or desist rights granted or in the process of being granted, and admit the facts in legal proceedings;
6. Ratify or not the acts undertaken by a self-appointed agent;



TRADUCCION OFICIAL N.º 009/2003
 ALICIA GROOT
 INTERPRETE OFICIAL
 RESOLUCION NO- 2198, ABRIL 17, 1999
 DEL MINISTERIO DE JUSTICIA

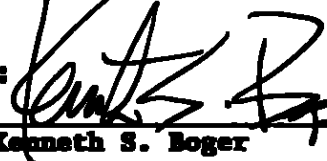
9

7. Receive service of process and appoint attorneys, resign and substitute the present power of attorney and revoke the substitutions.

The grantor shall pay each and every one of the attorneys for the costs, claims, expenses and liabilities of any nature, incurred by each one of the attorneys in relation with this power of attorney and shall ratify and confirm all acts undertaken by the attorneys in relation with this power of attorney, except in the cases of default, negligence or acts carried out in contradiction to the express instructions of the grantor.

This power of attorney shall be ruled and interpreted by the laws of the Republic of Colombia, which governs it in its entirety.

Given and signed at CAMBRIDGE, MA today, the 3rd of APRIL of 2003

By: 

Kenneth S. Boger
Senior Vice President and General Counsel
Vertex Pharmaceuticals, Inc.

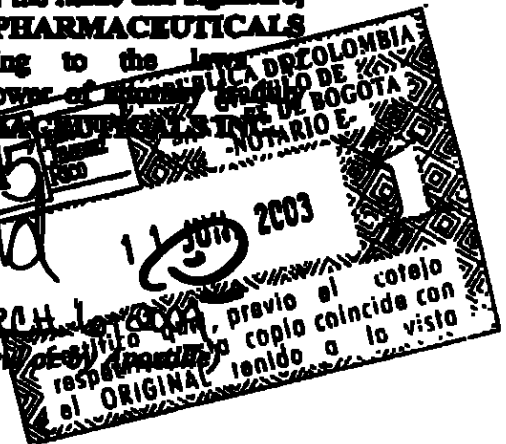
NOTARY PUBLIC CERTIFICATE

I Victoria M. Clifford the undersigned Notary Public, duly authorized admitted and sworn, practicing in do hereby certify: (1) that on the 3rd day of APRIL 2003, personally appeared before me Kenneth S. Boger who did produce to me the preceding power of attorney and did identify to me under his name and signature; (2) that I have seen the documents that attest that VERTEX PHARMACEUTICALS INC. exists and exercises its legal purpose according to the laws of Massachusetts; (3) that the person who signs the power of attorney is Kenneth S. Boger authorized to do it, as legal representative of VERTEX PHARMACEUTICALS INC.

Signature and seal:

BY COMMISSION EXPIRES MARCH 1, 2004

(This document should be duly legalized up to a Colombian Consulate)



TRADUCCION OFICIAL 009/2003
ALICIA GROOT
INTERPRETE OFICIAL
RESOLUCION No. 2193, ABRIL 17, 1999
DEL MINISTERIO DE JUSTICIA

The Commonwealth of Massachusetts

William Francis Galvin
Secretary of the Commonwealth

APOSTILLE

(Convention de la Haye du 5 octobre 1961)

1. Country: United States of America

This public document...

2. has been signed by Ms. Victoria M. Clifford

3. acting in the capacity Notary Public

4. in the state of Massachusetts, whose commission expires on

3/6/2008

CERTIFIED

5. at Boston, Massachusetts

6. on 4/23/2003 3:06:14 PM

7. by the secretary of the Commonwealth

8. 44638

9. signed under the

Great Seal of the Commonwealth

10. Signature

William Francis Galvin
William Francis Galvin
Secretary of the Commonwealth



TRADUCCION OFICIAL 009/2003
ALICIA GROOT
INTERPRETE OFICIAL
RESOLUCION NO. 2108, ABRIL 17, 1989
DEL MINISTERIO DE JUSTICIA

11

Título : INHIBIDORES IMIDAZOL Y BENZIMIDAZOL DE CASPASA
Y USOS DE LOS MISMOS

Resumen: Esta invención proporciona inhibidores de caspasa que tienen la fórmula: en donde R^1 es CO_2H , CH_2CO_2H , o ésteres, amidas o isoésteres de los mismos; R^2 y R^3 son cada uno independientemente seleccionados de hidrógeno o un grupo alifático C_1-C_6 opcionalmente sustituido; y R^4 y R^5 son cada uno independientemente seleccionados de hidrógeno, un grupo alifático C_1-C_6 opcionalmente sustituido, o R^4 y R^5 tomados junto con el anillo al cual ellos están unidos forman un anillo bicíclico opcionalmente sustituido. Los inhibidores de caspasa son útiles para tratar un número de enfermedades tales como cáncer, desórdenes inflamatorios y autoinmunes agudos, enfermedades isquémicas y ciertos desórdenes neurodegenerativos.

ANEXO INVENTORES

KAY, David, un ciudadano inglés domiciliado en 4 Church Path, Purton, Wiltshire SN5 9DR, Reino Unido de la Gran Bretaña; y **GOLEC, Julian**, un ciudadano inglés, domiciliado en 8 Manor Farm, Chapel Road, Ashbury, Wiltshire SN6 8LS, Reino Unido de la Gran Bretaña.

INHIBIDORES IMIDAZOL Y BENZIMIDAZOL DE CASPASA Y USOS DE
LOS MISMOS

REFERENCIA CRUZADA A SOLICITUDES RELACIONADAS

Esta solicitud reivindica prioridad de la Solicitud de Patente Provisional US 60/252,252. presentada el 21 de Noviembre de 2000.

CAMPO DE LA INVENCION

Esta invención se encuentra en el campo de la química medicinal y se relaciona con compuestos novedosos, y composiciones farmacéuticas de los mismos, que inhiben las caspasas que sirven de intermediarias para llegar a la apoptosis celular y la inflamación. La invención también se relaciona con métodos para usar los compuestos y las composiciones farmacéuticas de esta invención para tratar enfermedades en donde la actividad de la caspasa se encuentra implicada.

ANTECEDENTES DE LA INVENCION

La apoptosis, o muerte celular programada, es un mecanismo por medio del cual los organismos eliminan células indeseadas. La desregulación de la apoptosis, ya

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sea la apoptosis excesiva o la falla que esta sufre, ha estado implicada en un número de enfermedades tales como el cáncer, los desórdenes inflamatorios y autoinmunes agudos, las enfermedades isquémicas y ciertos desórdenes neurodegenerativos (ver por lo general *Science*, 1998, 281, 1283-1312; Ellis y otros, *Ann. Rev. Cell. Biol.*, 1991, 7, 663).

Las caspasas son una familia de enzimas cisteína proteasas que son intermediarias clave en las rutas de señalización para la apoptosis y el desensamble celular (Thornberry, *Chem. Biol.*, 1998, 5, R97-R103). Estas rutas de señalización varían dependiendo del tipo de célula de del estímulo, pero todas las rutas de apoptosis parecen converger en una ruta efectora común que conduce a la proteólisis de proteínas clave. Las caspasas están involucradas tanto en la fase efectora de la ruta de señalización como en la parte ascendente adicional de su iniciación. Las caspasas de la parte ascendente involucradas en los eventos de iniciación llegan a ser activadas y a su vez activan otras caspasas que están involucradas en las fases posteriores de la apoptosis.

La caspasa-1, la primera caspasa identificada, es también conocida como la enzima que convierte la interleucina o "ICE". La caspasa-1 convierte el precursor de interleucina-1 β ("pIL-1 β ") a la forma activa pro-inflamatoria por medio de la escisión específica de pIL-1 β entre Asp-116 y Ala-117. Además de la caspasa-1,

también hay otras once caspasas humanas conocidas, y todas ellas realizan escisiones específicamente en los residuos aspartilo. También se ha observado que ellas tienen rigurosos requerimientos de al menos cuatro residuos de aminoácidos en el lado N-terminal del sitio de escisión.

Las caspasas han sido clasificadas en tres grupos dependiendo de la secuencia de aminoácidos que es preferida o principalmente reconocida. Se ha demostrado que el grupo de caspasas, que incluye las caspasas 1, 4, y 5, prefiere los aminoácidos aromáticos hidrofóbicos en la posición 4 en el lado N-terminal del sitio de escisión. Otro grupo que incluye las caspasas 2, 3 y 7, reconocen los residuos aspartilo tanto en la posición 1 como en la 4 del lado N-terminal del sitio de escisión, y preferiblemente una secuencia de Asp-Glu-X-Asp. Un tercer grupo, que incluye las caspasas 6, 8, 9 y 10, soporta muchos aminoácidos en la secuencia de reconocimiento primaria, pero parece preferir los residuos con cadenas laterales alifáticas, ramificadas, tales como valina y leucina en la posición 4.

Las caspasas también han sido agrupadas de acuerdo a su función putativa. La primera subfamilia consiste de las caspasas-1 (ICE), 4, y 5. Se ha demostrado que estas caspasas están involucradas en el procesamiento de citoquina pro-inflamatoria y, por lo tanto, juegan un papel importante en la inflamación. La caspasa-1, la

enzima mas estudiada de esta clase, activa el precursor IL-1 β por medio de la escisión proteolítica. Esta enzima, por lo tanto, juega un papel clave en la respuesta inflamatoria. La caspasa-1 esta también involucrada en el procesamiento del factor de inducción gamma interferón (IGIF o IL-18), el cual estimula la producción de gamma interferón, un inmunoregulador clave que modula la presentación de antígeno, la activación de las células T y la adhesión de células.

Las caspasas remanentes integran la segunda y tercera de las subfamilias. Estas enzimas son de importancia central en las rutas de señalización intracelular que conducen a la apoptosis. Una subfamilia consiste de las enzimas involucradas en los eventos de iniciación en la ruta apoptótica, que incluye la transducción de señales desde la membrana de plasmática. Los miembros de esta subfamilia incluyen las caspasas-2, 8, 9 y 10. La otra subfamilia, que consiste de las caspasas efectoras 3, 6 y 7, están involucradas en los eventos de escisión finales de la parte descendente que resultan en la degradación sistemática y en la muerte de la célula por apoptosis. Las caspasas involucradas en la transducción de señales de la parte ascendente activan las caspasas de la parte descendente, las cuales luego inutilizan los mecanismos de reparación del ADN, fragmentan el ADN, desmantelan el citoesqueleto de la célula, y finalmente fragmentan la célula.

El conocimiento de la secuencia de cuatro aminoácidos principalmente reconocida por las caspasas ha sido empleado para diseñar los inhibidores de caspasa. Los inhibidores tetrapéptidos reversibles han sido preparados teniendo la estructura $\text{CH}_3\text{CO}-[\text{P4}]-[\text{P3}]-[\text{P2}]-\text{CH}(\text{R})\text{CH}_2\text{CO}_2\text{H}$ en donde de P2 a P4 representan una secuencia de reconocimiento de aminoácidos óptima y R es un aldehído, nitrilo o cetona capaz de unirse a la caspasa sulfhidril cisteína caspasa. Rano y Thornberry, *Chem. Biol.* 4, 149-155 (1997) ; Mjalli, y otros, *Bioorg. Med. Chem. Lett.* 3, 2689-2692 (1993); Nicholson y otros, *Nature* 376, 37-43 (1995). Se han preparado los inhibidores irreversibles basados en la secuencia de reconocimiento de tetrapéptidos análoga, en donde R es una aciloximetilcetona $-\text{COCH}_2\text{OCOR}'$. R' es ejemplificada por un fenilo opcionalmente substituido tal como 2,6-diclorobenzoiłoxi y en donde R es COCH_2X en donde X es un grupo saliente tal como F o Cl. Thornberry y otros, *Biochemistry* 33, 3934 (1994); Dolle y otros, *J Med. Chem.* 37, 563-564 (1994).

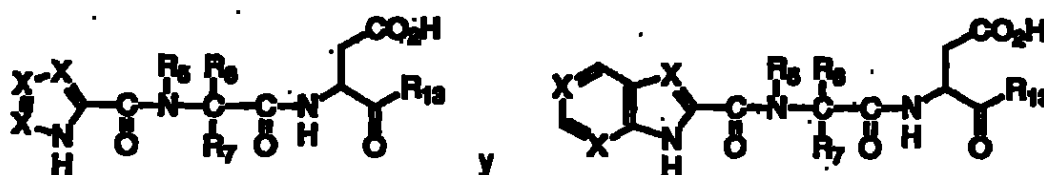
La utilidad de los inhibidores de caspasa para tratar una variedad de estados de enfermedad de mamíferos asociados con un incremento en la apoptosis celular ha sido demostrada empleando inhibidores de caspasa peptídicos. Por ejemplo, en modelos de roedores, se ha demostrado que los inhibidores de caspasa reducen el tamaño del infarto e inhiben la apoptosis del cardiomiocito después del infarto al miocardio, reducen el volumen de la lesión y

el déficit neurológico resultante del ataque, reducen la apoptosis post-traumática y el déficit neurológico en la lesión cerebral traumática, son efectivos en el tratamiento de la destrucción fulminante del hígado, y mejoran la supervivencia después de la conmoción endotóxica. Yaoita y otros, *Circulation*, **97**, 276 (1998); Endres y otros, *J Cerebral Blood Flow and Metabolism*, **18**, 238, (1998); Cheng y otros, *J. Clin. Invest.*, **101**, 1992 (1998); Yakovlev y otros, *J Neuroscience*, **17**, 7415 (1997); Rodríguez y otros, *J. Exp. Med.*, **184**, 2067 (1996); Grobmyer y otros, *Mol. Med.*, **5**, 585 (1999).

En general, los inhibidores peptídicos descritos anteriormente son muy potentes en contra de algunas de las enzimas caspasas. Sin embargo, esta potencia no siempre se ha reflejado en modelos celulares de apoptosis. Además, los inhibidores peptídicos son típicamente caracterizados por propiedades farmacológicas indeseables tales como una pobre absorción oral, una pobre estabilidad y un rápido metabolismo. Plattner y Norbeck, en *Drug Discovery Technologies*, Clark y Moos, Eds. (Ellis Horwood, Chichester, Inglaterra, 1990).

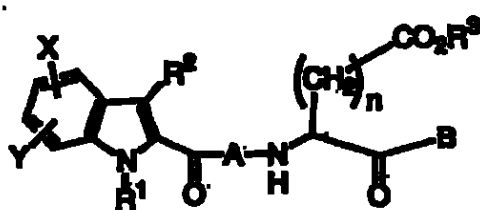
Se ha reportado el reconocimiento de la necesidad de mejorar las propiedades farmacológicas de los inhibidores peptídicos de caspasa, inhibidores peptidomiméticos y peptídicos con aminoácidos no-naturales.

La solicitud WO 95/35308 revela inhibidores de la enzima que convierte la interleucina-1 β incluyendo, *inter alia*, los compuestos de las formulas:



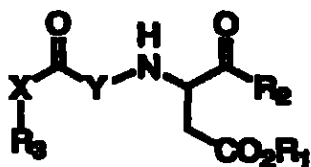
en donde X es independientemente seleccionado de =N- o =CH-; R₅ incluye hidrógeno; R₇ es hidrógeno y R₆ incluye una cadena lateral de aminoácidos- α ; y R₁₃ incluye hidrógeno, un anillo aromático o heteroaromático, un grupo alquilo C₁-C₆ recto o ramificado, opcionalmente substituido por F de manera simple o de manera múltiple. La solicitud WO 95/35308 reporta aquellos compuestos que son activos en contra de ICE y no reporta la actividad en contra de otras caspasas.

La solicitud WO 98/10778 revela la inhibición de apoptosis empleando inhibidores de la familia de la enzima que convierte la interleucina-1 β (ICE)/CED-3 de la fórmula:



en donde n es 1 o 2; R¹, R², y R³ son varios grupos; A es un aminoácido natural o antinatural; B incluye, *inter alia*, un grupo halometilo; y X e Y son substituyentes varios.

La solicitud WO 00/061542 revela inhibidores dipéptidos de la apoptosis que tienen la fórmula:



en donde R_1 es grupo alquilo opcionalmente substituido o hidrógeno; R_2 es hidrógeno o alquilo opcionalmente substituido; Y es un residuo de un aminoácido natural o no-natural y R_3 es un grupo alquilo, carbocíclico saturado, carbocíclico parcialmente saturado, arilo, heterocíclico saturado, heterocíclico parcialmente saturado o heteroarilo, en donde dicho grupo se encuentra opcionalmente substituido; X es O, S, NR_4 , o $(CR_4R_5)_n$ en donde R_4 y R_5 son, en cada caso, independientemente seleccionados del grupo que consiste de hidrógeno, alquilo y cicloalquilo, y n es 0, 1, 2, o 3; o X es NR_4 , y R_3 y R_4 son tomados junto con el átomo de nitrógeno al cual ellos están unidos para formar un grupo heterocíclico saturado, heterocíclico parcialmente saturado o heteroarilo, en donde dicho grupo se encuentra opcionalmente substituido o X es CR_4R_5 , y R_3 y R_4 son

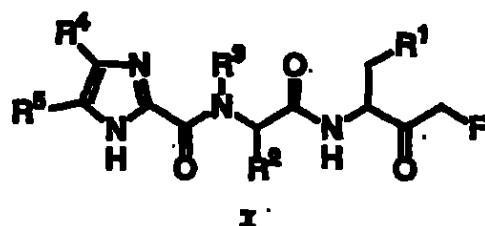
tomados junto con el átomo de carbono al cual ellos están unidos para formar un grupo carbocíclico saturado, carbocíclico parcialmente saturado, arilo, heterocíclico saturado, heterocíclico parcialmente saturado o heteroarilo que contiene oxígeno, en donde dicho grupo se encuentra opcionalmente substituido; y a condición de que cuando X es O, entonces R₃ no es bencilo o t-butilo no substituidos; y cuando X es CH₂, entonces R₃ no es H.

Aunque varios inhibidores de caspasa han sido reportados, no es claro si ellos poseen las propiedades farmacológicas apropiadas para ser terapéuticamente útiles. Por lo tanto, hay una continua necesidad por pequeñas moléculas inhibidoras de caspasa que sean potentes, estables, y que penetren membranas para proporcionar la inhibición efectiva de la apoptosis *in vivo*. Tales compuestos sería extremadamente útiles para tratar las enfermedades anteriormente mencionadas en donde las enzimas caspasa juegan un papel.

RESUMEN DE LA INVENCION

Ahora se ha encontrado que los compuestos de esta invención y las composiciones farmacéuticas de los mismos son especialmente efectivos como inhibidores de caspasas y de la apoptosis celular. Estos compuestos tienen la fórmula general I:

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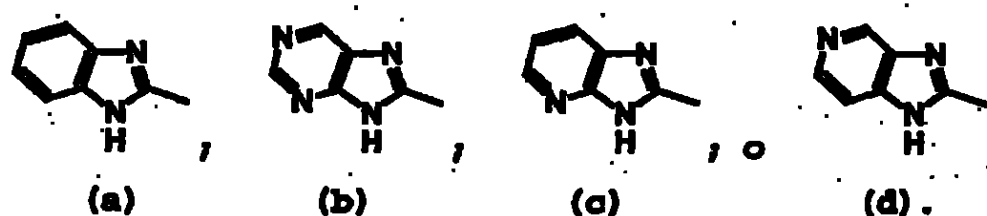
en donde:

R^1 es CO_2H , $\text{CH}_2\text{CO}_2\text{H}$, o ésteres, amidas o isoésteres de los mismos;

R^2 es hidrógeno o un grupo alifático $\text{C}_1\text{-C}_6$ opcionalmente sustituido;

R^3 es hidrógeno o un grupo alifático $\text{C}_1\text{-C}_6$ opcionalmente sustituido; y

R^4 y R^5 son cada uno independientemente seleccionados de hidrógeno, un grupo alifático $\text{C}_1\text{-C}_6$ opcionalmente sustituido, o R^4 y R^5 tomados junto con el anillo al cual ellos están unidos forman un anillo bicíclico opcionalmente sustituido, dicho anillo seleccionado a partir de los siguientes:

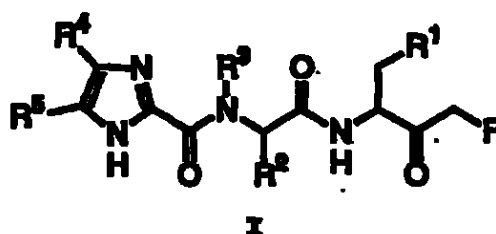


Los compuestos de esta invención tienen potentes propiedades de inhibición dentro de un rango de caspasas objetivo con buena eficacia en modelos celulares de apoptosis. Además, se espera que estos compuestos tengan

penetración celular mejorada y propiedades farmacocinéticas y, como una consecuencia de su potencia, que tengan eficacia mejorada en contra de enfermedades en donde las caspasas se encuentra implicadas.

DESCRIPCION DETALLADA DE LA INVENCION

Esta invención proporciona novedosos compuestos, y derivados farmacéuticamente aceptables de los mismos, que son especialmente efectivos como inhibidores de caspasa. La invención también proporciona métodos para emplear los compuestos para tratar los estados de enfermedad en los cuales las caspasas actúan como intermediarias. Los compuestos tienen la fórmula general I:



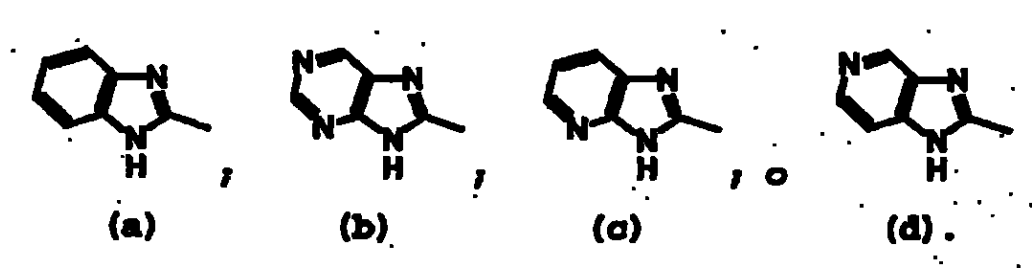
en donde:

R¹ es CO₂H, CH₂CO₂H, o ésteres, amidas o isoésteres de los mismos;

R² es hidrógeno o un grupo alifático C₁-C₆ opcionalmente sustituido;

R³ es hidrógeno o un grupo alifático C₁-C₆ opcionalmente sustituido; y

R^4 y R^5 son cada uno independientemente seleccionados de hidrógeno, un grupo alifático C_1-C_6 opcionalmente sustituido, o R^4 y R^5 tomados junto con el anillo al cual ellos están unidos forman un anillo bicíclico opcionalmente sustituido, dicho anillo seleccionado a partir de los siguientes:



El término "alifático", tal como se usa aquí, significa hidrocarburos C_1-C_{12} de cadena recta, ramificados o cíclicos, que se encuentran completamente saturados o que contienen una o más unidades de insaturación pero que no son aromáticos. Por ejemplo, grupos alifáticos adecuados incluyen los grupos alquilo, alquenilo, alquinilo, sustituidos o sin sustituir, lineales, ramificados o cíclicos, e híbridos de los mismos tales como (cicloalquil)alquilo, (cicloalquenil)alquilo o (cicloalquil)alquenilo. Los términos "alquilo", "alcoxi", "hidroxialquilo", "alcoxialquilo", y "alcoxycarbonilo", usados solos o como parte de un radical más grande, incluyen cadenas tanto rectas como ramificadas, que contienen de uno a doce átomos de carbono. Los términos "alquenilo" y "alquinilo" usados solos o como parte de un

radical más grande incluirán cadenas tanto rectas como ramificadas que contienen de dos a doce átomos de carbono. El término "cicloalquilo" usado solo o como parte de un radical más grande incluirán hidrocarburos C_3-C_{12} cíclicos que se encuentran completamente saturados, o que contienen una o más unidades de insaturación, pero que no son aromáticos.

Los términos "haloalquilo", "haloalquenilo" y "haloalcoxi" significan alquilo, alquenilo o alcoxi, según el caso, substituidos con uno o más átomos de halógeno. El término "halógeno" significa F, Cl, Br, o I.

El término "heteroátomo" significa nitrógeno, oxígeno, o azufre e incluye cualquier forma oxidada de nitrógeno y azufre, y la forma cuaternizada de cualquier nitrógeno básico. El término "nitrógeno" también incluye un nitrógeno sustituible de un anillo heterocíclico. Como un ejemplo, en un anillo saturado o parcialmente insaturado que tenga 0-3 heteroátomos seleccionados a partir de oxígeno, azufre o nitrógeno, el nitrógeno puede ser N (como en 3,4-dihidro-2H-pirrolilo), NH (como en pirrolidinilo) o NR^+ (como en pirrolidinilo N-substituido).

Los términos "carbociclo", "carbociclilo", "carbociclo-", o "carbocíclico", tal como se usan aquí, significan un sistema de anillo alifático que tiene de tres a catorce miembros. Los términos "carbociclo", "carbociclilo",

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"carbociclo-", o "carbocíclico", ya sea saturados o parcialmente insaturados, también hacen referencia a anillos que se encuentran opcionalmente substituidos. Los términos "carbociclo", "carbociclilo", "carbociclo-", o "carbocíclico" también incluyen anillos alifáticos que se encuentran fusionados a uno o más anillos aromáticos o no-aromáticos, tal como en un decahidronaftilo, tetrahidronaftilo, o indanilo, en donde el radical o punto de unión se encuentra sobre el anillo alifático.

El término "arilo" usado solo o como parte de un radical más grande como en "aralquilo", "aralcoxi", o "ariloxialquilo", hace referencia a los grupos de anillos aromáticos que tienen de cinco a catorce miembros, tales como fenilo, bencilo, fenetilo, 1-naftilo, 2-naftilo, 1-antracilo y 2-antracilo. El término "arilo" también hace referencia a anillos que se encuentran opcionalmente substituidos. El término "arilo" puede ser usado de manera intercambiable con el término "anillo arilo". "Arilo" también incluye sistemas de anillos aromáticos policíclicos fusionados en los cuales un anillo aromático se encuentra fusionado a uno o más anillos. Los ejemplos incluyen 1-naftilo, 2-naftilo, 1-antracilo y 2-antracilo. Incluido también dentro del alcance del término "arilo", tal como se usa aquí, se encuentra un grupo en el cual un anillo aromático se encuentra fusionado a uno o más anillos no-aromáticos, tal como en un indanilo, fenantridinilo, o tetrahidronaftilo, en donde el radical o punto de unión se encuentra sobre el anillo aromático.

El término "heterociclo", "heterociclilo", o "heterocíclico", tal como se emplea aquí, incluye sistemas de anillos no-aromáticos que tienen de cinco a catorce miembros, preferiblemente de cinco a diez, en los cuales uno o más carbonos del anillo, preferiblemente de uno a cuatro, son cada uno reemplazados por un heteroátomo tal como N, O, o S. Ejemplos de anillos heterocíclicos incluyen 3-1H-benzimidazol-2-ona, (1-substituido)-2-oxo-benzimidazol-3-ilo, 2-tetrahidrofuranilo, 3-tetrahidrofuranilo, 2-tetrahidropiranilo, 3-tetrahidropiranilo, 4-tetrahidropiranilo, [1,3]-dioxalanilo, [1,3]-ditiolanilo, [1,3]-dioxanilo, 2-tetrahidrotiofenilo, 3-tetrahidrotiofenilo, 2-morfolinilo, 3-morfolinilo, 4-morfolinilo, 2-tiomorfolinilo, 3-tiomorfolinilo, 4-tiomorfolinilo, 1-pirrolidinilo, 2-pirrolidinilo, 3-pirrolidinilo, 1-piperazinilo, 2-piperazinilo, 1-piperidinilo, 2-piperidinilo, 3-piperidinilo, 4-piperidinilo, 4-tiazolidinilo, diazolonilo, diazolonilo N-substituido, 1-ftalimidinilo, benzoxanilo, benzopirrolidinilo, benzopiperidinilo, benzoxolanilo, benzotiolanilo, y benzotianilo. Incluido también dentro del alcance del término "heterociclilo" o "heterocíclico", tal como se emplea aquí, se encuentra un grupo en el cual un anillo no-aromático que contiene heteroátomos se encuentra fusionado a uno o más anillos aromáticos o no-aromáticos, tal como en un indolinilo, cromanilo, fenantridinilo, tetrahydroquinolinilo, o

tetrahidroisoquinolinilo en donde el radical o punto de unión se encuentra sobre el anillo no-aromático que contiene heteroátomos. El término "heterociclo", "heterociclilo", o "heterocíclico" ya sea saturado o parcialmente insaturado, también hace referencia a anillos que se encuentran opcionalmente substituidos.

El término "heteroarilo", usado solo o como parte de un radical más grande como en "heteroaralquilo" o "heteroarilalcoxi" hace referencia a grupos de anillos heteroaromáticos que tienen de cinco a catorce miembros. Los ejemplos de anillos heteroarilo incluyen 2-furanilo, 3-furanilo, N-imidazolilo, 2-imidazolilo, 4-imidazolilo, 5-imidazolilo, 3-furazanilo, 3-isoxazolilo, 4-isoxazolilo, 5-isoxazolilo, 2-oxadiazolilo, 5-oxadiazolilo, 2-oxazolilo, 4-oxazolilo, 5-oxazolilo, 1-pirrolilo, 2-pirrolilo, 3-pirrolilo, 1-pirazolilo, 3-pirazolilo, 4-pirazolilo, 2-piridilo, 3-piridilo, 4-piridilo, 2-pirimidilo, 4-pirimidilo, 5-pirimidilo, 3-piridazinilo, 2-tiazolilo, 4-tiazolilo, 5-tiazolilo, 5-tetrazolilo, 2-triazolilo, 5-triazolilo, 2-tienilo, 3-tienilo, carbazolilo, benzimidazolilo, benztienilo, benzofuranilo, indolilo, quinolinilo, benzotriazolilo, benzotiazolilo, benzooxazolilo, benzimidazolilo, isoquinolinilo, indolilo, isoindolilo, acridinilo, o benzoisoxazolilo. Incluido también dentro del alcance del término "heteroarilo", tal como se usa aquí, se encuentra un grupo en el cual un anillo heteroatómico se encuentra fusionado a uno o más anillos aromáticos o no-aromáticos

en donde el radical o punto de unión se encuentra sobre el anillo heteroaromático.. Los ejemplos incluyen tetrahydroquinolinilo, tetrahydroisoquinolinilo, y pirido [3,4-d] pirimidinilo. El término "heteroarilo" también hace referencia a anillos que se encuentran opcionalmente substituidos. El término "heteroarilo" puede ser usado de manera intercambiable con el término "anillo heteroarilo" o "el término "heteroaromático".

Un grupo árido (que incluye aralquilo, aralcoxi, ariloxialquilo y similares) o heteroarilo (que incluye heteroaralquilo y heteroarilalcoxi y similares) puede contener uno o más substituyentes. Los ejemplos de substituyentes adecuados sobre el átomo de carbono insaturado de un grupo arilo, heteroarilo, aralquilo, o heteroaralquilo incluyen un halógeno, $-R^\circ$, $-OR^\circ$, $-SR^\circ$, 1,2-metileno-dioxi, 1,2-etilenodioxi, OH protegido (tal como aciloxi), fenilo (Ph), Ph substituido, $-O(Ph)$, $-O(Ph)$ substituido, $-CH_2(Ph)$, $-CH_2(Ph)$ substituido, $-CH_2CH_2(Ph)$, $-CH_2CH_2(Ph)$ substituido, $-NO_2$, $-CN$, $-N(R^\circ)_2$, $-NR^\circ C(O)R^\circ$, $-NR^\circ C(O)N(R^\circ)_2$, $-NR^\circ CO_2R^\circ$, $-NR^\circ NR^\circ C(O)R^\circ$, $-NR^\circ NR^\circ C(O)N(R^\circ)_2$, $-NR^\circ NR^\circ CO_2R^\circ$, $-C(O)C(O)R^\circ$, $-C(O)CH_2C(O)R^\circ$, $-CO_2R^\circ$, $-C(O)R^\circ$, $-C(O)N(R^\circ)_2$, $-OC(O)N(R^\circ)_2$, $-S(O)_2R^\circ$, $-SO_2N(R^\circ)_2$, $-S(O)R^\circ$, $-NR^\circ SO_2N(R^\circ)_2$, $-NR^\circ SO_2R^\circ$, $-C(=S)N(R^\circ)_2$, $-C(=NH)-N(R^\circ)_2$, $-(CH_2)_yNHC(O)R^\circ$, $-(CH_2)_yNHC(O)CH(V-R^\circ)(R^\circ)$; en donde R° es hidrógeno, un grupo alifático substituido o no substituido, un anillo heteroarilo o heterocíclico no substituido, fenilo (Ph), Ph substituido, $-O(Ph)$, $-O(Ph)$ substituido, $-CH_2(Ph)$, o -

$\text{CH}_2(\text{Ph})$ substituido; y es 0-6; y V es un grupo ligante. Los ejemplos de substituyentes sobre el grupo alifático o sobre el anillo fenilo de R^* incluyen amino, alquilamino, dialquilamino, aminocarbonilo, halógeno, alquilo, alquilaminocarbonilo, dialquilaminocarbonilo, alquilaminocarboniloxi, dialquilaminocarboniloxi, alcoxi, nitro, ciano, carboxi, alcoxicarbonilo, alquilcarbonilo, hidroxí, haloalcoxi, o haloalquilo.

Un grupo alifático o un anillo heterocíclico no-aromático puede contener uno o más substituyentes. Los ejemplos de substituyentes adecuados sobre el carbono saturado de un grupo alifático o de un anillo heterocíclico no-aromático incluyen aquellos listados anteriormente para el carbono insaturado de un grupo arilo o heteroarilo y los siguientes: $=\text{O}$, $=\text{S}$, $=\text{NNHR}^*$, $=\text{NN}(\text{R}^*)_2$, $=\text{N}-$, $=\text{NNHC}(\text{O})\text{R}^*$, $=\text{NNHC}\text{O}_2(\text{alquilo})$, $=\text{NNHSO}_2(\text{alquilo})$, o $=\text{NR}^*$, en donde cada R^* se selecciona de manera independiente a partir de hidrógeno, un grupo alifático no substituido o un grupo alifático substituido. Ejemplos de substituyentes sobre el grupo alifático incluyen, amino, alquilamino, dialquilamino, aminocarbonilo, halógeno, alquilo, alquilaminocarbonilo, dialquilaminocarbonilo, alquilaminocarboniloxi, dialquilaminocarboniloxi, alcoxi, nitro, ciano, carboxi, alcoxicarbonilo, alquilcarbonilo, hidroxí, haloalcoxi, o haloalquilo.

Los substituyentes adecuados sobre el nitrógeno de un anillo heterocíclico no-aromático incluyen $-\text{R}^*$, $-\text{N}(\text{R}^*)_2$, -

$C(O)R^+$, $-CO_2R^+$, $-C(O)C(O)R^+$, $-C(O)CH_2C(O)R^+$, $-SO_2R^+$, $-SO_2N(R^+)_2$, $-C(=S)N(R^+)_2$, $-C(=NH)-N(R^+)_2$, y $-NR^+SO_2R^+$; en donde R^+ es hidrógeno, un grupo alifático, un grupo alifático sustituido, fenilo (Ph), Ph sustituido, $-O(Ph)$, $-O(Ph)$ sustituido, $CH_2(Ph)$, $CH_2(Ph)$ sustituido, o un anillo heteroarilo o heterocíclico no sustituido. Los ejemplos de sustituyentes sobre el grupo alifático o el anillo fenilo incluyen amino, alquilamino, dialquilamino, aminocarbonilo, halógeno, alquilo, alquilaminocarbonilo, dialquilaminocarbonilo, alquilaminocarboniloxi, dialquilaminocarboniloxi, alcoxi, nitro, ciano, carboxi, alcoxicarbonilo, alquilcarbonilo, hidroxilo, haloalcoxi, o haloalquilo.

Una combinación de sustituyentes o variables es permisible solo si tal combinación resulta en un compuesto estable o químicamente factible. Un compuesto estable o un compuesto químicamente factible es aquel en el cual la estructura química no se encuentra substancialmente alterada cuando se guarda a una temperatura de 40 °C o menos, en la ausencia de humedad u otras condiciones químicamente reactivas, por al menos una semana.

Cuando el grupo R^1 se encuentra en la forma de un éster o amida, los compuestos de la presente invención sufren escisión metabólica en un mamífero a los ácidos carboxílicos correspondientes, los cuales son los inhibidores activos de las caspasas. Debido a que ellos

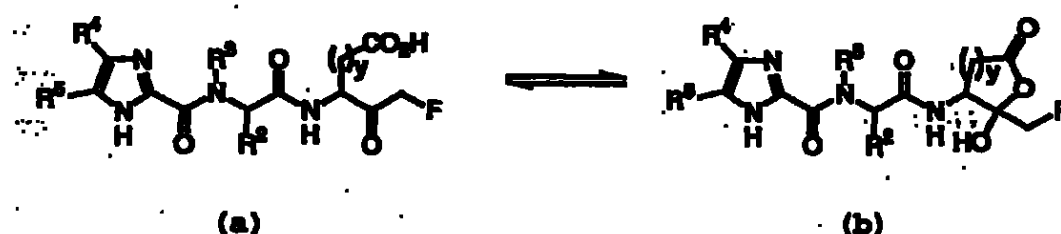
sufren escisión metabólica, la naturaleza precisa del grupo éster o amida no es crítica para el funcionamiento de esta invención. La estructura del grupo R^1 puede variar desde la relativamente simple dietil amida hasta un éster esteroideal. Los ejemplos de ésteres de ácidos carboxílicos de R^1 incluyen, pero no están limitados a, alifático C_1-C_{12} , tal como alquilo C_{1-6} o cicloalquilo C_3-10 , arilo, tal como fenilo, aralquilo, tal como bencilo o fenetilo, heterociclilo, heterociclilalquilo, heteroarilo, y heteroaralquilo. Ejemplos de anillos heterociclicos adecuados de R^1 incluyen, pero no están limitados a, anillos heterocíclicos de 5-6 miembros que tienen uno o dos heteroátomos tales como piperidinilo, piperazinilo, o morfolinilo. Los ejemplos de anillos heteroarilo de R^1 adecuados incluyen, pero no están limitados a, anillos heteroarilo de 5-6 miembros que tienen uno o dos heteroátomos tales como piridilo, pirimidinilo, furanilo y tienilo.

Las amidas de los ácidos carboxílicos de R^1 pueden ser primarias, secundarias o terciarias. Los substituyentes adecuados sobre el nitrógeno de la amida incluyen, pero no están limitadas a, uno o dos grupos seleccionados de manera independiente a partir de los grupos alifático, arilo, aralquilo, heterociclilo, heterociclilalquilo, heteroarilo y heteroaralquilo descritos anteriormente para el éster alcohol de R^1 . Asimismo, otros profármacos se encuentran incluidos dentro del alcance de esta invención. Ver Bradley D. Anderson, "Prodrugs for

Improved CNS Delivery" (Profármacos para la entrega mejorada del SNC) en Advanced Drug Delivery Reviews (1996), 19, 171-202.

Los isoésteres o bioisoésteres de los ácidos carboxílicos de R^1 , los ésteres y las amidas resultan del intercambio de un átomo o grupo de átomos para crear un nuevo compuesto con propiedades biológicas similares al ácido carboxílico o éster precursor. El reemplazo bioisostérico puede estar fisicoquímicamente o topológicamente basado. Un ejemplo de un reemplazo isostérico para un ácido carboxílico es $\text{CONHSO}_2(\text{alquilo})$ tal como CONHSO_2Me .

Los compuestos de esta invención en donde R^1 es CO_2H o $\text{CH}_2\text{CO}_2\text{H}$, γ -cetoácidos o δ -cetoácidos respectivamente pueden existir en solución, ya sea como la forma abierta (a) o la forma hemiacetal ciclizada (b) ($y=1$ para γ -cetoácidos, $y=2$ para δ -cetoácidos). La representación aquí de cualquier forma isomérica tiene la intención de incluir la otra.



Asimismo, será claro para una persona versada en la materia que ciertos compuestos de esta invención pueden

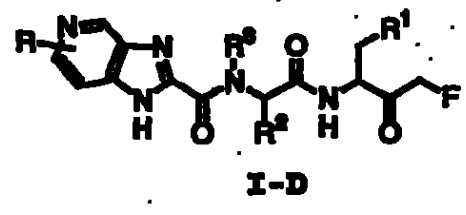
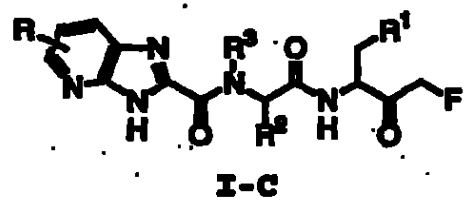
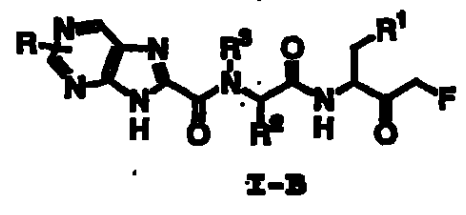
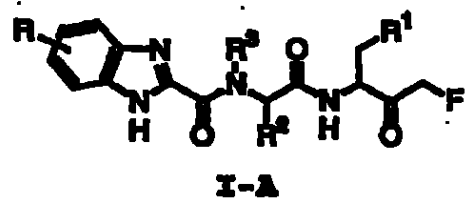
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existir en formas tautoméricas o formas hidratadas, estando todas esas formas de los compuestos dentro del alcance de la invención. A menos que otra cosa se establezca, las estructuras representadas aquí tienen también la intención de incluir todas las formas estereoquímicas de la estructura; esto es, las configuraciones R y S para cada centro asimétrico. Por lo tanto, los isómeros estereoquímicos simples al igual que las mezclas enantioméricas y diastereoméricas de los compuestos de la presente invención se encuentran dentro del alcance de la invención. A menos que otra cosa se establezca, las estructuras representadas aquí tienen también la intención de incluir los compuestos que solo difieren en la presencia de uno o más átomos isotópicamente enriquecidos. Por ejemplo, los compuestos que tienen las estructuras de la presente invención excepto por el reemplazo de un hidrógeno por un deuterio o tritio, o el reemplazo de un carbono por un carbono enriquecido ^{13}C - o ^{14}C - están dentro del alcance de esta invención.

Una cantidad de inhibidores dipeptídicos ICE/caspasa que fueron descritos de manera genérica en la solicitud WO 95/35308 han sido ahora analizados en busca de actividad en contra de las caspasas en los ensayos enzimáticos y basados en células descritos más adelante. Dentro de los compuestos analizados, se encontró que los nuevos compuestos de fórmula I tenían una actividad

inesperadamente buena en contra de un número de enzimas caspasa.

Cuando R⁴ y R⁵ son tomados juntos para formar un anillo fusionado al imidazol, se proporcionan las siguientes realizaciones de esta invención:



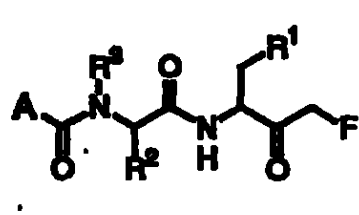
en donde R¹, R² y R³ son tal como se describió anteriormente y R representa uno o más substituyentes opcionales. Los ejemplos de los grupos R, cuando se encuentran unidos a una posición no-adyacente a un nitrógeno del anillo, incluyen alquilo C₁₋₆, alcoxi C₁₋₆, halógeno, alquilamino, dialquilamino, alquilaminocarbonilo, dialquilaminocarbonilo, y alquilcarbonilo. Los ejemplos de los grupos R, cuando se encuentran unidos a una posición adyacente a un nitrógeno del anillo, incluyen alquilo C₁₋₆, alquilamino, y dialquilamino.

Los compuestos preferidos de esta invención son los compuestos de fórmula I que tienen una o más de las siguientes características y más preferiblemente todas las siguientes características:

- (a) R^1 es CO_2H o ésteres, amidas o isoésteres del mismo;
- (b) R^2 es un grupo alquilo C_1-C_6 de cadena recta o ramificada;
- (c) R^3 es hidrógeno; y
- (d) R^4 y R^5 son cada uno hidrógeno, o R^4 y R^5 junto con el anillo al cual ellos se encuentran unidos forman un anillo benzimidazol.

Cuando R^2 se encuentra substituido, los substituyentes preferidos incluyen hidroxí, tio, amino o halógeno.

Los ejemplos representativos específicos de los compuestos de fórmula I se muestran en la Tabla 1.

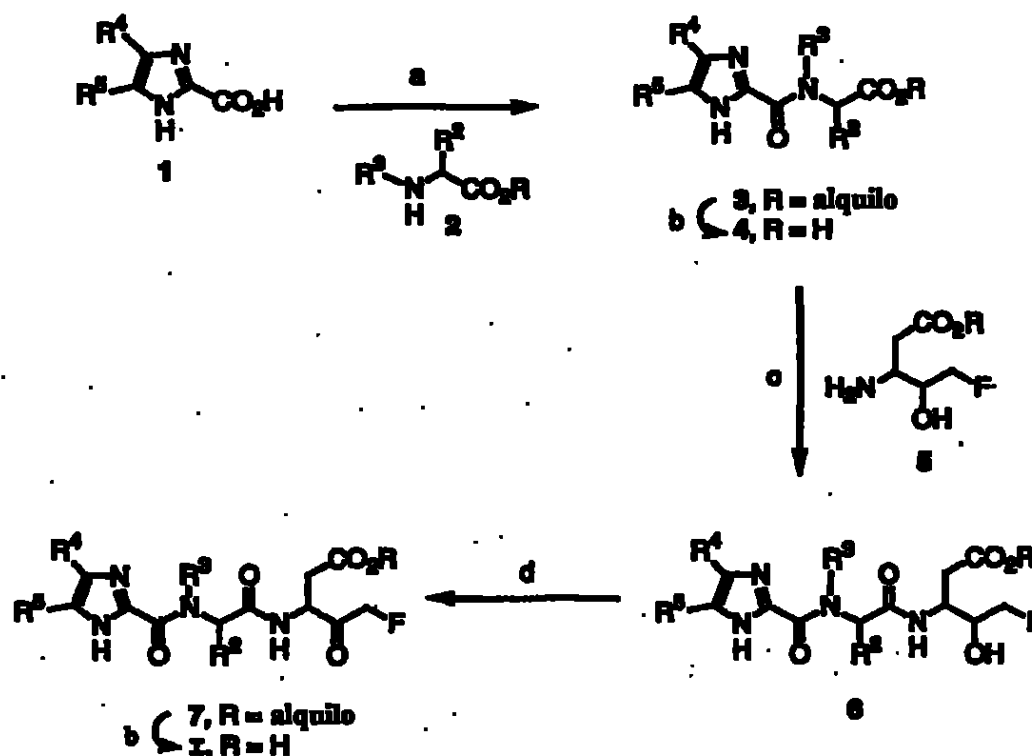


No.	Anillo A	R^1	R^2	R^3
1	Imidazol-2-ilo	CO_2H	CH_3-	H
2	Benzimidazol-2-ilo	CO_2H	CH_3-	H
3	Imidazol-2-ilo	CO_2H	CH_3CH_2-	H

4	Imidazol-2-ilo	CO ₂ H	(CH ₃) ₂ CH-	H
5	Benzimidazol-2-ilo	CO ₂ H	(CH ₃) ₂ CH-	H
6	9H-Purin-8-ilo	CO ₂ H	(CH ₃) ₂ CH-	H
7	3H-Imidazo[4,5-b]piridin-2-ilo	CO ₂ H	(CH ₃) ₂ CH-	H

Los compuestos de esta invención pueden ser preparados en general por medio de métodos conocidos para aquellas personas versadas en la materia para compuestos análogos, tal como se ilustra por medio del Esquema general I siguiente y por medio de los ejemplos preparativos que le siguen.

Esquema I



Reactivos: (a) EDC, diisopropiletilamina, HOBT, 2; (b) hidrólisis o TFA/DCM; (c) DMAP, EDC,

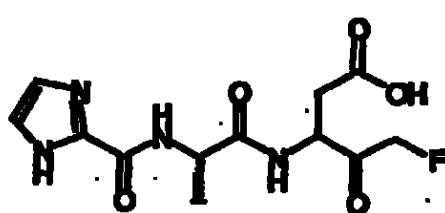
diisopropiletilamina, HOBT, 4; (d) Periodinano Dess-Martin

El Esquema I muestra una ruta general para preparar los compuestos de la presente invención. Las siguientes abreviaciones son empleadas: EDC es 1-(3-dimetilaminopropil)-3-etilcarbodiimida; HOBT es 1-hidroxibenzotriazol ; TFA es ácido trifluoroacético; DCM es diclorometano; y DMAP es 4-dimetilaminopiridina. La reacción del conocido ácido imidazol-2-carboxílico o del conocido ácido benzimidazol-2-carboxílico (representado por medio de la estructura genérica 1) con el derivado amino éster 2 proporciona la amida 3. Si el éster 3 es un éster tert-butilo, El TFA en DCM es empleado para proporcionar el ácido 4. Para los otros grupos R, la hidrólisis standard puede ser empleada. El ácido 4 es luego acoplado con el amino alcohol 5 para proporcionar 6. Dependiendo de la naturaleza de R, una amino cetona puede ser empleada en lugar del amino alcohol 5, lo cual evita la etapa de oxidación subsecuente. En el caso de las fluorometil cetonas en donde CO_2R es CO_2tBu , el amino alcohol 5 puede ser obtenido de acuerdo con el método de Revesz y otros, *Tetrahedron Lett.*, 1994, 35, 9693. Finalmente, el hidroxilo en el compuesto 6 es oxidado y el compuesto es luego tratado apropiadamente de acuerdo con la naturaleza de R^1 . Por ejemplo, si el producto I requiere que R^1 sea un ácido carboxílico, entonces R^1 en 7 es preferiblemente un éster y la etapa final en el esquema es la hidrólisis.

Experimental

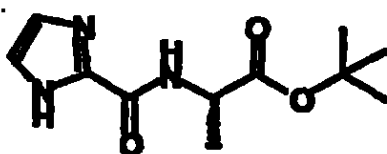
Ejemplo 1

Sal trifluoroacetato del ácido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-propionilamino}-4-oxo-pentanoico (Compuesto 1)



Método A:

Ester *tert*-butilo del ácido (2S)-2-[(1H-Imidazol-2-carbonil)-amino]-propiónico

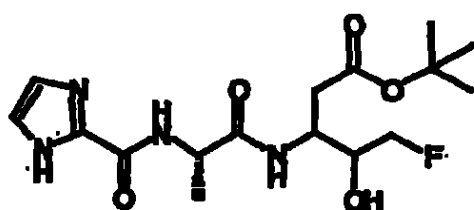


A una solución del ácido 1H-imidazol-2-carboxílico (0.17g) en N,N-dimetilformamida (DMF) (3mL) se adicionó clorhidrato de éster *tert*-butil de alanina (0.22g), diisopropiletil amina (0.27mL) y HOBT (0.41g) antes de enfriar a 0° C y la mezcla de reacción fue tratada con EDC.HCl (0.32g). El baño de enfriamiento fue retirado y

la mezcla de reacción se agitó a temperatura ambiente por 18 hrs antes de ser diluida con etil acetato y lavada con agua y salmuera, secada (MgSO_4) y concentrada a presión reducida. El residuo fue purificado por medio de cromatografía de sílica gel (acetato de etilo al 30% en hexanos) para proporcionar el compuesto del subtítulo como un aceite incoloro (0.263g, 73%): ^1H RMN 400 MHz CDCl_3 1.50 (9H, s), 1.51 (3H, d, J 7.2), 3.70 (1H, m), 7.28 (2H, s), 7.78 (1H, d, J 7.6), 11.49 (1H, br s).

Método B:

Ester tert-butilo del ácido [3S/R, 4S/R, (2S)]-5-Fluoro-4-hidroxi-3-{2-[(1H-imidazol-2-carbonil)-amino]-propionilamino}-pentanoico



Una solución de éster tert-butilo del ácido (2S)-2-[(1H-imidazol-2-carbonil)-amino]-propionico (0.257g) en diclorometano (2mL) fue enfriada a 0° C antes de la adición gota a gota de ácido trifluoroacético y la mezcla de reacción se calentó a temperatura ambiente y se agitó por 2 hr antes de la evaporación bajo presión reducida. El residuo fue co-evaporado con diclorometano (dos veces) y tolueno (dos veces) para dejar el ácido (2S)-2-[(1H-

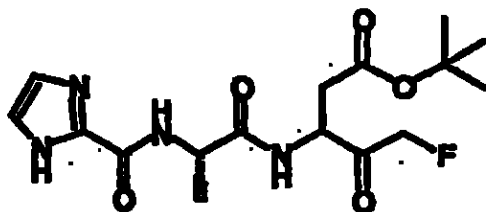
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imidazol-2-carbonil)-amino]-propionico requerido que fue usado sin purificación adicional (0.40g).

Una solución del ácido (2S)-2-[(1H-imidazol-2-carbonil)-amino]-propionico y del éster tert-butilo del ácido 3-amino-5-fluoro-4-hidroxi-pentanoico (0.254g) en THF (7mL) fue enfriada a 0° C antes de la adición de DMAP (0.151g), diisopropiletil amina (0.56mL), HOBT (0.16g) y EDC.HCl (0.23g). La mezcla de reacción se agitó a temperatura ambiente por 18 hrs antes de ser concentrada a presión reducida. El residuo fue purificado por medio de cromatografía de sílica gel (Metanol al 5% en diclorometano) para proporcionar el compuesto del subtítulo como un sólido incoloro (0.386g, 97%): ¹H RMN 400MHz CDC13/CD3OD 1.40 (12H, m), 3.92 (1H, m), 4.20-4.55 (4H, m), 7.11 (2H, d, J 15) ¹⁹F RMN CDC13 -229.74 (m), -229.84 (m), -230.54 (m), -230.87 (m).

Método C:

Ester tert-butilo del ácido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-propionilamino}-4-oxo-pentanoico

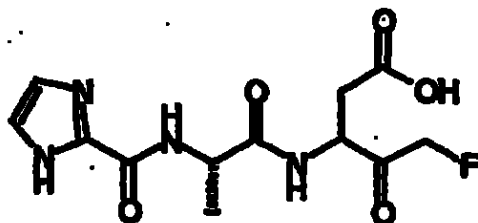


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Una solución del éster tert-butilo del ácido [3S/R, (2S)]-5-fluoro-4-hidroxi-3-{2-[(1H-imidazol-2-carbonil)-amino]-propionilamino}-pentanoico (0.381g) en diclorometano fue enfriada a 0° C antes de la adición de 1,1,1 triacetoxi-1,1-dihidro-1,2-benziodoxol-3(1H)-ona (0.476g). La mezcla fue agitada a temperatura ambiente por 2 h antes de la adición de una porción adicional de 1,1,1 triacetoxi-1,1-dihidro-1,2-benziodoxol-3(1H)-ona (0.05g) y la mezcla de reacción se agitó por 90 min. antes de ser concentrada a presión reducida. El residuo fue disuelto en acetato de etilo y lavado con una mezcla 1:1 de NaHSO₄ acuoso y Na₂S₂O₃ acuoso. La capa orgánica fue recolectada, secada (MgSO₄) y concentrada. El residuo fue purificado por cromatografía instantánea (flash) (metanol al 5% en diclorometano) para dar el compuesto del subtítulo como una espuma incolora (319mg, 84%): ¹H RMN 400MHz CDCl₃ 1.37+1.43 (9H, 2xs), 1.54 (3H, m), 2.85 (1H, m), 3.03 (1H, m), 4.85-5.30 (4H, m), 7.18 (2H, d, J 16), 7.90 (1H, m), 7.98 (1H, m), 11.37+11.45 (1H, 2xs) ; ¹⁹F RMN 376 MHz CDCl₃ -231.85 (t, J 48), -232.12 (t, J 48).

Método D:

Compuesto 1

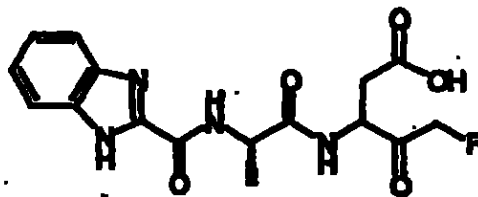


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Una solución del éster tert-butilo del ácido [3S/R, (2S)]-5-Fluoro-3-(2-[(1H-imidazol-2-carbonil)-amino]-propionilamino)-4-oxo-pentanoico (0.31g) en diclorometano (2mL) fue enfriada a 0 °C antes de la adición gota a gota de ácido trifluoroacético y la mezcla de reacción se calentó a temperatura ambiente y se agitó por 2 hr antes de la evaporación bajo presión reducida. El residuo fue co-evaporado con diclorometano (dos veces) y triturado bajo éter para dar el compuesto del título como un sólido incoloro (0.35g): IR 1785.7, 1730.1, 1653.7, 1538.1, 1458.2, 1384.2, 1268.7, 1188.4, 1150.9, 1053.3, 992.13, 931.8, 867.9, 847.0, 768.5 cm^{-1} ; ^1H RMN 400MHz DMSO D_6 1.37 (3H, d), 2.40-2.85 (2H, m, asp CH_2); 4.34-4.75 (2.5H, m, $2\times\text{CH}+0.5\text{CH}_2\text{F}$); 5.13-5.41 (1.5H, m, CH_2F); 7.50 (2H, s, imidazol CHs); 8.58-8.79 (2H, m, NHs); ^{13}C RMN 100 MHz DMSO D_6 18.13, 18.85 (ala CH_3); 33.13, 34.75 (asp CH_2); 48.68, 52.41 (CHs); 83.46, 85.21 (CH_2F); 123.67 (CH imidazol); 139.57, 158.86, 172.35 (m) ($\text{C}=\text{O}$ s); 202.70 (5 picos de cetona); ^{19}F RMN 376 MHz DMSO D_6 desacoplado - 75.19 (3F, s, CF_3COOH); -(226.89, 226.96, 230.80, 231.59, 232.95, 233.06 (1F, 6xs, COCH_2F anillo abierto y anillo cerrado).

Ejemplo 2

Sal trifluoroacetato del ácido [3S/R, (2S)]-3-(2-[(1H-Benzoimidazol-2-carbonil)-amino]-propionilamino)-5-fluoro-4-oxo-pentanoico (Compuesto 2)

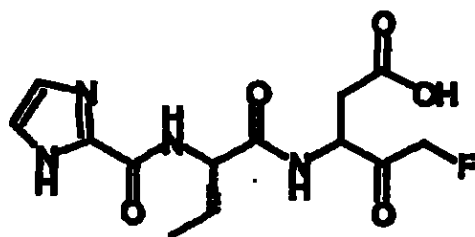


Este compuesto fue preparado a partir del ácido 1H-benzoimidazol-2-carboxílico empleando procedimientos similares a aquellos descritos en los anteriores Métodos A-D (142 mg, 90% para la etapa final): (el compuesto se aisló como la sal TFA) sólido de color hueso; IR (sólido, cm^{-1}) 3277.9, 1654.6, 1526.6, 1188.6, 1142.5, 1050.4, 927.5, 748.2, 712.4; ^1H RMN ($\text{DMSO}-\text{D}_6$) 1.42 (3H, d), 2.51-2.95 (2H, m), 4.21-4.75 (2H, m), 4.76-5.60 (3H, brm), 7.41 (2H, m), 7.65 (2H, m), 8.21-9.05 (2H, m); ^{13}C RMN ($\text{DMSO}-\text{D}_6$) 18.0, 18.7, 18.8 (Ala CH_3), 37.2, 34.6, 34.7 (Asp CH_2), 47.6, 48.8, 48.85, 49.1 (Asp CH), 52.0, 52.5 (Ala CH), 83.5, 85.2, 85.3, 103.8, 106.0 (CH_2F), 116.6, 123.9 (Arilo CH), 145.3, 145.4, (Arilo C), 158.4, 158.7, 158.8, 172.1, 172.2, 172.4, 172.5, 172.6, 172.7, 173.2 (NC=O), 202.6, 202.7, 202.8, 202.9 (C=O) ; M^+ encontrado 364.1177.

$\text{C}_{16}\text{H}_{17}\text{FN}_4\text{O}_5$ requiere M^+ 364.1183 (1.8 ppm).

Ejemplo 3

Sal trifluoroacetato del ácido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-butirilamino}-4-oxo-pentanoico (Compuesto 3)

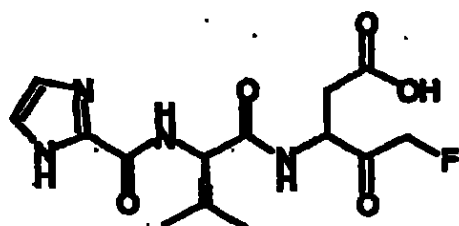


Este compuesto fue preparado a partir del ácido 1H-benzoimidazol-2-carboxílico empleando procedimientos similares a aquellos descritos en los anteriores métodos A-D (147 mg, 64% para la etapa final): IR 3280.0, 1659.5, 157.9, 1192.5, 1141.6, 784.7, 721.1 cm^{-1} ; ^1H RMN 400MHz DMSO D_6 0.95 (3H, m), 1.78 (2H, m), 2.58-2.98 (2H, m), 4.30-4.78 (2.5H, m), 5.10-5.42 (1.5H, m), 7.41 (2H, s), 8.44+8.75 (2H, 2xm); ^{13}C RMN 100 MHz DMSO D_6 10.19, 10.29, 15.52 (CH₃), 25.42, 25.49, 26.03, 33.06, 33.13, 34.65, 34.80 (CH₂), 47.45, 47.53, 52.0, 53.96, 54.13 (CH) 65.27 (CH₂), 84.36 (d, J 177, CH₂F), 103.81, 104.00 (C), 123.89 (CH), 139.74 (C=O), 156.90, 158.39, 158.74, 171.51, 171.80, 171.83, 172.02, 173.11 (C=O), 202.51, 202.66, 202.76, 202.90 (CH₂FC=O); ^{19}F RMN 376 MHz DMSO D_6 -226.82 (t, J 45), -226.84 (t, J 45), -230.67 (t, J 45), -231.43 (t, J 45), -232.79 (t, J 45), -232.82 (t, J45).

Ejemplo 4

Acido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-3-metilbutirilamino}-4-oxo-pentanoico

(Compuesto 4)

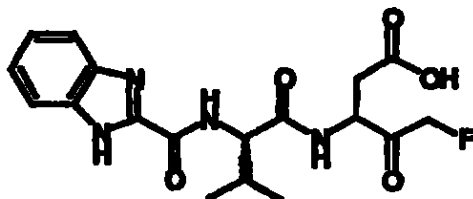


Este compuesto fue preparado a partir del ácido 1H-benzoimidazol-2-carboxílico empleando procedimientos similares a aquellos descritos en los anteriores Métodos A-D (80g, 85% para la etapa final): polvo blanco, IR (sólido, cm^{-1}) 1736, 1649, 1557, 1511, 1455, 1434, 1393; ^1H RMN (DMSO+TFA) 0.92-0.95 (6H, m), 2.06-2.15 (1H, m), 2.56-2.90 (2H, m), 4.33-5.36 (4H, m), 7.79 (2H, s), 8.58-8.90 (2H, m); ^{19}F RMN (DMSO+TFA) -226.8 (t), -230.6 (t), -231.0 (t), -232.5 (t), -232.6 (t); ^{13}C RMN (DMSO+TFA) 18.1/18.4 (CH₃), 19.2/19.3 (CH₃), 34.5/34.8 (CH₂), 51.9/52.2 (CH), 58.5/58.8 (CH), 84.3/84.4 (2d, J 178.7/178.7, CH₂F), 122.0 (CH), 137.5 (C), 153.7 (C), 170.6 (C), 171.9/172.0 (C), 202.5/202.8 (2d, J 14.6/14.6, CO).

Ejemplo5

Ácido [3S/R, (2S)]-3-{2-[(1H-Benzoimidazol-2-carbonil)-amino]-3-metilbutirilamino}-5-fluoro-4-oxo-pentanoico

(Compuesto 5)



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Este compuesto fue preparado a partir del ácido 1H-benzoimidazol-2-carboxílico empleando procedimientos similares a aquellos descritos en los anteriores Métodos A-D (90mg, 87% para la etapa final): polvo blanco, IR (sólido, cm^{-1}) 1737, 1665, 1527, 1373, 1194, 1137; ^1H RMN (DMSO) 0.90-0.95 (6H, m), 2.15-2.18 (1H, m), 2.59-2.92 (2H, m), 4.33-4.76 y 5.12-5.38 (4H, 2m), 7.31-7.35 (2H, m), 7.66-7.68 (2H, m), 8.36-8.82 (2H, m); ^{19}F RMN (DMSO+TFA) -226.7 (t), -226.9 (t), -232.4 (t), -232.6 (t); ^{13}C RMN (DMSO) 18.3/18.4/18.5/18.7 (CH₃), 19.4/19.5 (CH₃), 31.0/31.1/31.6 (CH), 34.7/34.8 (CH₂), 51.8/52.1 (CH), 57.9/58.3/58.6 (CH), 84.3/84.4 (2d, J 178.7/178.7, CH₂F), 124.0 (CH), 145.2/145.2 (C), 158.4/158.5/158.7/158.8 (C), 170.9/171.1/171.2 (C), 172.0/172.0 (C), 173.1 (C), 173.9 (C), 202.06/202.6 (2d, J 13.8, CO).

Los compuestos de esta invención están diseñados para inhibir las caspasas. Por lo tanto, los compuestos de esta invención pueden ser analizados en cuanto a su habilidad para inhibir la apoptosis, a la liberación de IL-1 β o a la actividad de las caspasas directamente. Los ensayos para cada una de las actividades son conocidos en la técnica y son descritos más adelante en detalle en la Sección de pruebas.

De acuerdo a otra realización, la invención proporciona una composición que comprende un compuesto de esta

invención o una sal farmacéuticamente aceptable del mismo, como se describió anteriormente, y un portador farmacéuticamente aceptable.

Si las sales farmacéuticamente aceptables de los compuestos de esta invención son utilizadas en estas composiciones, esas sales son preferiblemente derivadas de ácidos y bases inorgánicas u orgánicas. Incluidas dentro de dichas sales ácidas se encuentran las siguientes: acetato, adipato, alginato, aspartato, benzoato, benceno sulfonato, bisulfato, butirato, citrato, camforato, camfor sulfonato, ciclopentanopropionato, digluconato, dodecilsulfato, etanosulfonato, fumarato, glucoheptanoato, glicerofosfato, hemisulfato, heptanoato, hexanoato, clorhidrato, bromhidrato, yodhidrato, 2-hidroxietanosulfonato, lactato, maleato, metanosulfonato, 2-naftalenosulfonato, nicotinato, oxalato, pamoato, pectinato, persulfato, 3-fenil-propionato, picrato, pivalato, propionato, succinato, tartrato, tiocianato, tosilato y undecanoato. Las sales básicas incluyen las sales de amonio, las sales de metales alcalinos, tales como las sales de sodio y potasio, las sales de metales alcalinotérreos, tales como las sales de calcio y magnesio, las sales con bases orgánicas, tales como las sales de diciclohexilamina, N-metil-D-glucamina y las sales con aminoácidos tales como arginina, lisina, y así sucesivamente.

También, los grupos básicos que contienen nitrógeno pueden ser cuaternizados con agentes tales como haluros de alquilo inferior, tales como metilo, etilo, propilo, y cloruro, bromuros y yoduros de butilo; dialquil sulfatos, tales como dimetil, dietil, dibutil y diamil sulfatos, haluros de cadena larga tales como los cloruros, bromuros y yoduros de decilo, laurilo, miristilo y estearilo, aralquil haluros, tales como los bromuros de bencilo y fenetilo y otros. Los productos solubles en agua o en aceite o dispersables son obtenidos de ese modo.

Los compuestos utilizados en las composiciones y métodos de esta invención pueden también ser modificados anexando funcionalidades apropiadas para incrementar las propiedades biológicas selectivas. Tales modificaciones son conocidas en la técnica e incluyen aquellas que incrementan la penetración biológica dentro de un sistema biológico determinado (por ejemplo, la sangre, el sistema linfático, el sistema nervioso central), que incrementan la disponibilidad oral, que incrementan la solubilidad para permitir la administración por medio de inyección, que alteran el metabolismo y que alteran la velocidad de excreción.

Los portadores farmacéuticamente aceptables que pueden ser empleados en estas composiciones incluyen, pero no están limitados a, intercambiadores iónicos, alúmina, estearato de aluminio, lecitina, proteínas del suero, tales como la albúmina sérica humana, sustancias tampón

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tales como fosfatos, glicina, ácido sórbico, sorbato de potasio, mezclas parciales de glicéridos de ácidos grasos vegetales saturados, agua, sales o electrolitos, tales como sulfato de protamina, fosfato ácido disódico, fosfato ácido de potasio, cloruro de sodio, sales de zinc, sílica coloidal, trisilicato de magnesio, polivinil pirrolidona, sustancias basadas en celulosa, polietilen glicol, carboximetilcelulosa de sodio, poliacrilatos, ceras, polímeros de bloque polietileno-polioxipropileno, polietilen glicol y grasa de lana.

De acuerdo con una realización preferida, las composiciones de esta invención están formuladas para la administración farmacéutica a un paciente, preferiblemente a un ser humano. Un aspecto de esta invención se relaciona con un método para el tratamiento de una enfermedad relacionada con la caspasa en un paciente en necesidad del mismo, método que comprende administrar al paciente una cantidad terapéuticamente efectiva de un compuesto de fórmula I o una composición farmacéutica del mismo. El término "paciente" incluye sujetos humanos y veterinarios.

Tales composiciones farmacéuticas de la presente invención pueden ser administradas oralmente, parenteralmente, por medio de un spray de inhalación, tópicamente, rectalmente, nasalmente, bucalmente, vaginalmente o por medio de un reservorio implantado. El término "parenteral", tal como se usa aquí, incluye

inyección subcutánea, intravenosa, intramuscular, intra-articular, intra-sinovial, intraesternal, intratecal, intrahepática, intralesional e intracraneal, o técnicas de infusión. Preferiblemente, las composiciones son administradas oralmente o intravenosamente.

Las formas inyectables estériles de las composiciones de esta invención pueden ser suspensiones acuosas u oleaginosas. Estas suspensiones pueden ser formuladas de acuerdo con las técnicas conocidas en la técnica usando agentes dispersantes o humectantes adecuados y agentes de suspensión. La preparación inyectable estéril también puede ser una solución o suspensión inyectable estéril en un diluyente o solvente no tóxico parenteralmente aceptable, tal como por ejemplo una solución en 1,3-butanodiol. Dentro de los vehículos y solventes aceptables que puede ser empleados se encuentran el agua, la solución de Ringer y la solución de cloruro de sodio isotónico. Además, los aceites fijos estériles son tradicionalmente empleados como un solvente o medio de suspensión. Para este propósito, cualquier aceite fijo suave puede ser empleado incluyendo mono- o di-glicéridos sintéticos. Los ácidos grasos, tales como el ácido oleico y sus derivados de glicérido son útiles en la preparación de inyectables, tanto como lo son los aceites farmacéuticamente aceptables, tales como el aceite de oliva o el aceite de ricino, especialmente en sus versiones polioxietiladas. Estas soluciones o suspensiones oleosas pueden también contener un diluyente

o dispersante de alcohol de cadena larga, tal como la carboximetil celulosa o agentes dispersantes similares que son normalmente empleados en la formulación de formas de dosificación farmacéuticamente aceptables que incluyen las emulsiones y las suspensiones. Otros tensioactivos normalmente empleados, tales como los Tweens, Spans y otros agentes emulsificantes o incrementadores de biodisponibilidad que son normalmente empleados en la fabricación de sólidos, líquidos u otras formas de dosificación farmacéuticamente aceptables también pueden ser usados para los propósitos de la formulación.

Las composiciones farmacéuticas de esta invención pueden ser oralmente administradas en cualquier forma de dosificación oralmente aceptable que incluyen, pero no se limitan a, cápsulas, tabletas, suspensiones o soluciones acuosas. En el caso de las tabletas para uso oral, los portadores que son normalmente empleados incluyen la lactosa y el almidón de maíz. Los agentes lubricantes, tal como el estearato de magnesio, también son típicamente adicionados. Para la administración oral en una forma de cápsula, los diluyentes útiles incluyen lactosa y almidón de maíz seco. Cuando las suspensiones acuosas son requeridas para el uso oral, el ingrediente activo es combinado con agentes emulsificantes y de suspensión. Si se desea, también pueden ser adicionados ciertos agentes endulzantes, saborizantes o colorantes.

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De manera alternativa, las composiciones farmacéuticas de esta invención pueden ser administradas en la forma de supositorios para la administración rectal. Estos pueden ser preparados mediante la mezcla del agente con un excipiente adecuado no irritante que es sólido a temperatura ambiente pero líquido a la temperatura rectal y, por lo tanto, se fundirá en el recto liberando el medicamento. Tales materiales incluyen la manteca de cacao, la cera de abejas y los polietilen glicoles.

Las composiciones farmacéuticas de esta invención también pueden ser administradas tópicamente, especialmente cuando el objetivo del tratamiento incluye áreas u órganos fácilmente accesible por medio de la aplicación tópica, incluyendo las enfermedades de los ojos, de la piel, o del tracto intestinal inferior. Las formulaciones tópicas adecuadas son fácilmente preparadas para cada una de estas áreas u órganos.

La aplicación tópica para el tracto intestinal inferior puede ser efectuada en una formulación rectal de supositorio (ver anteriormente) o en una formulación adecuada de enema. También pueden ser usados los parches Tópicamente-transdérmicos.

Para las aplicaciones tópicas, las composiciones farmacéuticas pueden ser formuladas en un ungüento adecuado que contenga el componente activo suspendido o disuelto en uno o más portadores. Los portadores para la

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administración tópica de los compuestos de esta invención incluyen, pero no se limitan a, aceite mineral, petrolato líquido, petrolato blanco, propilen glicol, polioxietileno, compuesto de polioxipropileno, cera emulsificante y agua. De manera alternativa, las composiciones farmacéuticas pueden ser formuladas en una loción o crema adecuadas que contengan los componentes activos suspendidos o disueltos en uno o más portadores farmacéuticamente aceptables. Los portadores adecuados incluyen, pero no están limitados a, aceite mineral, monoestearato de sorbitan, polisorbato 60, cera de cetil ésteres, cetearil alcohol, 2-octildodecanol, bencil alcohol y agua.

Para uso oftálmico, las composiciones farmacéuticas pueden ser formuladas como suspensiones micronizadas en salina estéril con el pH ajustado, isotónica, o, preferiblemente, como soluciones en salina estéril con el pH ajustado, isotónica, ya sea con o sin un conservante tal como cloruro de bencilalconio. De manera alternativa, para usos oftálmicos, las composiciones farmacéuticas pueden ser formuladas en un ungüento tal como petrolato.

Las composiciones farmacéuticas de esta invención también pueden ser administradas por medio de aerosol nasal o inhalación. Tales composiciones son preparadas de acuerdo con técnicas bien conocidas en el arte de la formulación farmacéutica y pueden ser preparadas como soluciones en salina, empleando bencil alcohol u otros conservantes

adecuados, promotores de absorción para incrementar la biodisponibilidad, fluorocarbonos, y/u otros agentes solubilizantes o dispersantes convencionales.

Las composiciones anteriormente descritas son particularmente útiles para el tratamiento de las enfermedades relacionadas con la caspasa. El término "enfermedad relacionada con la caspasa" hace referencia a una enfermedad mediada por IL-1, una enfermedad mediada por la apoptosis, una enfermedad inflamatoria, una enfermedad autoinmune, un trastorno destructivo del hueso, un trastorno proliferativo, una enfermedad infecciosa, una enfermedad degenerativa, una enfermedad asociada con la muerte celular, una enfermedad por exceso en la toma de alcohol dietético, una enfermedad mediada por virus, uveítis, peritonitis inflamatoria, osteoartritis, pancreatitis, asma, síndrome de dolor respiratorio de adultos, glomerulonefritis, artritis reumatoide, lupus eritematoso sistémico, escleroderma, tiroiditis crónica, enfermedad de Grave, gastritis autoinmune, diabetes, anemia hemolítica autoinmune, neutropenia autoinmune, trombocitopenia, hepatitis activa crónica, miastenia grave, enfermedad inflamatoria del intestino, enfermedad de Crohn, psoriasis, dermatitis atópica, cicatrización, enfermedad de injerto contra anfitrión, rechazo de trasplante de órganos, osteoporosis, leucemias y trastornos relacionados, síndrome mielodisplástico, trastorno múltiple de huesos relacionado con el mieloma, leucemia mielogena aguda,

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melanoma metastático, sarcoma de Kaposi, mieloma múltiple, shock hemorrágico, sepsia, shock séptico, quemaduras, Shigellosis, enfermedad de Alzheimer, enfermedad de Parkinson, enfermedad de Huntington, enfermedad de Kennedy, enfermedad de priones, isquemia cerebral, epilepsia, isquemia del miocardio, enfermedad del corazón aguda y crónica, infarto al miocardio, falla cardíaca congestiva, aterosclerosis, injerto de derivaciones de la arteria coronaria, atrofia muscular espinal, esclerosis amiotrófica lateral, esclerosis múltiple, encefalitis relacionada con el VIH, envejecimiento, alopecia, daño neurológico debido a ataque, colitis ulcerativa, lesión cerebral traumática, lesión de la columna vertebral, hepatitis B, hepatitis C, hepatitis G, fiebre amarilla, fiebre del dengue, o encefalitis japonesa, varias formas de enfermedades del hígado, enfermedad renal, enfermedad poliáptica del riñón, enfermedad de úlcera gástrica y duodenal asociada con el H. pílora, infección del VIH, tuberculosis, y meningitis.

Los usos preferidos de las composiciones de la presente invención incluyen una enfermedad mediada por IL-1, una enfermedad mediada por la apoptosis, una enfermedad inflamatoria, una enfermedad autoinmune, un trastorno destructivo del hueso, una enfermedad infecciosa, una enfermedad degenerativa, una enfermedad asociada con la muerte celular, una enfermedad por exceso en la toma de alcohol dietético, una enfermedad mediada por virus,

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uveítis, peritonitis inflamatoria, osteoartritis, pancreatitis, síndrome de dolor respiratorio de adultos, glomerulonefritis, artritis reumatoide, diabetes, trombocitopenia, enfermedad inflamatoria del intestino, enfermedad de Crohn, psoriasis, cicatrización, rechazo de transplante de órganos, osteoporosis, shock hemorrágico, sepsia, shock séptico, quemaduras, Shigellosis, enfermedad de Alzheimer, enfermedad de Parkinson, enfermedad de Huntington, enfermedad de Kennedy, enfermedad de priones, isquemia cerebral, epilepsia, enfermedad del corazón aguda y crónica, injerto de derivaciones de la arteria coronaria, esclerosis amiotrófica lateral, esclerosis múltiple, alopecia, colitis ulcerativa, lesión cerebral traumática, lesión de la columna vertebral, varias formas de enfermedades del hígado, enfermedad renal, enfermedad de úlcera gástrica y duodenal asociada con el H. píloro, y meningitis.

Los compuestos y las composiciones también son útiles en el tratamiento de complicaciones asociadas con injertos de derivaciones de la arteria coronaria. Los compuestos y las composiciones también son particularmente útiles para el tratamiento del cáncer, ya sean solos o en combinación con otra terapia tal como quimioterapia o radioterapia. Los compuestos y las composiciones también pueden ser empleados como un componente de la inmunoterapia para el tratamiento de varias formas de cáncer.

La cantidad de compuesto de la presente invención en las composiciones anteriormente descritas debe ser suficiente para ocasionar una disminución detectable en la gravedad de la enfermedad o en la actividad de la caspasa y/o en la apoptosis celular, tal como es medida por medio de cualquiera de los análisis descritos en los ejemplos.

De acuerdo con otra realización, las composiciones de esta invención pueden además comprender otro agente terapéutico. Tales agentes incluyen, pero no se limitan a, agentes trombolíticos tales como el activador de plasminógenos tisulares y la estreptocinasa. Cuando se emplea un segundo agente, el segundo agente puede ser administrado ya sea como una forma de dosificación separada o como parte de una única forma de dosificación con los compuestos o las composiciones de esta invención.

También debe entenderse que un régimen específico de dosificación y tratamiento para cualquier paciente particular dependerá de una variedad de factores, que incluyen la actividad del compuesto específico empleado, la edad, el peso corporal, la salud general, el sexo, la dieta, el tiempo de administración, la velocidad de excreción, la combinación de medicamentos, y el juicio del médico tratante y la gravedad de la enfermedad particular a ser tratada. La cantidad de los ingredientes activos también dependerá del compuesto particular y del otro agente terapéutico, si esta presente, en la composición.

En una realización preferida, la invención proporciona un método para el tratamiento de un mamífero, que tiene una de las enfermedades anteriormente mencionadas, que comprende la etapa de administrar a dicho mamífero una composición farmacéuticamente aceptable descrita con anterioridad. En esta realización, si al paciente también se le administra otro agente terapéutico o inhibidor de caspasa, este puede ser entregado junto con el compuesto de esta invención en una única forma de dosificación, o, como una forma de dosificación separada. Cuando se administra como una forma de dosificación separada, el otro agente o inhibidor de caspasa puede ser administrado antes, al mismo tiempo, o después de la administración de una composición farmacéuticamente aceptable que comprende un compuesto de esta invención.

Para que esta invención sea mas completamente entendida, los siguientes ejemplos preparativos y de ensayo son proporcionados a continuación. Estos ejemplos son solo para propósitos ilustrativos y no son para ser interpretados como limitantes del alcance de la invención desde ningún punto de vista.

Ejemplo 6

Análisis enzimáticos

Los análisis de la inhibición de caspasa están basados en la escisión de un sustrato fluorogénico por medio de las Caspasas humanas -1, -3, u -8, purificadas,

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recombinantes. Los análisis son llevados a cabo esencialmente de la misma manera como se describe en la solicitud W00142216.

Cada uno de los compuestos 1-5 poseen un valor de k_{inact} superior a $20000 \text{ M}^{-1}\text{s}^{-1}$ en contra de cada una de las caspasa-1, caspasa-3 y caspasa-8.

Ejemplo 7

Inhibición de la secreción de IL-1 β a partir de la población mezclada de células mononucleares sanguíneas periféricas (PBMC)

El procesamiento de la pre-IL-1 β por medio de la caspasa-1 puede ser medido en el cultivo celular empleando una variedad de fuentes celulares. La PBMC humana obtenida a partir de donantes saludables proporciona una población mezclada de linfocitos y células mononucleares que producen un espectro de interleucinas y citoquinas como respuesta a muchas clases de estimuladores fisiológicos. Las condiciones del análisis empleadas para la inhibición de la secreción de IL-1 β a partir de la población mezclada de células mononucleares sanguíneas periféricas puede ser encontrada en la solicitud W00142216.

La potencia inhibidora de los compuestos puede ser representada por medio de un valor IC_{50} , el cual es la concentración del inhibidor a la cual el 50% del IL-1 β maduro es detectado en el sobrenadante cuando se compara

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con los controles positivos. Se encontró que los compuestos ensayados proporcionan un valor IC_{50} menor a 1μ para la inhibición de la secreción de IL- 1β a partir de PBMC.

EJEMPLO 8

Análisis de la Apoptosis inducida por Anti-Fas

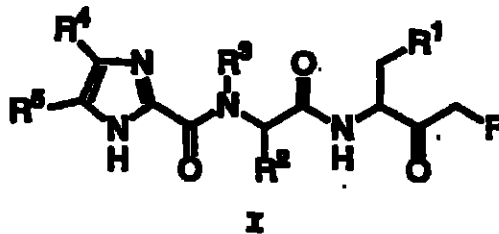
La apoptosis celular puede ser inducida por medio de la unión del ligando Fas (FasL) a su receptor, CD95 (Fas). Las condiciones de un análisis para medir el efecto de los compuestos en la inhibición de la ruta apoptótica mediada por la Caspasa-8 pueden ser encontradas en la solicitud W00142216.

Se encontró que cada uno de los compuestos 1-5 proporcionan un valor IC_{50} menor a 200nM para la actividad en el análisis de la apoptosis inducida por FAS.

Si bien nosotros hemos descrito diferentes realizaciones de esta invención, es claro que nuestros ejemplos básicos pueden ser alterados para proporcionar otras realizaciones, que utilizarán los compuestos y los métodos de esta invención. Por lo tanto, será apreciado que el alcance de esta invención se define por medio de las reivindicaciones anexas más que por las realizaciones específicas que han sido representadas a manera de ejemplo.

Lo que se reivindica es:

1. Un compuesto de fórmula I:



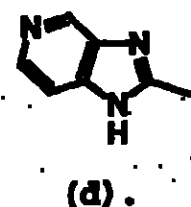
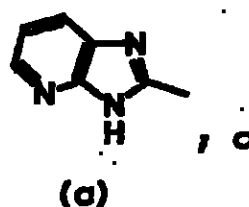
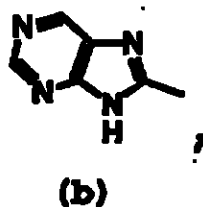
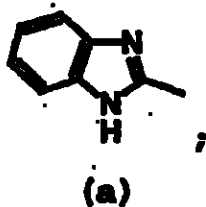
en donde:

R^1 es CO_2H , $\text{CH}_2\text{CO}_2\text{H}$, o ésteres, amidas o isoésteres de los mismos;

R^2 es hidrógeno o un grupo alifático $\text{C}_1\text{-C}_6$ opcionalmente sustituido;

R^3 es hidrógeno o un grupo alifático $\text{C}_1\text{-C}_6$ opcionalmente sustituido; y

R^4 y R^5 son cada uno independientemente seleccionados de hidrógeno, un grupo alifático $\text{C}_1\text{-C}_6$ opcionalmente sustituido, o R^4 y R^5 tomados junto con el anillo al cual ellos están unidos forman un anillo bicíclico opcionalmente sustituido, dicho anillo bicíclico seleccionado a partir de los siguientes:



2. El compuesto de la reivindicación 1 en donde R^2 es un grupo alquilo C_{1-6} recto o ramificado opcionalmente substituido.

3. El compuesto de la reivindicación 1 que tiene una o más características seleccionadas del grupo que consiste de:

- a) R^1 es CO_2H o ésteres, amidas o isoésteres del mismo;
- b) R^2 es un grupo alquilo C_1-C_6 de cadena recta o ramificada;
- c) R^3 es hidrógeno; y
- d) R^4 y R^5 son cada uno hidrógeno, o R^4 y R^5 junto con el anillo al cual ellos están unidos forman un anillo benzimidazol.

4. El compuesto de la reivindicación 3 que tiene las siguientes características:

- a) R^1 es CO_2H o ésteres, amidas o isoésteres del mismo;
- b) R^2 es un grupo alquilo C_1-C_6 de cadena recta o ramificada;
- c) R^3 es hidrógeno; y
- d) R^4 y R^5 son cada uno hidrógeno, o R^4 y R^5 junto con el anillo al cual ellos están unidos forman un anillo benzimidazol.

5. Un compuesto seleccionado del grupo que consiste de:

Acido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-propionilamino}-4-oxo-pentanoico;

Ester tert-butilo del ácido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-propionilamino}-4-oxo-pentanoico;

Acido [3S/R, (2S)]-3-{2-[(1H-Benzimidazol-2-carbonil)-amino]-propionilamino}-5-fluoro-4-oxo-pentanoico;

Acido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-butirilamino}-4-oxo-pentanoico;

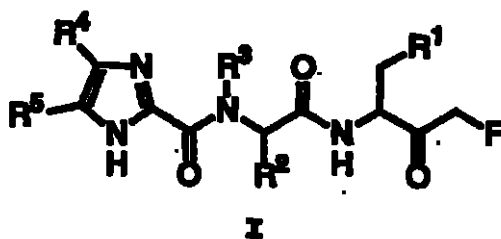
Acido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-3-metilbutirilamino}-4-oxo-pentanoico;

Acido [3S/R, (2S)]-3-{2-[(1H-Benzimidazol-2-carbonil)-amino]-3-metilbutirilamino}-5-fluoro-4-oxo-pentanoico;

o una sal de adición de los mismos.

6. Una composición farmacéutica que comprende un compuesto de acuerdo con cualquiera de las reivindicaciones 1-5 y un portador farmacéuticamente aceptable.

7. Un método para tratar una enfermedad mediada por la caspasa en un paciente, método que comprende administrar al paciente una cantidad terapéuticamente efectiva de un compuesto de fórmula I:



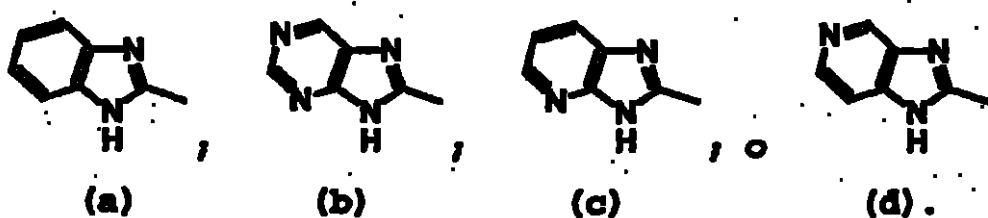
en donde:

R^1 es CO_2H , $\text{CH}_2\text{CO}_2\text{H}$, o ésteres, amidas o isoésteres de los mismos;

R^2 es hidrógeno o un grupo alifático $\text{C}_1\text{-C}_6$ opcionalmente sustituido;

R^3 es hidrógeno o un grupo alifático $\text{C}_1\text{-C}_6$ opcionalmente sustituido; y

R^4 y R^5 son cada uno independientemente seleccionados de hidrógeno, un grupo alifático $\text{C}_1\text{-C}_6$ opcionalmente sustituido, o R^4 y R^5 tomados junto con el anillo al cual ellos están unidos forman un anillo bicíclico opcionalmente sustituido, dicho anillo bicíclico seleccionado a partir de los siguientes:



8. El método de acuerdo con la reivindicación 7 en donde la enfermedad o tratamiento se selecciona de una enfermedad mediada por IL-1, una enfermedad mediada por la apoptosis, una enfermedad inflamatoria, una enfermedad

autoinmune, un trastorno destructivo del hueso, un trastorno proliferativo, una enfermedad infecciosa, una enfermedad degenerativa, una enfermedad asociada con la muerte celular, una enfermedad por exceso en la toma de alcohol dietético, una enfermedad mediada por virus, uveítis, peritonitis inflamatoria, osteoartritis, pancreatitis, asma, síndrome de dolor respiratorio de adultos, glomerulonefritis, artritis reumatoide, lupus eritematoso sistémico, escleroderma, tiroiditis crónica, enfermedad de Grave, gastritis autoinmune, diabetes, anemia hemolítica autoinmune, neutropenia autoinmune, trombocitopenia, hepatitis activa crónica, miastenia grave, enfermedad inflamatoria del intestino, enfermedad de Crohn, psoriasis, dermatitis atópica, cicatrización, enfermedad de injerto contra anfitrión, rechazo de trasplante de órganos, osteoporosis, leucemias y trastornos relacionados, síndrome mielodisplástico, trastorno múltiple de huesos relacionado con el mieloma, leucemia mielogena aguda, melanoma metastático, sarcoma de Kaposi, mieloma múltiple, shock hemorrágico, sepsia, shock séptico, quemaduras, Shigellosis, enfermedad de Alzheimer, enfermedad de Parkinson, enfermedad de Huntington, enfermedad de Kennedy, enfermedad de priones, isquemia cerebral, epilepsia, isquemia del miocardio, enfermedad del corazón aguda y crónica, infarto al miocardio, falla cardíaca congestiva, aterosclerosis, injerto de derivaciones de la arteria coronaria, atrofia muscular espinal, esclerosis amiotrófica lateral, esclerosis múltiple, encefalitis relacionada con el VIH,

envejecimiento, alopecia, daño neurológico debido a ataque, colitis ulcerativa, lesión cerebral traumática, lesión de la columna vertebral, hepatitis B, hepatitis C, hepatitis G, fiebre amarilla, fiebre del dengue, o encefalitis japonesa, varias formas de enfermedades del hígado, enfermedad renal, enfermedad poliáptica del riñón, enfermedad de úlcera gástrica y duodenal asociada con el H. pílora, infección del VIH, tuberculosis, meningitis, un tratamiento de complicaciones asociadas con injertos de derivaciones de la arteria coronaria, o una inmunoterapia para el tratamiento de varias formas de cáncer.

9. El método de la reivindicación 8, en donde la enfermedad se selecciona de una enfermedad mediada por IL-1, una enfermedad mediada por la apoptosis, una enfermedad inflamatoria, una enfermedad autoinmune, un trastorno destructivo del hueso, una enfermedad infecciosa, una enfermedad degenerativa, una enfermedad asociada con la muerte celular, una enfermedad por exceso en la toma de alcohol dietético, una enfermedad mediada por virus, uveítis, peritonitis inflamatoria, osteoartritis, pancreatitis, síndrome de dolor respiratorio de adultos, glomerulonefritis, artritis reumatoide, diabetes, trombocitopenia, enfermedad inflamatoria del intestino, enfermedad de Crohn, psoriasis, cicatrización, rechazo de transplante de órganos, osteoporosis, shock hemorrágico, sepsia, shock séptico, quemaduras, Shigellosis, enfermedad de

Alzheimer, enfermedad de Parkinson, enfermedad de Huntington, enfermedad de Kennedy, enfermedad de priones, isquemia cerebral, epilepsia, enfermedad del corazón aguda y crónica, injerto de derivaciones de la arteria coronaria, esclerosis amiotrófica lateral, esclerosis múltiple, alopecia, colitis ulcerativa, lesión cerebral traumática, lesión de la columna vertebral, varias formas de enfermedades del hígado, enfermedad renal, enfermedad de úlcera gástrica y duodenal asociada con el H. píloro, y meningitis.

10. El método de la reivindicación 7, en donde la enfermedad o condición es una complicación asociada con injertos de derivaciones de la arteria coronaria.

11. El método de la reivindicación 7, en donde la enfermedad es cáncer.

12. El método de la reivindicación 11, en donde el método es usado en combinación con quimioterapia o radioterapia.

13. El método de la reivindicación 11, en donde el compuesto es un componente de la inmunoterapia para el tratamiento del cáncer.

14. El método de acuerdo con cualquiera de las reivindicaciones 7-13, en donde el compuesto tiene una o

más características seleccionadas del grupo que consiste de:

- a) R^1 es CO_2H o ésteres, amidas o isoésteres del mismo;
- b) R^2 es un grupo alquilo C_1-C_6 de cadena recta o ramificada;
- c) R^3 es hidrógeno; y
- d) R^4 y R^5 son cada uno hidrógeno, o R^4 y R^5 junto con el anillo al cual ellos están unidos forman un anillo benzimidazol.

15. El método de acuerdo con cualquiera de las reivindicaciones 7-13, en donde el compuesto tiene las siguientes características:

- a) R^1 es CO_2H o ésteres, amidas o isoésteres del mismo;
- b) R^2 es un grupo alquilo C_1-C_6 de cadena recta o ramificada;
- c) R^3 es hidrógeno; y
- d) R^4 y R^5 son cada uno hidrógeno, o R^4 y R^5 junto con el anillo al cual ellos están unidos forman un anillo benzimidazol.

16. El método de acuerdo con cualquiera de las reivindicaciones 7-13, en donde el compuesto es seleccionado del grupo que consiste de:

Acido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-propionilamino}-4-oxo-pentanoico;

Ester tert-butilo del ácido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-propionilamino}-4-oxo-pentanoico;

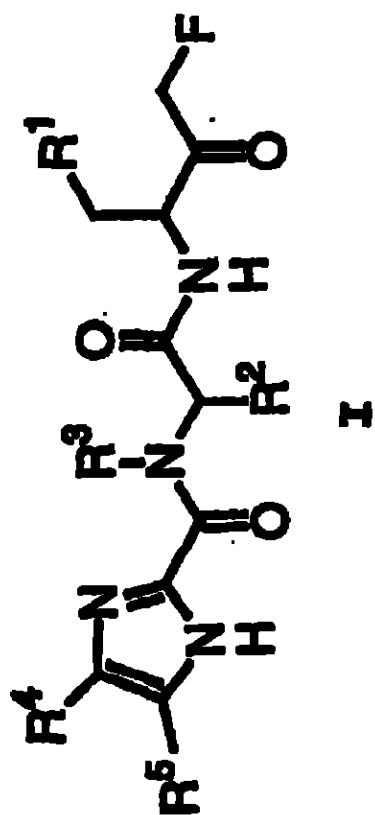
Acido [3S/R, (2S)]-3-{2-[(1H-Benzimidazol-2-carbonil)-amino]-propionilamino}-5-fluoro-4-oxo-pentanoico;

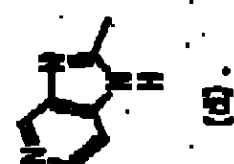
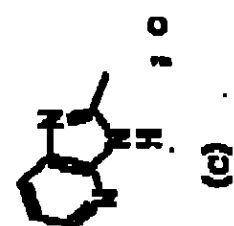
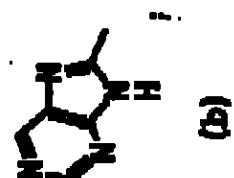
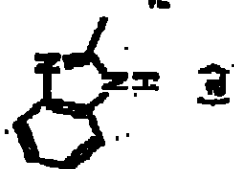
Acido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-butirilamino}-4-oxo-pentanoico;

Acido [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazol-2-carbonil)-amino]-3-metilbutirilamino}-4-oxo-pentanoico;

Acido [3S/R, (2S)]-3-{2-[(1H-Benzimidazol-2-carbonil)-amino]-3-metilbutirilamino}-5-fluoro-4-oxo-pentanoico;

o una sal de adición de los mismos.





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IMIDAZOLE AND BENZIMIDAZOLE CASPASE INHIBITORS AND USES
THEREOF.

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to US Provisional Patent Application 60/252,252 filed November 21, 2000.

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Field of the Invention

This invention is in the field of medicinal chemistry and relates to novel compounds, and pharmaceutical compositions thereof, that inhibit caspases that mediate cell apoptosis and inflammation.

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The invention also relates to methods of using the compounds and pharmaceutical compositions of this invention to treat diseases where caspase activity is implicated.

Background of the Invention

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Apoptosis, or programmed cell death, is a principal mechanism by which organisms eliminate unwanted cells. The deregulation of apoptosis, either excessive apoptosis or the failure to undergo it, has been implicated in a number of diseases such as cancer, acute inflammatory and autoimmune disorders, ischemic diseases and certain neurodegenerative disorders (see generally Science, 1998, 281, 1283-1312; Ellis et al., Ann. Rev. Cell. Biol., 1991, 7, 663).

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Caspases are a family of cysteine protease enzymes that are key mediators in the signaling pathways

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for apoptosis and cell disassembly (Thornberry, Chem. Biol., 1998, 5, R97-R103). These signaling pathways vary depending on cell type and stimulus, but all apoptosis pathways appear to converge at a common effector pathway leading to proteolysis of key proteins. Caspases are involved in both the effector phase of the signaling pathway and further upstream at its initiation. The upstream caspases involved in initiation events become activated and in turn activate other caspases that are involved in the later phases of apoptosis.

Caspase-1, the first identified caspase, is also known as interleukin converting enzyme or "ICE." Caspase-1 converts precursor interleukin-1 β ("pIL-1 β ") to the pro-inflammatory active form by specific cleavage of pIL-1 β between Asp-116 and Ala-117. Besides caspase-1 there are also eleven other known human caspases, all of which cleave specifically at aspartyl residues. They are also observed to have stringent requirements for at least four amino acid residues on the N-terminal side of the cleavage site.

The caspases have been classified into three groups depending on the amino acid sequence that is preferred or primarily recognized. The group of caspases, which includes caspases 1, 4, and 5, has been shown to prefer hydrophobic aromatic amino acids at position 4 on the N-terminal side of the cleavage site. Another group which includes caspases 2, 3 and 7, recognize aspartyl residues at both positions 1 and 4 on the N-terminal side of the cleavage site, and preferably a sequence of Asp-Glu-X-Asp. A third group, which includes caspases 6, 8, 9 and 10, tolerate many amino acids in the primary

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recognition sequence, but seem to prefer residues with branched, aliphatic side chains such as valine and leucine at position 4.

The caspases have also been grouped according to their putative function. The first subfamily consists of caspases-1 (ICE), 4, and 5. These caspases have been shown to be involved in pro-inflammatory cytokine processing and therefore play an important role in inflammation. Caspase-1, the most studied enzyme of this class, activates the IL-1 β precursor by proteolytic cleavage. This enzyme therefore plays a key role in the inflammatory response. Caspase-1 is also involved in the processing of interferon gamma inducing factor (IGIF or IL-18) which stimulates the production of interferon- γ , a key immunoregulator that modulates antigen presentation, T-cell activation and cell adhesion.

The remaining caspases make up the second and third subfamilies. These enzymes are of central importance in the intracellular signaling pathways leading to apoptosis. One subfamily consists of the enzymes involved in initiating events in the apoptotic pathway, including transduction of signals from the plasma membrane. Members of this subfamily include caspases-2, 8, 9 and 10. The other subfamily, consisting of the effector caspases 3, 6 and 7, are involved in the final downstream cleavage events that result in the systematic breakdown and death of the cell by apoptosis. Caspases involved in the upstream signal transduction activate the downstream caspases, which then disable DNA repair mechanisms, fragment DNA, dismantle the cell cytoskeleton and finally fragment the cell.

Knowledge of the four amino acid sequence primarily recognized by the caspases has been used to design caspase inhibitors. Reversible tetrapeptide inhibitors have been prepared having the structure

5 $\text{CH}_3\text{CO}-[\text{P4}]-[\text{P3}]-[\text{P2}]-\text{CH}(\text{R})\text{CH}_2\text{CO}_2\text{H}$ where P2 to P4 represent an optimal amino acid recognition sequence and R is an aldehyde, nitrile or ketone capable of binding to the caspase cysteine sulfhydryl. Rano and Thornberry, *Chem. Biol.* 4, 149-155 (1997); Mjalli, et al., *Bioorg. Med. Chem. Lett.* 3, 2689-2692 (1993); Nicholson et al., *Nature* 376, 37-43 (1995). Irreversible inhibitors based on the analogous tetrapeptide recognition sequence have been prepared where R is an acyloxymethylketone $-\text{COCH}_2\text{OCOR}'$. R' is exemplified by an optionally substituted phenyl

10 such as 2,6-dichlorobenzoyloxy and where R is COCH_2X where X is a leaving group such as F or Cl. Thornberry et al., *Biochemistry* 33, 3934 (1994); Dolle et al., *J Med. Chem.* 37, 563-564 (1994).

The utility of caspase inhibitors to treat a

20 variety of mammalian disease states associated with an increase in cellular apoptosis has been demonstrated using peptidic caspase inhibitors. For example, in rodent models, caspase inhibitors have been shown to reduce infarct size and inhibit cardiomyocyte apoptosis

25 after myocardial infarction, to reduce lesion volume and neurological deficit resulting from stroke, to reduce post-traumatic apoptosis and neurological deficit in traumatic brain injury, to be effective in treating fulminant liver destruction, and to improve survival

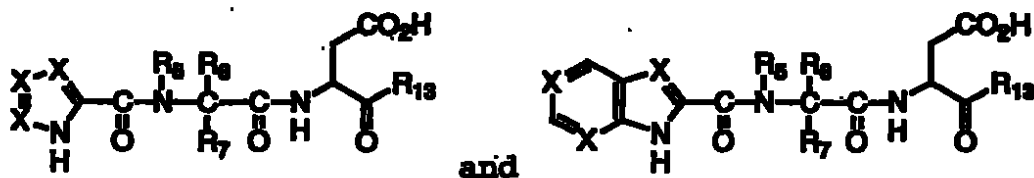
30 after endotoxic shock. Yaoita et al., *Circulation*, 97, 276 (1998); Endres et al., *J Cerebral Blood Flow and*

Metabolism, 18, 238, (1998); Cheng et al., J. Clin. Invest., 101, 1992 (1998); Yakovlev et al., J. Neuroscience, 17, 7415 (1997); Rodriguez et al., J. Exp. Med., 184, 2067 (1996); Grobmyer et al., Mol. Med., 5, 585 (1999).

In general, the peptidic inhibitors described above are very potent against some of the caspase enzymes. However, this potency has not always been reflected in cellular models of apoptosis. In addition peptide inhibitors are typically characterized by undesirable pharmacological properties such as poor oral absorption, poor stability and rapid metabolism. Plattner and Norbeck, in *Drug Discovery Technologies*, Clark and Moos, Eds. (Ellis Horwood, Chichester, England, 1990).

Recognizing the need to improve the pharmacological properties of the peptidic caspase inhibitors, peptidomimetic and non-natural amino acid peptide inhibitors have been reported.

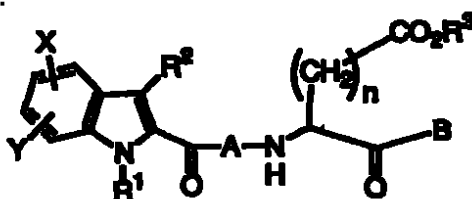
WO 95/35308 discloses inhibitors of interleukin-1 β converting enzyme including, inter alia, compounds of the formulae:



wherein X is independently selected from =N- or =CH-; R₅ includes hydrogen; R₇ is hydrogen and R₆ includes an α -amino acid side chain; and R₁₃ includes hydrogen, an aromatic or heteroaromatic ring, a C₁-C₆ straight or branched alkyl group singly or multiply optionally

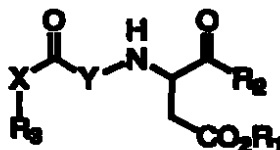
substituted by F. WO 95/35308 reports those compounds to be active against ICE and does not report activity against other caspases.

WO 98/10778 discloses inhibition of apoptosis
5 using interleukin-1 β -converting enzyme (ICE)/CED-3 family inhibitors of formula:



wherein n is 1 or 2; R¹, R², and R³ are various groups; A
10 is a natural or unnatural amino acid; B includes, *inter alia*, a halomethyl group; and X and Y are various substituents.

WO 00/061542 discloses dipeptide apoptosis inhibitors having the formula:



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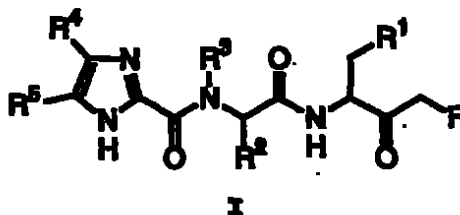
where R₁ is an optionally substituted alkyl or hydrogen group; R₂ is hydrogen or optionally substituted alkyl; Y is a residue of a natural or non-natural amino acid and R₃
20 is an alkyl, saturated carbocyclic, partially saturated carbocyclic, aryl, saturated heterocyclic, partially saturated heterocyclic or heteroaryl group, wherein said group is optionally substituted; X is O, S, NR₄, or (CR₄R₅)_n where R₄ and R₅ are, at each occurrence,
25 independently selected from the group consisting of hydrogen, alkyl and cycloalkyl, and n is 0, 1, 2, or 3;

or X is NR_4 , and R_3 and R_4 are taken together with the nitrogen atom to which they are attached to form a saturated heterocyclic, partially saturated heterocyclic or heteroaryl group, wherein said group is optionally substituted or X is CR_4R_5 , and R_3 and R_4 are taken together with the carbon atom to which they are attached to form a saturated carbocyclic, partially saturated carbocyclic, aryl, saturated heterocyclic, partially saturated heterocyclic or oxygen-containing heteroaryl group, wherein said group is optionally substituted; and provided that when X is O, then R_3 is not unsubstituted benzyl or t-butyl; and when X is CH_2 , then R_3 is not H.

While a number of caspase inhibitors have been reported, it is not clear whether they possess the appropriate pharmacological properties to be therapeutically useful. Therefore, there is a continued need for small molecule caspase inhibitors that are potent, stable, and penetrate membranes to provide effective inhibition of apoptosis *in vivo*. Such compounds would be extremely useful in treating the aforementioned diseases where caspase enzymes play a role.

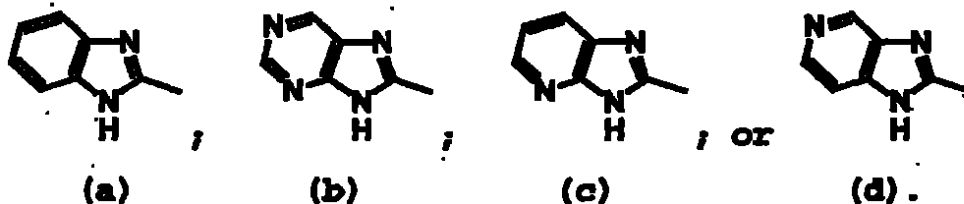
Summary of the Invention

It has now been found that compounds of this invention and pharmaceutical compositions thereof are particularly effective as inhibitors of caspases and cellular apoptosis. These compounds have the general formula I:



wherein:

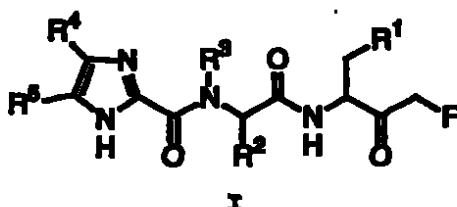
- R¹** is CO₂H, CH₂CO₂H, or esters, amides or isosteres thereof;
- R²** is hydrogen or an optionally substituted C₁-C₆ aliphatic group;
- R³** is hydrogen or an optionally substituted C₁-C₆ aliphatic group; and
- R⁴** and **R⁵** are each independently selected from hydrogen, an optionally substituted C₁-C₆ aliphatic group, or **R⁴** and **R⁵** taken together with the ring to which they are attached form an optionally substituted bicyclic ring, said bicyclic ring selected from the following:



The compounds of this invention have potent inhibition properties across a range of caspase targets with good efficacy in cellular models of apoptosis. In addition, these compounds are expected to have improved cell penetration and pharmacokinetic properties and, as a consequence of their potency, have improved efficacy against diseases where caspases are implicated.

Detailed Description of the Invention

This invention provides novel compounds, and pharmaceutically acceptable derivatives thereof, that are particularly effective as caspase inhibitors. The invention also provides methods for using the compounds to treat caspase-mediated disease states in mammals. The compounds have the general formula I:



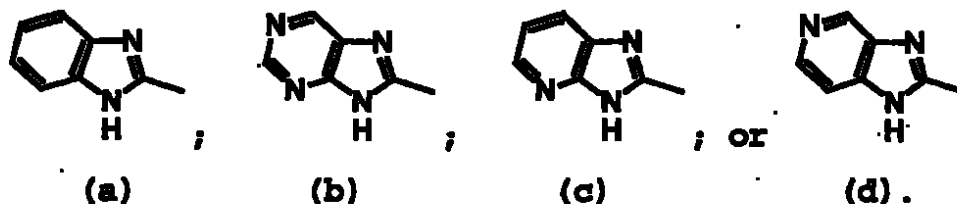
10 wherein:

R^1 is CO_2H , $\text{CH}_2\text{CO}_2\text{H}$, or esters, amides or isosteres thereof;

R^2 is hydrogen or an optionally substituted $\text{C}_1\text{-C}_6$ aliphatic group;

15 R^3 is hydrogen or an optionally substituted $\text{C}_1\text{-C}_6$ aliphatic group; and

R^4 and R^5 are each independently selected from hydrogen, an optionally substituted $\text{C}_1\text{-C}_6$ aliphatic group, or R^4 and R^5 taken together with the ring to which they are attached form an optionally substituted bicyclic ring, said bicyclic ring selected from the following:



The term "aliphatic" as used herein means straight-chain, branched or cyclic $\text{C}_1\text{-C}_{12}$ hydrocarbons

which are completely saturated or which contain one or more units of unsaturation but which are not aromatic. For example, suitable aliphatic groups include substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl. The terms "alkyl", "alkoxy", "hydroxyalkyl", "alkoxyalkyl", and "alkoxycarbonyl", used alone or as part of a larger moiety includes both straight and branched chains containing one to twelve carbon atoms. The terms "alkenyl" and "alkynyl" used alone or as part of a larger moiety shall include both straight and branched chains containing two to twelve carbon atoms. The term "cycloalkyl" used alone or as part of a larger moiety shall include cyclic C₃-C₁₂ hydrocarbons which are completely saturated or which contain one or more units of unsaturation, but which are not aromatic.

The terms "haloalkyl", "haloalkenyl" and "haloalkoxy" means alkyl, alkenyl or alkoxy, as the case may be, substituted with one or more halogen atoms. The term "halogen" means F, Cl, Br, or I.

The term "heteroatom" means nitrogen, oxygen, or sulfur and includes any oxidized form of nitrogen and sulfur, and the quaternized form of any basic nitrogen. Also the term "nitrogen" includes a substitutable nitrogen of a heterocyclic ring. As an example, in a saturated or partially unsaturated ring having 0-3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4-dihydro-2H-pyrrolyl), NH (as

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in pyrrolidinyl) or NR⁺ (as in N-substituted pyrrolidinyl).

The terms "carbocycle", "carbocyclyl", "carbocyclo", or "carbocyclic" as used herein means an aliphatic ring system having three to fourteen members. The terms "carbocycle", "carbocyclyl", "carbocyclo", or "carbocyclic" whether saturated or partially unsaturated, also refers to rings that are optionally substituted. The terms "carbocycle", "carbocyclyl", "carbocyclo", or "carbocyclic" also include aliphatic rings that are fused to one or more aromatic or nonaromatic rings, such as in a decahydronaphthyl, tetrahydronaphthyl, or indanyl where the radical or point of attachment is on the aliphatic ring.

The term "aryl" used alone or as part of a larger moiety as in "aralkyl", "aralkoxy", or "aryloxyalkyl", refers to aromatic ring groups having five to fourteen members, such as phenyl, benzyl, phenethyl, 1-naphthyl, 2-naphthyl, 1-anthracyl and 2-anthracyl. The term "aryl" also refers to rings that are optionally substituted. The term "aryl" may be used interchangeably with the term "aryl ring". "Aryl" also includes fused polycyclic aromatic ring systems in which an aromatic ring is fused to one or more rings. Examples include 1-naphthyl, 2-naphthyl, 1-anthracyl and 2-anthracyl. Also included within the scope of the term "aryl", as it is used herein, is a group in which an aromatic ring is fused to one or more non-aromatic rings, such as in an indanyl, phenanthridinyl, or tetrahydronaphthyl, where the radical or point of attachment is on the aromatic ring.

The term "heterocycle", "heterocyclyl", or "heterocyclic" as used herein includes non-aromatic ring systems having five to fourteen members, preferably five to ten, in which one or more ring carbons, preferably one to four, are each replaced by a heteroatom such as N, O, or S. Examples of heterocyclic rings include 3-1H-benzimidazol-2-one, (1-substituted)-2-oxo-benzimidazol-3-yl, 2-tetrahydrofuranyl, 3-tetrahydrofuranyl, 2-tetrahydropyranyl, 3-tetrahydropyranyl, 4-tetrahydropyranyl, [1,3]-dioxalanyl, [1,3]-dithiolanyl, [1,3]-dioxanyl, 2-tetrahydrothiophenyl, 3-tetrahydrothiophenyl, 2-morpholinyl, 3-morpholinyl, 4-morpholinyl, 2-thiomorpholinyl, 3-thiomorpholinyl, 4-thiomorpholinyl, 1-pyrrolidinyl, 2-pyrrolidinyl, 3-pyrrolidinyl, 1-piperazinyl, 2-piperazinyl, 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-piperidinyl, 4-thiazolidinyl, diazolonyl, N-substituted diazolonyl, 1-phthalimidinyl, benzoxanyl, benzopyrrolidinyl, benzopiperidinyl, benzoxolanyl, benzothiolanyl, and benzothianyl. Also included within the scope of the term "heterocyclyl" or "heterocyclic", as it is used herein, is a group in which a non-aromatic heteroatom-containing ring is fused to one or more aromatic or non-aromatic rings, such as in an indolinyl, chromanyl, phenanthridinyl, tetrahydroquinolinyl, or tetrahydroisoquinolinyl where the radical or point of attachment is on the non-aromatic heteroatom-containing ring. The term "heterocycle", "heterocyclyl", or "heterocyclic" whether saturated or partially unsaturated, also refers to rings that are optionally substituted.

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The term "heteroaryl", used alone or as part of a larger moiety as in "heteroaralkyl" or "heteroarylalkoxy", refers to heteroaromatic ring groups having five to fourteen members. Examples of heteroaryl rings include 2-furanyl, 3-furanyl, N-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl, 3-furazanyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-oxadiazolyl, 5-oxadiazolyl, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 1-pyrazolyl, 3-pyrazolyl, 4-pyrazolyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-pyrimidyl, 3-pyridazinyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 5-tetrazolyl, 2-triazolyl, 5-triazolyl, 2-thienyl, 3-thienyl, carbazolyl, benzimidazolyl, benzothienyl, benzofuranyl, indolyl, quinolinyl, benzotriazolyl, benzothiazolyl, benzooxazolyl, benzimidazolyl, isoquinolinyl, indolyl, isoindolyl, acridinyl, or benzoisoxazolyl. Also included within the scope of the term "heteroaryl", as it is used herein, is a group in which a heteroatomic ring is fused to one or more aromatic or nonaromatic rings where the radical or point of attachment is on the heteroaromatic ring. Examples include tetrahydroquinolinyl, tetrahydroisoquinolinyl, and pyrido[3,4-d]pyrimidinyl. The term "heteroaryl" also refers to rings that are optionally substituted. The term "heteroaryl" may be used interchangeably with the term "heteroaryl ring" or the term "heteroaromatic".

An aryl (including aralkyl, aralkoxy, aryloxyalkyl and the like) or heteroaryl (including heteroaralkyl and heteroarylalkoxy and the like) group may contain one or more substituents. Examples of

suitable substituents on the unsaturated carbon atom of an aryl, heteroaryl, aralkyl, or heteroaralkyl group include a halogen, $-R^o$, $-OR^o$, $-SR^o$, 1,2-methylene-dioxy, 1,2-ethylenedioxy, protected OH (such as acyloxy), phenyl (Ph), substituted Ph, $-O(Ph)$, substituted $-O(Ph)$, $-CH_2(Ph)$, substituted $-CH_2(Ph)$, $-CH_2CH_2(Ph)$, substituted $-CH_2CH_2(Ph)$, $-NO_2$, $-CN$, $-N(R^o)_2$, $-NR^oC(O)R^o$, $-NR^oC(O)N(R^o)_2$, $-NR^oCO_2R^o$, $-NR^oNR^oC(O)R^o$, $-NR^oNR^oC(O)N(R^o)_2$, $-NR^oNR^oCO_2R^o$, $-C(O)C(O)R^o$, $-C(O)CH_2C(O)R^o$, $-CO_2R^o$, $-C(O)R^o$, $-C(O)N(R^o)_2$, $-OC(O)N(R^o)_2$, $-S(O)_2R^o$, $-SO_2N(R^o)_2$, $-S(O)R^o$, $-NR^oSO_2N(R^o)_2$, $-NR^oSO_2R^o$, $-C(=S)N(R^o)_2$, $-C(=NH)-N(R^o)_2$, $-(CH_2)_yNHC(O)R^o$, $-(CH_2)_yNHC(O)CH(V-R^o)(R^o)$; wherein R^o is hydrogen, a substituted or unsubstituted aliphatic group, an unsubstituted heteroaryl or heterocyclic ring, phenyl (Ph), substituted Ph, $-O(Ph)$, substituted $-O(Ph)$, $-CH_2(Ph)$, or substituted $-CH_2(Ph)$; y is 0-6; and V is a linker group. Examples of substituents on the aliphatic group or the phenyl ring of R^o include amino, alkylamino, dialkylamino, aminocarbonyl, halogen, alkyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylaminocarbonyloxy, dialkylaminocarbonyloxy, alkoxy, nitro, cyano, carboxy, alkoxy carbonyl, alkyl carbonyl, hydroxy, haloalkoxy, or haloalkyl.

An aliphatic group or a non-aromatic heterocyclic ring may contain one or more substituents. Examples of suitable substituents on the saturated carbon of an aliphatic group or of a non-aromatic heterocyclic ring include those listed above for the unsaturated carbon of an aryl or heteroaryl group and the following:

$=O$, $=S$, $=NHR^*$, $=NN(R^*)_2$, $=N-$, $=NNHC(O)R^*$, $=NNHCO_2(alkyl)$,

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-NNHSO₂(alkyl), or -NR^{*}, where each R^{*} is independently selected from hydrogen, an unsubstituted aliphatic group or a substituted aliphatic group. Examples of substituents on the aliphatic group include amino, alkylamino, dialkylamino, aminocarbonyl, halogen, alkyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylaminocarbonyloxy, dialkylaminocarbonyloxy, alkoxy, nitro, cyano, carboxy, alkoxycarbonyl, alkylcarbonyl, hydroxy, haloalkoxy, or haloalkyl.

10 Suitable substituents on the nitrogen of a non-aromatic heterocyclic ring include -R^{*}, -N(R^{*})₂, -C(O)R^{*}, -CO₂R^{*}, -C(O)C(O)R^{*}, -C(O)CH₂C(O)R^{*}, -SO₂R^{*}, -SO₂N(R^{*})₂, -C(=S)N(R^{*})₂, -C(=NH)-N(R^{*})₂, and -NR^{*}SO₂R^{*}; wherein R^{*} is hydrogen, an aliphatic group, a substituted aliphatic group, phenyl (Ph), substituted Ph, -O(Ph), substituted -O(Ph), CH₂(Ph), substituted CH₂(Ph), or an unsubstituted heteroaryl or heterocyclic ring. Examples of substituents on the aliphatic group or the phenyl ring include amino, alkylamino, dialkylamino, aminocarbonyl, halogen, alkyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylaminocarbonyloxy, dialkylaminocarbonyloxy, alkoxy, nitro, cyano, carboxy, alkoxycarbonyl, alkylcarbonyl, hydroxy, haloalkoxy, or haloalkyl.

25 A combination of substituents or variables is permissible only if such a combination results in a stable or chemically feasible compound. A stable compound or chemically feasible compound is one in which the chemical structure is not substantially altered when kept at a temperature of 40 °C or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

When the R^1 group is in the form of an ester or amide, the present compounds undergo metabolic cleavage in a mammal to the corresponding carboxylic acids, which are the active caspase inhibitors. Because they undergo
5 metabolic cleavage, the precise nature of the ester or amide group is not critical to the working of this invention. The structure of the R^1 group may range from the relatively simple diethyl amide to a steroidal ester. Examples of esters of R^1 carboxylic acids include, but are
10 not limited to, C_{1-12} aliphatic, such as C_{1-6} alkyl or C_{3-10} cycloalkyl, aryl, such as phenyl, aralkyl, such as benzyl or phenethyl, heterocyclyl, heterocyclylalkyl, heteroaryl, and heteroaralkyl. Examples of suitable R^1 heterocyclyl rings include, but are not limited to, 5-6
15 membered heterocyclic rings having one or two heteroatoms such as piperidinyl, piperazinyl, or morpholinyl. Examples of suitable R^1 heteroaryl rings include, but are not limited to, 5-6 membered heteroaryl rings having one or two heteroatoms such as pyridyl, pyrimidinyl, furanyl
20 and thienyl.

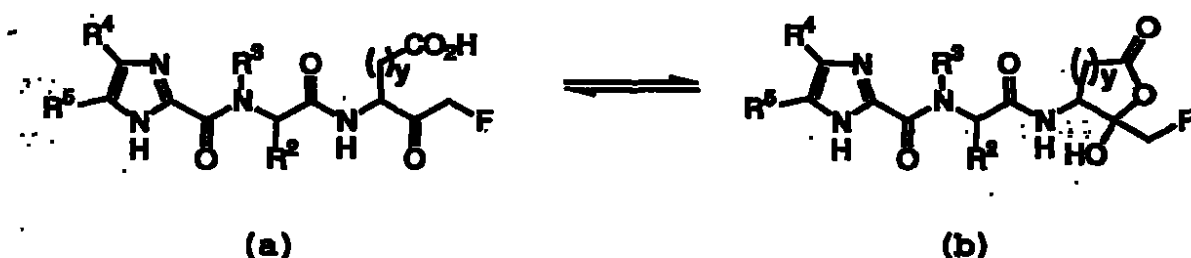
Amides of R^1 carboxylic acids may be primary, secondary or tertiary. Suitable substituents on the amide nitrogen include, but are not limited to, one or
25 two groups independently selected from the aliphatic, aryl, aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl and heteroaralkyl groups described above for the R^1 ester alcohol. Likewise, other prodrugs are included within the scope of this invention. See Bradley
D. Anderson, "Prodrugs for Improved CNS Delivery" in
30 Advanced Drug Delivery Reviews (1996), 19, 171-202.

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Isosteres or bioisosteres of R^1 carboxylic acids, esters and amides result from the exchange of an atom or group of atoms to create a new compound with similar biological properties to the parent carboxylic acid or ester. The bioisosteric replacement may be physicochemically or topologically based. An example of an isosteric replacement for a carboxylic acid is $CONHSO_2(alkyl)$ such as $CONHSO_2Me$.

Compounds of this invention where R^1 is CO_2H or CH_2CO_2H , γ -ketoacids or δ -ketoacids respectively may exist in solution as either the open form (a) or the cyclized hemiketal form (b) ($y=1$ for γ -ketoacids, $y=2$ for δ -ketoacids). The representation herein of either isomeric form is meant to include the other.

15



Likewise it will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms or hydrated forms, all such forms of the compounds being within the scope of the invention. Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of

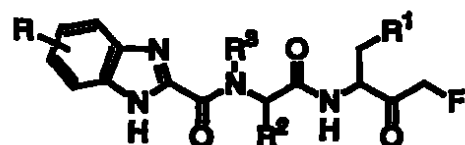
the invention. Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the

5 present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by a ^{13}C - or ^{14}C -enriched carbon are within the scope of this invention.

A number of dipeptidic ICE/caspase inhibitors that were generically described in WO 95/35308 have now been tested for activity against caspases in the enzymatic and cell-based assays described below. Among the compounds tested, the new compounds of formula I were found to have unexpectedly good activity against a number

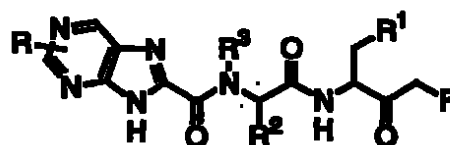
15 of caspase enzymes.

When R^4 and R^5 are taken together to form a ring fused to the imidazole, the following embodiments of this invention are provided:

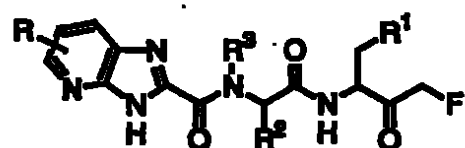


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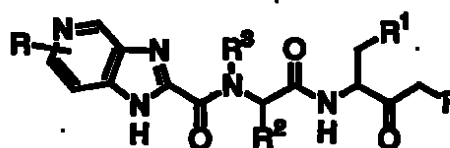
I-A



I-B



I-C



I-D

where R^1 , R^2 and R^3 are as described above and R

25 represents one or more optional substituents. Examples of R groups, when attached to a position nonadjacent to a ring nitrogen, include C_{1-6} alkyl, C_{1-6} alkoxy, halo,

84
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alkylamino, dialkylamino, alkylaminocarbonyl, dialkylaminocarbonyl, and alkylcarbonyl. Examples of R groups, when attached to a position adjacent to a ring nitrogen, include C₁₋₆, alkyl, alkylamino, and dialkylamino.

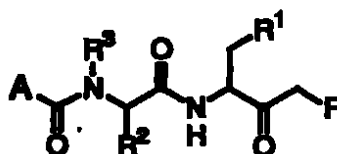
5 Preferred compounds of this invention are compounds of formula I that have one or more of the following features and more preferably all of the following features:

- 10 (a) R¹ is CO₂H or esters, amides or isosteres thereof;
 (b) R² is a C₁-C₆ straight chain or branched alkyl group;
 (c) R³ is hydrogen, and
 (d) R⁴ and R⁵ are each hydrogen, or R⁴ and R⁵ together with the ring to which they are attached form a
 15 benzimidazole ring.

When R² is substituted, preferred substituents include hydroxy, thio, amino or halogen.

Specific representative examples of compounds of formula I are shown in Table 1.

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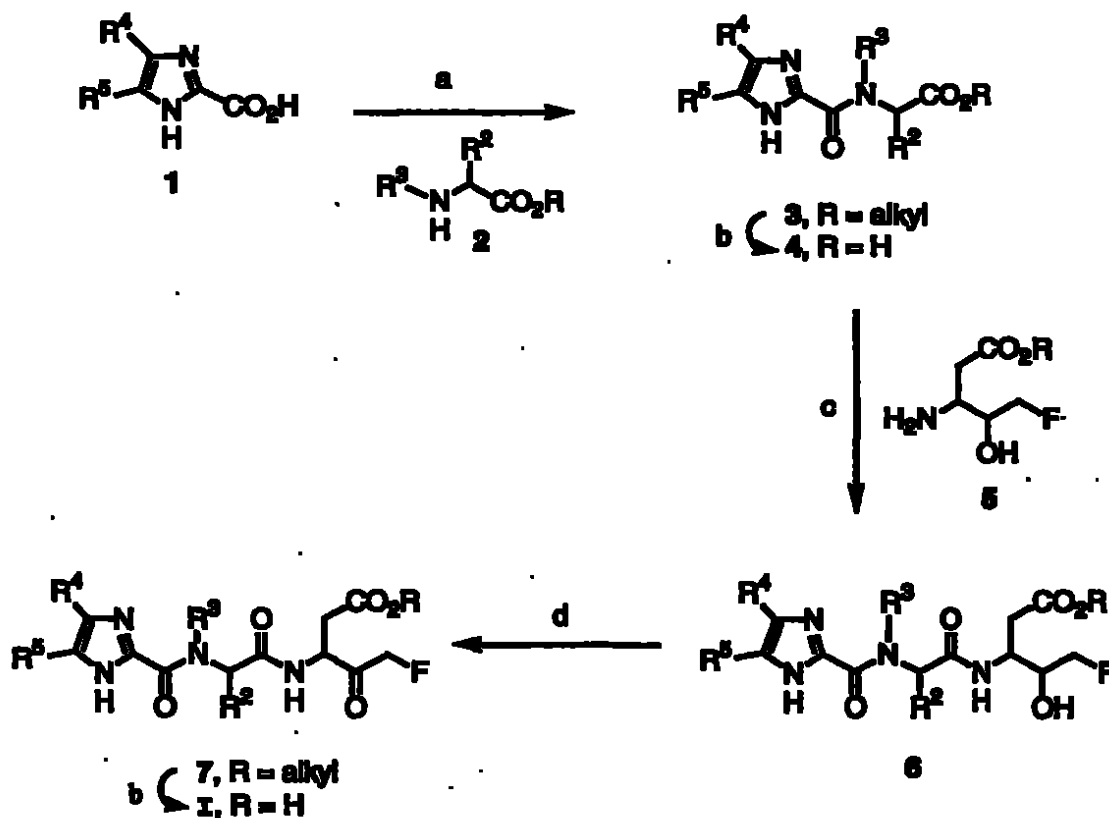


No.	A Ring	R ¹	R ²	R ³
1	Imidazol-2-yl	CO ₂ H	CH ₃ -	H
2	Benzimidazol-2-yl	CO ₂ H	CH ₃ -	H
3	Imidazol-2-yl	CO ₂ H	CH ₃ CH ₂ -	H
4	Imidazol-2-yl	CO ₂ H	(CH ₃) ₂ CH-	H
5	Benzimidazol-2-yl	CO ₂ H	(CH ₃) ₂ CH-	H

No.	A Ring	R ¹	R ²	R ³
6	9H-Purin-8-yl	CO ₂ H	(CH ₃) ₂ CH-	H
7	3H-Imidazo[4,5-b]pyridin-2-yl	CO ₂ H	(CH ₃) ₂ CH-	H

The compounds of this invention may be prepared in general by methods known to those skilled in the art for analogous compounds, as illustrated by the general Scheme I below and by the preparative examples that follow.

Scheme I



10

Reagents: (a) EDC, diisopropylethylamine, HOBT, 2;

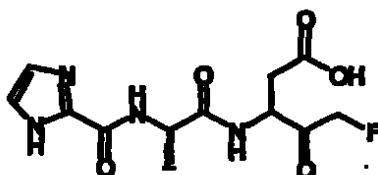
(b) hydrolysis or TFA/DCM; (c) DMAP, EDC, diisopropylethylamine, HOBT, 4; (d) Dess-Martin periodinane

5 Scheme I shows a general route to prepare the present compounds. The following abbreviations are used: EDC is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; HOBT is 1-hydroxybenzotriazole; TFA is trifluoroacetic acid; DCM is dichloromethane; and DMAP is 4-
10 dimethylaminopyridine. Reaction of the known imidazole-2-carboxylic acid or the known benzimidazole-2-carboxylic acid (represented by generic structure 1) with amino ester derivative 2 provides amide 3. If the ester 3 is a tert-butyl ester, TFA in DCM is used to provide the acid
15 4. For other R groups, standard hydrolysis may be used. The acid 4 is then coupled with the amino alcohol 5 to provide 6. Depending on the nature of R an amino ketone may be used, in place of the amino alcohol 5, which avoids the subsequent oxidation step. In the case of
20 fluoromethyl ketones where CO_2R is CO_2tBu , the amino alcohol 5 may be obtained according to the method of Revesz et al., *Tetrahedron Lett.*, 1994, 35, 9693. Finally the hydroxyl in compound 6 is oxidized and the compound then treated appropriately according to the
25 nature of R^1 . For example, if the product I requires R^1 to be a carboxylic acid, then R^1 in 7 is preferably an ester and the final step in the scheme is hydrolysis.

Experimental

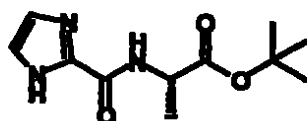
Example 1

[3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-
5 amino]-propionylamino}-4-oxo-pentanoic acid,
trifluoroacetate salt (Compound 1)



Method A:

(2S)-2-[(1H-Imidazole-2-carbonyl)-amino]-propionic acid
10 tert-butyl ester

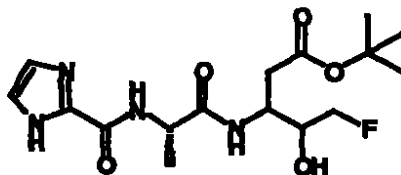


To a solution of 1H-imidazole-2-carboxylic acid (0.17g) in *N,N*-dimethylformamide (DMF) (3mL) was added alanine tert-butyl ester hydrochloride (0.22g), diisopropylethyl amine (0.27mL) and HOBt (0.41g) before cooling to 0° C and reaction mixture treated with EDC.HCl (0.32g). The cooling bath was removed and the reaction mixture stirred at ambient temperature for 18 hrs before being diluted with ethyl acetate and washed with water and brine, dried (MgSO₄) and concentrated at reduced pressure. The residue was purified by silica gel chromatography (30% ethyl acetate in hexanes) to afford the sub-title compound as a colourless oil (0.263g, 73%): ¹H NMR 400 MHz CDCl₃ 1.50 (9H, s), 1.51 (3H, d, J 7.2), 3.70 (1H, m), 7.28 (2H, s), 7.78 (1H, d, J 7.6), 11.49 (1H, br s).

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Method B:

[3S/R, 4S/R, (2S)]-5-Fluoro-4-hydroxy-3-{2-[(1H-imidazole-2-carbonyl)-amino]-propionylamino}-pentanoic acid tert-butyl ester



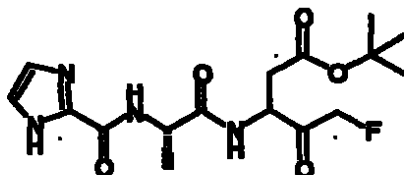
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A solution of (2S)-2-[(1H-imidazole-2-carbonyl)-amino]-propionic acid tert-butyl ester (0.257g) in dichloromethane (2mL) was cooled to 0° C before dropwise addition of trifluoroacetic acid and reaction mixture warmed to room temperature and stirred for 2hr before evaporation under reduced pressure. The residue was co-evaporated with dichloromethane (twice) and toluene (twice) to leave the required (2S)-2-[(1H-imidazole-2-carbonyl)-amino]-propionic acid that was used without further purification (0.40g).

A solution of (2S)-2-[(1H-imidazole-2-carbonyl)-amino]-propionic acid and 3-amino-5-fluoro-4-hydroxy-pentanoic acid tert-butyl ester (0.254g) in THF (7mL) was cooled to 0° C before addition of DMAP (0.151g), diisopropylethyl amine (0.56mL), HOBT (0.16g) and EDC.HCl (0.23g). The reaction mixture stirred at ambient temperature for 18 hrs before being concentrated at reduced pressure. The residue was purified by silica gel chromatography (5% methanol in dichloromethane) to afford the sub-title compound as a colourless solid (0.386g, 97%): ¹H NMR 400MHz CDCl₃/CD₃OD 1.40 (12H, m), 3.92 (1H, m), 4.20-4.55 (4H, m), 7.11 (2H, d, J 15) ¹⁹F NMR CDCl₃, -229.74 (m), -229.84 (m), -230.54 (m), -230.87 (m).

Method C:

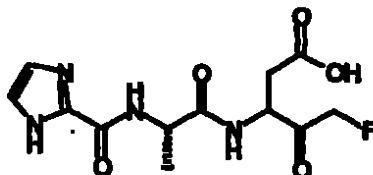
[3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-
amino]-propionylamino}-4-oxo-pentanoic acid tert-butyl
5 ester



A solution of [3S/R, (2S)]-5-fluoro-4-hydroxy-3-{2-[(1H-imidazole-2-carbonyl)-amino]-propionylamino}-pentanoic acid tert-butyl ester (0.381g) in dichloromethane was
10 cooled to 0°C before addition of 1,1,1 triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one (0.476g). The mixture was stirred at room temperature for 2h before addition of an additional portion of 1,1,1 triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one (0.05g) and reaction mixture
15 stirred for 90 min before being concentrated at reduced pressure. The residue was dissolved in ethyl acetate and washed with a 1:1 mixture of aqueous NaHSO₄ and aqueous Na₂S₂O₃. The organic layer was collected, dried (MgSO₄) and concentrated. The residue was purified by flash
20 chromatography (5% methanol in dichloromethane) to give the sub-title compound as a colourless foam (319mg, 84%):
¹H NMR 400MHz CDCl₃ 1.37+1.43 (9H, 2xs), 1.54 (3H, m), 2.85 (1H, m), 3.03 (1H, m), 4.85-5.30 (4H, m), 7.18 (2H, d, J 16), 7.90 (1H, m), 7.98 (1H, m), 11.37+11.45 (1H, 2xs);
25 ¹⁹F NMR 376 MHz CDCl₃ -231.85 (t, J 48), -232.12 (t, J 48).

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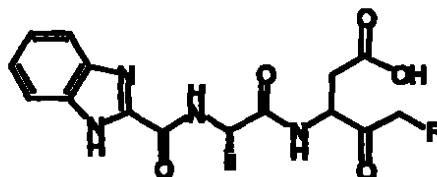
Method D:

Compound 1

A solution of [38/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-
5 carbonyl)-amino]-propionylamino}-4-oxo-pentanoic acid
tert-butyl ester (0.31g) in dichloromethane (2mL) was
cooled to 0° C before dropwise addition of
trifluoroacetic acid and reaction mixture warmed to room
temperature and stirred for 2hr before evaporation under
10 reduced pressure. The residue was co-evaporated with
dichloromethane (twice) and triturated under ether to
give the title compound as a colourless solid (0.35g): IR
1785.7, 1730.1, 1653.7, 1538.1, 1458.2, 1384.2, 1268.7,
1188.4, 1150.9, 1053.3, 992.13, 931.8, 867.9, 847.0, ...
15 768.5 cm⁻¹; ¹H NMR 400MHz DMSO D₆ 1.37 (3H, d), 2.40-
2.85 (2H, m, asp CH₂); 4.34-4.75 (2.5H, m, 2xCH + 0.5CH₂F); 5.13-
5.41 (1.5H, m, CH₂F); 7.50 (2H, s, imidazole CHs); 8.58-
8.79 (2H, m, NHs); ¹³C NMR 100 MHz DMSO D₆ 18.13, 18.85 (ala
CH₃); 33.13, 34.75 (asp CH₂); 48.68, 52.41 (CHs); 83.46,
20 85.21 (CH₂F); 123.67 (CH imidazole);
139.57, 158.86, 172.35 (m) (C=Os); 202.70 (5 peaks ketone);
¹⁹F NMR 376 MHz DMSO D₆ decoupled -75.19 (3F, s, CF₃COOH);
-(226.89, 226.96, 230.80, 231.59, 232.95, 233.06 (1F, 6xs,
COCH₂F ring opened and ring closed)).

Example 2

[3S/R, (2S)]-3-{2-[(1H-Benzimidazole-2-carbonyl)-amino]-propionylamino}-5-fluoro-4-oxo-pentanoic acid, trifluoroacetate salt (Compound 2)



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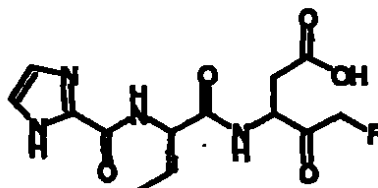
This was prepared from 1H-benzimidazole-2-carboxylic acid using procedures similar to those described in Methods A-D above (142 mg, 90% for final step): (compound isolated as the TFA salt) Off-white solid; IR (solid, cm^{-1})

- 10 3277.9, 1654.6, 1526.6, 1188.6, 1142.5, 1050.4, 927.5, 748.2, 712.4; ^1H NMR ($\text{DMSO}-d_6$) 1.42 (3H, d), 2.51-2.95 (2H, m), 4.21-4.75 (2H, m), 4.76-5.60 (3H, brm), 7.41 (2H, m), 7.65 (2H, m), 8.21-9.05 (2H, m); ^{13}C NMR ($\text{DMSO}-d_6$) 18.0, 18.7, 18.8 (Ala CH_3), 37.2, 34.6, 34.7 (Asp CH_2),
 15 47.6, 48.8, 48.85, 49.1 (Asp CH); 52.0, 52.5 (Ala CH), 83.5, 85.2, 85.3, 103.8, 106.0 (CH_2F), 116.6, 123.9 (Aryl CH), 145.3, 145.4, (Aryl C), 158.4, 158.7, 158.8, 172.1, 172.2, 172.4, 172.5, 172.6, 172.7, 173.2 (NC=O), 202.6, 202.7, 202.8, 202.9 (C=O); Found M^+ 364.1177.
 20 $\text{C}_{16}\text{H}_{17}\text{FN}_4\text{O}_5$ requires M^+ 364.1183 (1.8 ppm).

Example 3

[3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-butyrylamino}-4-oxo-pentanoic acid, trifluoroacetate salt (Compound 3)

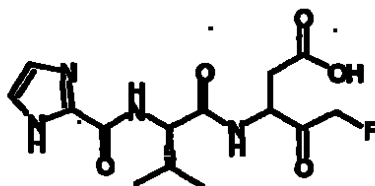
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This was prepared from 1H-benzoimidazole-2-carboxylic acid using procedures similar to those described in Methods A-D above (147 mg, 64% for final step): IR 3280.0, 1659.5, 157.9, 1192.5, 1141.6, 784.7, 721.1 cm^{-1} ,
5 ^1H NMR 400MHz DMSO D_6 0.95 (3H, m), 1.78 (2H, m), 2.58-2.98 (2H, m), 4.30-4.78 (2.5H, m), 5.10-5.42 (1.5H, m), 7.41 (2H, s), 8.44+8.75 (2H, 2xm); ^{13}C NMR 100 MHz DMSO D_6 10.19, 10.29, 15.52 (CH₃), 25.42, 25.49, 26.03, 33.06, 33.13, 34.65, 34.80 (CH₂), 47.45, 47.53, 52.0, 53.96,
10 54.13 (CH) 65.27 (CH₂), 84.36 (d, J 177, CH₂F), 103.81, 104.00 (C), 123.89 (CH), 139.74 (C=O), 156.90, 158.39, 158.74, 171.51, 171.80, 171.83, 172.02, 173.11 (C-O), 202.51, 202.66, 202.76, 202.90 (CH₂FC=O); ^{19}F NMR 376 MHz DMSO D_6 -226.82 (t, J 45), -226.84 (t, J 45), -230.67 (t, J 45), -231.43 (t, J 45), -232.79 (t, J 45), -232.82 (t, J 45).

Example 4

20 [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-3-methylbutyrylamino}-4-oxo-pentanoic acid
(Compound 4)

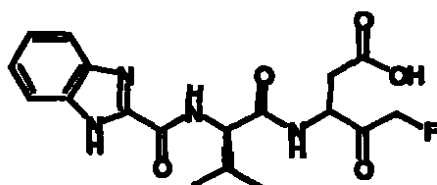


This was prepared from 1H-benzoimidazole-2-carboxylic acid using procedures similar to those described in Methods A-D above (80g, 85% for final step): white powder, IR (solid, cm^{-1}) 1736, 1649, 1557, 1511, 1455, 1434, 1393; ^1H NMR (DMSO+TFA) 0.92-0.95 (6H, m), 2.06-2.15 (1H, m), 2.56-2.90 (2H, m), 4.33-5.36 (4H, m), 7.79 (2H, s), 8.58-8.90 (2H, m); ^{19}F NMR (DMSO+TFA) -226.8 (t),
-27-

-230.6 (t), -231.0 (t), -232.5 (t), -232.6 (t), ¹³C NMR (DMSO+TFA) 18.1/18.4 (CH₃), 19.2/19.3 (CH₃), 34.5/34.8 (CH₂), 51.9/52.2 (CH), 58.5/58.8 (CH), 84.3/84.4 (2d, J 178.7/178.7, CH₂F), 122.0 (CH), 137.5 (C), 153.7 (C),
5 170.6 (C), 171.9/172.0 (C), 202.5/202.8 (2d, J 14.6/14.6, CO).

Example 5

[38/R, (2S)]-3-{2-[(1H-Benzimidazole-2-carbonyl)-amino]-
10 3-methylbutyrylamino}-5-fluoro-4-oxo-pentanoic acid
(Compound 5)



This was prepared from 1H-benzimidazole-2-carboxylic acid using procedures similar to those described in
15 Methods A-D above (90mg, 87% for final step): white powder, IR (solid, cm⁻¹) 1737, 1665, 1527, 1373, 1194, 1137.; ¹H NMR (DMSO) 0.90-0.95 (6H, m), 2.15-2.18 (1H, m), 2.59-2.92 (2H, m), 4.33-4.76 and 5.12-5.38 (4H, 2m), 7.31-7.35 (2H, m), 7.66-7.68 (2H, m), 8.36-8.82 (2H, m);
20 ¹⁹F NMR (DMSO+TFA) -226.7 (t), -226.9 (t), -232.4 (t), -232.6 (t); ¹³C NMR (DMSO) 18.3/18.4/18.5/18.7 (CH₃), 19.4/19.5 (CH₃), 31.0/31.1/31.6 (CH), 34.7/34.8 (CH₂), 51.8/52.1 (CH), 57.9/58.3/58.6 (CH), 84.3/84.4 (2d, J 178.7/178.7, CH₂F), 124.0 (CH), 145.2/145.2 (C),
25 158.4/158.5/158.7/158.8 (C), 170.9/171.1/171.2 (C), 172.0/172.0 (C), 173.1 (C), 173.9 (C), 202.06/202.6 (2d, J 13.8, CO).

The compounds of this invention are designed to inhibit caspases. Therefore, the compounds of this

invention can be assayed for their ability to inhibit apoptosis, the release of IL-1 β or caspase activity directly. Assays for each of the activities are known in the art and are described below in detail in the Testing
5 section.

According to another embodiment, the invention provides a composition comprising a compound of this invention or a pharmaceutically acceptable salt thereof, as described above, and a pharmaceutically acceptable
10 carrier.

If pharmaceutically acceptable salts of the compounds of this invention are utilized in these compositions, those salts are preferably derived from inorganic or organic acids and bases. Included among
15 such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzene sulfonate, bisulfate, butyrate, citrate, camphorate, camphor sulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate,
20 glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate,
25 3-phenyl-propionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts, such as sodium and potassium salts, alkaline earth metal salts, such as calcium and magnesium salts,
30 salts with organic bases, such as dicyclohexylamine

salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth.

Also, the basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates, such as dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides, such as benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The compounds utilized in the compositions and methods of this invention may also be modified by appending appropriate functionalities to enhance selective biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological system (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism and alter rate of excretion.

Pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers, alumina, aluminum stearat, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium

trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

According to a preferred embodiment, the compositions of this invention are formulated for pharmaceutical administration to a patient, preferably a human being. One aspect of this invention relates to a method of treating a caspase-related disease in a patient in need thereof, which method comprises administering to the patient a therapeutically effective amount of a compound of formula I or a pharmaceutical composition thereof. The term "patient" includes human and veterinary subjects.

Such pharmaceutical compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally or intravenously.

Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable

solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers that 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 cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with
5 emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the pharmaceutical compositions of this invention may be administered in the form of
10 suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug.
15 Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs
20 readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

Topical application for the lower intestinal
25 tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment
30 containing the active component suspended or dissolved in one or more carriers. Carriers for topical

administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

The above-described compositions are particularly useful for treating caspase-related

diseases. The term "caspase-related disease" refers to an IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, a proliferative disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, asthma, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Grave's disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis, scarring, graft vs host disease, organ transplant rejection, osteoporosis, leukemias and related disorders, myelodysplastic syndrome, multiple myeloma-related bone disorder, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, multiple myeloma, haemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia, epilepsy, myocardial ischemia, acute and chronic heart disease, myocardial infarction, congestive heart failure, atherosclerosis, coronary artery bypass graft, spinal muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, ulcerative colitis, traumatic brain injury,

spinal cord injury, hepatitis-B, hepatitis-C, hepatitis-G, yellow fever, dengue fever, or Japanese encephalitis, various forms of liver disease, renal disease, polyaptic kidney disease, H. pylori-associated gastric and duodenal ulcer disease, HIV infection, tuberculosis, and meningitis.

Preferred uses of the present compositions include an IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, diabetes, thrombocytopenia, inflammatory bowel disease, Crohn's disease, psoriasis, scarring, organ transplant rejection, osteoporosis, haemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia, epilepsy, acute and chronic heart disease, coronary artery bypass graft, amyotrophic lateral sclerosis, multiple sclerosis, alopecia, ulcerative colitis, traumatic brain injury, spinal cord injury, various forms of liver disease, renal disease, H. pylori-associated gastric and duodenal ulcer disease, and meningitis.

The compounds and compositions are also useful in treating complications associated with coronary artery bypass grafts. The compounds and compositions are also particularly useful for treating cancer, either alone or

in combination with another therapy such as chemotherapy or radiotherapy. The compounds and compositions also may be used as a component of immunotherapy for the treatment of various forms of cancer.

5 The amount of compound present in the above-described compositions should be sufficient to cause a detectable decrease in the severity of the disease or in caspase activity and/or cell apoptosis, as measured by any of the assays described in the examples.

10 According to another embodiment, the compositions of this invention may further comprise another therapeutic agent. Such agents include, but are not limited to, thrombolytic agents such as tissue plasminogen activator and streptokinase. When a second
15 agent is used, the second agent may be administered either as a separate dosage form or as part of a single dosage form with the compounds or compositions of this invention.

 It should also be understood that a specific
20 dosage and treatment regimen 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, sex, diet, time of
administration, rate of excretion, drug combination, and
25 the judgment of the treating physician and the severity of the particular disease being treated. The amount of active ingredients will also depend upon the particular compound and other therapeutic agent, if present, in the composition.

30 In a preferred embodiment, the invention provides a method of treating a mammal, having one of the

aforementioned diseases, comprising the step of administering to said mammal a pharmaceutically acceptable composition described above. In this embodiment, if the patient is also administered another therapeutic agent or caspase inhibitor, it may be delivered together with the compound of this invention in a single dosage form, or, as a separate dosage form. When administered as a separate dosage form, the other caspase inhibitor or agent may be administered prior to, at the same time as, or following administration of a pharmaceutically acceptable composition comprising a compound of this invention.

In order that this invention be more fully understood, the following preparative and testing examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

Example 6

Enzyme Assays

The assays for caspase inhibition are based on the cleavage of a fluorogenic substrate by recombinant, purified human Caspases -1, -3, or -8. The assays are run in essentially the same manner as described in WO0142216.

Compounds 1-5 each possess a k_{inact} value greater than $20000 \text{ M}^{-1}\text{s}^{-1}$ against each of caspase-1, caspase-3 and caspase-8.

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Example 7Inhibition of IL-1 β secretion from Mixed Population of Peripheral Blood Mononuclear Cells (PBMC)

Processing of pre-IL-1 β by caspase-1 may be measured in cell culture using a variety of cell sources. Human PBMC obtained from healthy donors provides a mixed population of lymphocyte and mononuclear cells that produce a spectrum of interleukins and cytokines in response to many classes of physiological stimulators.

10 The assay conditions used for inhibition of IL-1 β secretion from mixed population of peripheral blood mononuclear cells may be found in WO0142216.

The inhibitory potency of the compounds can be represented by an IC₅₀ value, which is the concentration of inhibitor at which 50% of the mature IL-1 β is detected in the supernatant as compared to the positive controls.

15 The compounds tested were found to provide an IC₅₀ value less than 1 μ M for inhibition of IL-1 β secretion from PBMC

EXAMPLE 8Anti-Fas Induced Apoptosis Assay

Cellular apoptosis may be induced by the binding of Fas ligand (FasL) to its receptor, CD95 (Fas).

20 Conditions for an assay to measure the effect of compounds on the inhibition of the caspase-8-mediated apoptotic pathway may be found in WO0142216.

Compounds 1-5 each were found to provide an IC₅₀ value less than 200nM for the activity in the FAS induced apoptosis assay.

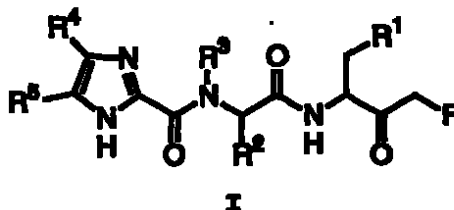
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While we have described a number of embodiments of this invention, it is apparent that our basic examples

may be altered to provide other embodiments, which
utilize the compounds and methods of this invention.
Therefore, it will be appreciated that the scope of this
invention is to be defined by the appended claims rather
5 than by the specific embodiments, which have been
represented by way of example.

What is claimed is:

1. A compound of formula I:



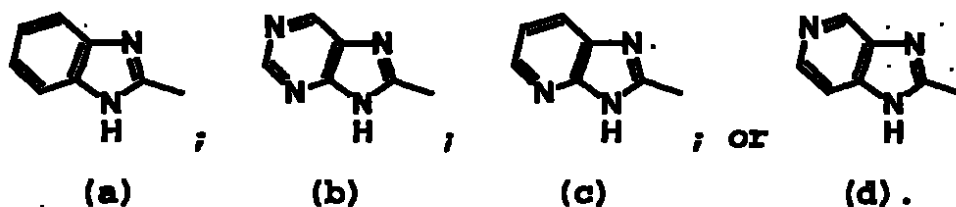
wherein:

R¹ is CO₂H, CH₂CO₂H, or esters, amides or isosteres thereof;

R² is hydrogen or an optionally substituted C₁-C₆ aliphatic group;

R³ is hydrogen or an optionally substituted C₁-C₆ aliphatic group; and

R⁴ and R⁵ are each independently selected from hydrogen, an optionally substituted C₁-C₆ aliphatic group, or R⁴ and R⁵ taken together with the ring to which they are attached form an optionally substituted bicyclic ring, said bicyclic ring selected from the following:



2. The compound of claim 1 where R² is an optionally substituted C₁-₆ straight or branched alkyl group.

3. The compound of claim 1 having one or more features selected from the group consisting of:

- a) R^1 is CO_2H or esters, amides or isosteres thereof;
- b) R^2 is a C_1-C_6 straight chain or branched alkyl group;
- c) R^3 is hydrogen; and
- d) R^4 and R^5 are each hydrogen, or R^4 and R^5 together with the ring to which they are attached form a benzimidazole ring.

4. The compound of claim 3 having the following features:

- a) R^1 is CO_2H or esters, amides or isosteres thereof;
- b) R^2 is a C_1-C_6 straight chain or branched alkyl group;
- c) R^3 is hydrogen; and
- d) R^4 and R^5 are each hydrogen, or R^4 and R^5 together with the ring to which they are attached form a benzimidazole ring.

5. A compound selected from the group consisting of:

[3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-propionylamino}-4-oxo-pentanoic acid;

5 [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-propionylamino}-4-oxo-pentanoic acid tert-butyl ester;

[3S/R, (2S)]-3-{2-[(1H-Benzimidazole-2-carbonyl)-amino]-propionylamino}-5-fluoro-4-oxo-pentanoic acid;

10 [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-butyrylamino}-4-oxo-pentanoic acid;

[3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-3-methylbutyrylamino}-4-oxo-pentanoic acid;

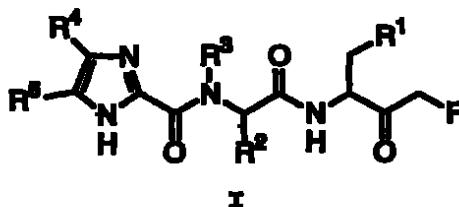
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[3S/R, (2S)]-3-{2-[(1H-Benzimidazole-2-carbonyl)-amino]-3-methylbutyrylamino}-5-fluoro-4-oxo-pentanoic acid;

or an addition salt thereof.

6. A pharmaceutical composition comprising a compound according to any one of claims 1-5 and a pharmaceutically acceptable carrier.

7. A method of treating a caspase-related disease in a patient, which method comprises administering to the patient a therapeutically effective amount of a compound of formula I:



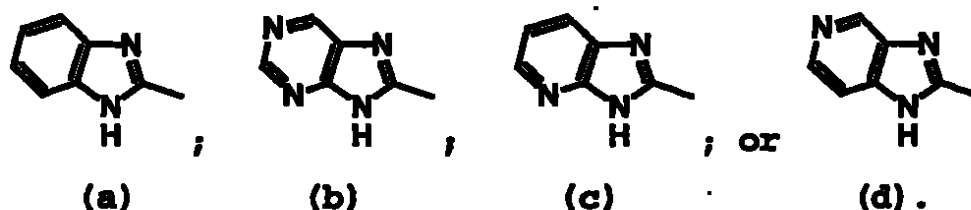
wherein:

R¹ is CO₂H, CH₂CO₂H, or esters, amides or isosteres thereof;

R² is hydrogen or an optionally substituted C₁-C₆ aliphatic group;

R³ is hydrogen or an optionally substituted C₁-C₆ aliphatic group; and

R⁴ and R⁵ are each independently selected from hydrogen, an optionally substituted C₁-C₆ aliphatic group, or R⁴ and R⁵ taken together with the ring to which they are attached form an optionally substituted bicyclic ring, said bicyclic ring selected from the following: ..



8. The method according to claim 7 wherein the disease or treatment is selected from an IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, a proliferative disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, asthma, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Grave's disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis, scarring, graft vs host disease, organ transplant rejection, osteoporosis, leukemias and related disorders, myelodysplastic syndrome, multiple myeloma-related bone disorder, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, multiple myeloma, haemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion diseases, cerebral ischemia, epilepsy, myocardial ischemia, acute and chronic heart disease, myocardial infarction,

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congestive heart failure, atherosclerosis, coronary artery bypass graft, spinal muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, ulcerative colitis, traumatic brain injury, spinal cord injury, hepatitis-B, hepatitis-C, hepatitis-G, yellow fever, dengue fever, or Japanese encephalitis, various forms of liver disease, renal disease, polyaptic kidney disease, H. pylori-associated gastric and duodenal ulcer disease, HIV infection, tuberculosis, meningitis, a treatment for complications associated with coronary artery bypass grafts, or an immunotherapy for the treatment of various forms of cancer.

9. The method of claim 8 wherein the disease is selected from IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, diabetes, thrombocytopenia, inflammatory bowel disease, Crohn's disease, psoriasis, scarring, organ transplant rejection, osteoporosis, haemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia, epilepsy, acute and chronic heart

disease, coronary artery bypass graft, amyotrophic lateral sclerosis, multiple sclerosis, alopecia, ulcerative colitis, traumatic brain injury, spinal cord injury, various forms of liver disease, renal disease, H. pylori-associated gastric and duodenal ulcer disease, and meningitis.

10. The method of claim 7 wherein the disease or condition is a complication associated with coronary artery bypass grafts.

11. The method of claim 7 wherein the disease is cancer.

12. The method of claim 11 wherein the method is used in combination with chemotherapy or radiotherapy.

13. The method of claim 11 wherein the compound is a component of immunotherapy for the treatment of cancer.

14. The method according to any of claims 7-13 wherein the compound has one or more features selected from the group consisting of:

(a) R^1 is CO_2H or esters, amides or isosteres thereof;

(b) R^2 is a C_1-C_6 straight chain or branched alkyl group;

(c) R^3 is hydrogen; and

(d) R^4 and R^5 are each hydrogen, or R^4 and R^5 together with the ring to which they are attached form a benzimidazole ring.

15. The method according to any of claims 7-13 wherein the compound has the following features:

- (a) R^1 is CO_2H or esters, amides or isosteres thereof;
- (b) R^2 is a C_1-C_6 straight chain or branched alkyl group;
- (c) R^3 is hydrogen; and
- (d) R^4 and R^5 are each hydrogen, or R^4 and R^5 together with the ring to which they are attached form a benzimidazole ring.

16. The method according to any of claims 7-13 wherein the compound is selected from the group consisting of:

[3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-propionylamino}-4-oxo-pentanoic acid;

[3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-propionylamino}-4-oxo-pentanoic acid

5 tert-butyl ester;

[3S/R, (2S)]-3-{2-[(1H-Benzimidazole-2-carbonyl)-amino]-propionylamino}-5-fluoro-4-oxo-pentanoic acid;

[3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-butyrylamino}-4-oxo-pentanoic acid;

10 [3S/R, (2S)]-5-Fluoro-3-{2-[(1H-imidazole-2-carbonyl)-amino]-3-methylbutyrylamino}-4-oxo-pentanoic acid;

[3S/R, (2S)]-3-{2-[(1H-Benzimidazole-2-carbonyl)-amino]-3-methylbutyrylamino}-5-fluoro-4-oxo-pentanoic acid;

or an addition salt thereof.

"DOCKETED FOR:

PCT

(PCT Rule 44.1)

To:
VERTEX PHARMACEUTICALS INC.
Attn. Silverman, Ian
130 Waverly Street
Cambridge, MA 02139-4242
UNITED STATES OF AMERICA

Date of mailing
(day/month/year) 17/06/2002

Applicant's or agent's file reference
00-125PCT

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/US 01/47688

International filing date
(day/month/year) 19/11/2001

Appendix

VERTEX PHARMACEUTICALS INCORPORATED

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 48):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Office.

- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

- 4. Further action(s):** The applicant is reminded of the following:

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within 18 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election. Within 12 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority

European Patent Office, P.B. 5818 Patenteaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 851 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Véronique Baillou

JUN 17 2002

VERTEX PHARMACEUTICALS INC.
LEGAL DEPARTMENT

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NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and these requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the letter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 26 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 48.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 48.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 49.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/selected Office, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/selected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 00-125PCT	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/US 01/ 47688	International filing date (day/month/year) 19/11/2001	(Earliest) Priority Date (day/month/year) 21/11/2000
Applicant VERTEX PHARMACEUTICALS INCORPORATED		

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 4 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 29.1(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:

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.

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PCT/US 01/47688

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D233/90 C07D235/24 C07D473/00 C07D471/04 C07K5/02
A61K31/4164 A61P29/00

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 C07D C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal; WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 11129 A (IDUN PHARMACEUTICALS INC) 19 March 1998 (1998-03-19) page 4, line 20 -page 5, line 5; claims 1,45-49; figures 1-3 page 21, line 22-31	1-16
Y	US 5 716 929 A (GOLEC JULIAN M C ET AL) 10 February 1998 (1998-02-10) column 2, line 49 -column 4, line 40; claims 1,54 column 50, line 25 column 72, line 40-60	1-16
A	US 5 714 484 A (BECKER MARK ET AL) 3 February 1998 (1998-02-03) column 5, line 5-14	1-16

☐ 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 doubt on priority claim(s) or which is cited to establish the publication date of another claim 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

"A" document member of the same patent family

Date of the actual completion of the international search

10 June 2002

Date of mailing of the international search report

17/06/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5610 Patentmann 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-8040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-8016

Authorized officer

Gavrillu, D

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The present claims 1, 3 and 4 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The functional term "isosteres" does not enable the skilled person to determine which technical feature are necessary to perform the stated function. It is thus unclear which specific compounds fall within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search to the claims impossible. Consequently, the search does not include isosteres of the compounds of formula 1.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/47688

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 No.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 7-16 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.
2. ☒ Claims No.:
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:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims No.:
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 No.:
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.

Information on patent family members

International Application No
PCT/US 01/47688

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9811129	A	19-03-1998	US 5869519 A	09-02-1999
			AT 215953 T	15-04-2002
			AU 739321 B2	11-10-2001
			AU 4413897 A	02-04-1998
			AU 738048 B2	06-09-2001
			AU 4481997 A	02-04-1998
			AU 739496 B2	11-10-2001
			AU 4583597 A	02-04-1998
			CA 2237314 A1	19-03-1998
			CN 1234038 A	03-11-1999
			DE 69711845 D1	16-05-2002
			EP 0920444 A1	09-06-1999
			EP 0929311 A1	21-07-1999
			EP 0874850 A1	04-11-1998
			JP 2001500377 T	16-01-2001
			JP 2000501428 T	08-02-2000
			JP 2001501923 T	13-02-2001
			NZ 330451 A	28-01-2000
			NZ 334605 A	25-08-2000
			WO 9811109 A1	19-03-1998
			WO 9811129 A1	19-03-1998
			WO 9810778 A1	19-03-1998
			US 2001044415 A1	22-11-2001
			US 6200969 B1	13-03-2001
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			US 2002025935 A1	28-02-2002
			US 5877197 A	02-03-1999
US 5716929	A	10-02-1998	US 5847135 A	08-12-1998
			US 5656627 A	12-08-1997
			US 5756466 A	26-05-1998
			AP 797 A	07-01-2000
			AU 709114 B2	19-08-1999
			AU 2944695 A	15-01-1996
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			CZ 9603698 A3	11-06-1997
			EP 0784628 A1	23-07-1997
			FI 965036 A	14-02-1997
			HU 76622 A2	28-10-1997
			JP 10504285 T	28-04-1998
			NO 965365 A	17-02-1997
			NZ 289560 A	29-09-1999
			PL 318220 A1	26-05-1997
			SK 160996 A3	10-09-1997
			WO 9535308 A1	28-12-1995
			US 6103711 A	15-08-2000
			US 5973111 A	26-10-1999
			US 6025147 A	15-02-2000
			ZA 9504988 A	17-12-1996
US 5714484	A	03-02-1998	US 5486623 A	23-01-1996
			AU 713934 B2	16-12-1999
			AU 6100996 A	30-12-1996
			CA 2223268 A1	19-12-1996
			EP 0863883 A1	16-09-1998

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INTERNATIONAL PATENT CLASSIFICATION
Information on patent family members

International Application No
PCT/US 01/47688

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5714484	A	JP 11507912 T	13-07-1999
		US 6147188 A	14-11-2000
		WO 9640647 A1	19-12-1996
		AU 1266495 A	27-06-1995
		CA 2177495 A1	15-06-1995
		EP 0731696 A1	18-09-1996
		JP 9506368 T	24-06-1997
		WO 9515749 A1	15-06-1995
		US 6297277 B1	02-10-2001
		US 5663380 A	02-09-1997
		US 5925772 A	20-07-1999

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 May 2002 (30.05.2002)

PCT

(10) International Publication Number
WO 02/42278 A2

(51) International Patent Classification: C07D 233/00

(21) International Application Number: PCT/US01/47688

(22) International Filing Date:
19 November 2001 (19.11.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/252,252 21 November 2000 (21.11.2000) US

(71) Applicant (for all designated States except US): VERTEX
PHARMACEUTICALS INCORPORATED [US/US];
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(72) Inventors; and

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(74) Agents: SILVERMAN, Ian et al.; Vertex Pharmaceuti-
cals Inc., 130 Waverly Street, Cambridge, MA 02139-4242
(US).

(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, GH, GI,
GM, HR, HU, ID, IL, IN, IS, JP, KR, KG, KP, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI,
SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU,
ZA, ZW.

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

Published:

— without international search report and to be republished
upon receipt of that report

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

(54) Title: IMIDAZOLE AND BENZIMIDAZOLE CASPASE INHIBITORS AND USES THEREOF

(57) Abstract: This invention provides caspase inhibitors having the formula: wherein R¹ is CO₂H, CH₂CO₂H, or esters, amides or isosteres thereof; R² and R³ are each independently selected from hydrogen or an optionally substituted C₁-C₄ aliphatic group; and R⁴ and R⁵ are each independently selected from hydrogen, an optionally substituted C₁-C₆ aliphatic group, or R⁴ and R⁵ taken together with the ring to which they are attached form an optionally substituted bicyclic ring. The caspase inhibitors are useful for treating a number of diseases such as cancer, acute inflammatory and autoimmune disorders, ischemic diseases and certain neurodegenerative disorders.

WO 02/42278 A2

PATENT COOPERATION TREATY

9/27/02 16
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From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Vossius & Partner
Siebertstr. 4
81676 Munich
ALLEMAGNE

EINGEGANGEN
Vossius & Partner
24. Sep. 2002

Frist
beurh.

PCT

WRITTEN OPINION

(PCT Rule 66)

Applicant's or agent's file reference G2071 PCT		Date of mailing (day/month/year) 23.09.2002
International application No. PCT/US01/47688		International filing date (day/month/year) 19/11/2001
International Patent Classification (IPC) or both national classification and IPC C07D233/80		Priority date (day/month/year) 21/11/2000
Applicant VERTEX PHARMACEUTICALS INCORPORATED et al.		

1. This written opinion is the second drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- 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 Rule 66.2(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain document cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.6 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 66.2 is: 21/03/2003.

Name and mailing address of the International Preliminary Examining Authority

Authorized officer / Examiner

no
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WRITTEN OPINION

International application No. PCT/US01/47688

I. Basis of the opinion

1. With regard to the elements of the International application (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"):

Description, pages:

1-40 as originally filed

Claims, No.:

1-18 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the International application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the International search (under Rule 23.1(b)).
- ☐ the language of publication of the International application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of International preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the International application, the International preliminary examination 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.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, No.:
- ☐ the drawings, sheets:

WRITTEN OPINION

International application No. **PCT/US01/47688**

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

- ☐ the entire international application,
- ☒ claims Nos. 7-16 with respect to industrial applicability and 1, 3 and 4 with respect to isosteres,

because:

- ☒ the said international application, or the said claims Nos. 7-16 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 1, 3 and 4.

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Rule 86.2(a)(II) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement
- | | | |
|-------------------------------|--------|-----------|
| Novelty (N) | Claims | 1-16(YES) |
| Inventive step (IS) | Claims | 1-16(NO) |
| Industrial applicability (IA) | Claims | 1-6(YES) |

WRITTEN OPINION

International application No. **PCT/US01/47688**

**2. Citations and explanations
see separate sheet**

**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/US01/47688

Re Item I

Should amendments be filed in response to this written opinion, these will only be taken into account when the applicant identifies clearly all amendments made (e.g. submitting one set of originally pages with hand-written amendments) and indicates on which passages of the original application these amendments are based (Rule 66.8 PCT)

Re Item III

Claims 7-16 relate to subject-matter considered by this Authority to be covered by the provision of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

For reasoning with regards to unsearched subject-matter, see Form PCT/ISA/210 of the International Search Report. No International Preliminary Examination will be carried out with respect to subject-matter which is not covered by the search report (Rule 66.1(e) PCT).

Re Item V

1. Reference is made to the following documents:

D1: WO 98/11129

D2: US 5 716 929

2. Novelty (Article 33(1) and (2) PCT)

The subject-matter of the present application is related to dipeptide derivatives having an imidazole derivative as C-amino acid, useful as inhibitors of caspase.

D1 discloses compounds differing from those claimed by the present application in that the terminal ring is an indole group. D2 discloses structures which differ from the present compounds in that they do not contain a terminal halomethyl function. The above-mentioned documents are not relevant to the novelty question.

3. Inventive step (Article 33(1) and (3) PCT).

**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/US01/47688

The technical problem underlying the application lies in the provision of further dipeptide-based caspase inhibitors.

D1, which discloses some C-terminal modified (N-substituted)-2-Indolyl dipeptides as inhibitors of caspase, is considered to represent the closest prior art.

Compound 61 (figure 2 of D1) differs from example 5 of the present application by the replacement of the benzimidazole ring with the N-methylindole ring. In the above-mentioned figure from D1 a series of fluoromethyl derivatives (having a C-terminal indolyl ring) are tested for their activity as caspase inhibitors.

D2 teaches the equivalence of terminal pyrrole- and imidazole-based heterocycles in a structurally very closely related group caspase inhibitors.

Considering the fact that D1 discloses compounds having a fluoromethyl group on the N-terminal amino acid, D2 discloses compounds having the same imidazole derivative as the present invention as C-terminal amino acid, as caspase inhibitors, the person skilled in the art would have arrived at the compounds of the present application with the expectation that they would possess the above-mentioned activity.

Consequently, in the absence of evidence of unexpected properties, the subject-matter of the present application cannot be regarded as involving an inventive step.

However, an inventive step could be acknowledged if evidence is provided showing unexpected effects of the compounds of the present application compared to the structurally closest compounds of the prior art.

Moreover it is pointed out that the definition of formula 1 is not considered to represent a reasonable generalization over the examples as far as the definition "optionally substituted" used for the substituents R2-R5 is concerned.

4. Industrial applicability (Article 33(4) PCT).

For the assessment of the present claims 7-16 on the question whether they are

**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/US01/47688

Industrial applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

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9/27/02

EINGEGANGEN
Vossius & Partner
26. Sep. 2002
Friedrich

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

VOSSIUS & PARTNER
Siebertstrasse 4
81675 Munich
Germany

INFORMATION CONCERNING ELECTED
OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

Date of mailing (day/month/year) 16 September 2002 (16.09.02)		IMPORTANT INFORMATION	
Applicant's or agent's file reference 00-125PCT			
International application No. PCT/US01/47688	International filing date (day/month/year) 19 November 2001 (19.11.01)	Priority date (day/month/year) 21 November 2000 (21.11.00)	
Applicant VERTEX PHARMACEUTICALS INCORPORATED 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, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR
National : AU, 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, GQ, GW, ML, MR, NE, SN, TD, TG
National : AE, AG, AL, AM, AT, 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, PH, PT, SD, SE, SG, SI, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, 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 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 39(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.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 740.14.35	Authorized officer: Denise POSPIEZNY Telephone No. (41-22) 338.53.38
--	--

VOSSIUS & PARTNER

Patentanwälte

Vossius & Partner POB 86 07 67 81634 Munich Germany

European Patent Office
Erhardtstr. 27

80298 MÜNCHEN

PATENTANWÄLTE
EUROPEAN PATENT ATTORNEYS
EUROPEAN TRADEMARK ATTORNEYS

Dr. VOLKER VOSSIUS, Dipl.-Chem.
(bis 1992; danach in anderer Kanzlei)
Dr. PAUL TAUCHNER, Dipl.-Chem.
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RECHTSANWÄLTE

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Dr. THURE SCHUBERT
BRIGITTE SCHÄFER

PCT/US01/47688
Vertex Pharmaceuticals Inc.
Our Ref.: G2071 PCT

August 20, 2002
UEX/RUD

This is in reply to the Written Opinion under Rule 66 PCT dated August 6, 2002:

Applicant herewith requests detailed substantive examination in accordance with the Notice of the President of the European Patent Office dated November 2, 2001 published in the Official Journal of the EPO 2001, 539. It is further requested that a second Written Opinion be issued.


Dr. Alexa von Ueckhill
European Patent Attorney

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PATENT COOPERATION TREATY

20/02

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

Vossius & Partner
Siebertstr. 4
81675 Munich
ALLEMAGNE

EINGEGANGEN
Vossius & Partner
07. Aug. 2002

WRITTEN OPINION

(PCT Rule 66)

Applicant's or agent's file reference G2071 PCT		Date of mailing (day/month/year) 06/08/2002
REPLY DUE within 1 / 00 months/days from the above date of mailing		
International application No. PCT/US 01/47688	International filing date (day/month/year) 19/11/2001	Priority date (day/month/year) 21/11/2000
International Patent Classification (IPC) or both national classification and IPC G07D233/00		
Applicant VERTEX PHARMACEUTICALS INCORPORATED et al.		

1. This written opinion is the first drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- 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 Rule 66.2(a)(ii) 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

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: **21/03/2003**

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epou d
Fax: (+49-89) 2399-4445

Authorized officer

Examiner

Formalities officer
(incl. extension of time limits)
Tel. (+49-89) 2399 2828



From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Vossius & Partner
Siebertstr. 4
81675 Munich
ALLEMAGNE

EINGEGANGEN
Vossius & Partner
28. Feb. 2003

File
date:

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing
(day/month/year) 27.02.2003

Applicant's or agent's file reference
G2071 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US01/47888

International filing date (day/month/year)
18/11/2001

Priority date (day/month/year)
21/11/2000

Applicant
VERTEX PHARMACEUTICALS INCORPORATED 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.

For the purpose of deciding whether the claimed invention is patentable or not, the elected Offices may apply criteria additional to or different from the criteria on which the international preliminary examination report is based (see Articles 27(5), 33(5)). Additional criteria may include e.g. exemptions from patentability and the requirements of enabling disclosure and of clarity and support of claims.

Name and mailing address of the IPEA/

 European Patent Office
D-80289 Munich
Tel. +49 89 2339 - 0 Tlx 523000 epmu d
Fax +49 89 2339 - 4465

Authorized officer

Ambros, J.R.

Tel. +49 89 2339-8012



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118

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference G2071 PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US01/47688	International filing date (day/month/year) 19/11/2001	Priority date (day/month/year) 21/11/2000
International Patent Classification (IPC) or national classification and IPC C07D233/90		
Applicant VERTEX PHARMACEUTICALS INCORPORATED 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 7 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 807 of the Administrative Instructions under the PCT).

These annexes consist 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 20/08/2002	Date of completion of this report 27.02.2003
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80289 Munich Tel. +49 89 2399 - 0 Tlx 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Gavriliu, D  Telephone No. +49 89 2399 8274

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US01/47688

I. Basis of the report

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
- Description, pages:

1-40 as originally filed

Claims, No.:

1-16 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination 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.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US01/47688

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☐ claims Nos. .

because:

- ☒ the said international application, or the said claims Nos. 7-16 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 1,3,4.
2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims 1-16
	No: Claims
Inventive step (IS)	Yes: Claims
	No: Claims 1-16
Industrial applicability (IA)	Yes: Claims 1-6

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US01/47688

No: Claims

2. Citations and explanations
see separate sheet

INTERNATIONAL PRELIMINARY

International application No. PCT/US01/47688

EXAMINATION REPORT - SEPARATE SHEET

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 7-16 relate to subject-matter considered by this Authority to be covered by the provision of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i)PCT).

For reasoning with regards to unsearched subject-matter, see Form PCT/ISA/210 of the International Search Report. No International Preliminary Examination will be carried out with respect to subject-matter which is not covered by the search report (Rule 66.1(e)PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: WO 98/11129

D2: US 5 716 929

2. Novelty (Article 33(1) and (2) PCT)

The subject-matter of the present application is related to dipeptide derivatives having an imidazole derivative as C-amino acid, useful as inhibitors of caspase.

D1 discloses compounds differing from those claimed by the present application in that the terminal ring is an indole group. D2 discloses structures which differ from the present compounds in that they do not contain a terminal halomethyl function. The above-mentioned documents are not relevant to the novelty question.

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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US01/47688

3. Inventive step (Article 33(1) and (3) PCT).

The technical problem underlying the application lies in the provision of further dipeptide-based caspase inhibitors.

D1, which discloses some C-terminal modified (N-substituted)-2-Indolyl dipeptides as inhibitors of caspase, is considered to represent the closest prior art.

Compound 61 (figure 2 of D1) differs from example 5 of the present application by the replacement of the benzimidazole ring with the N-methylindole ring. In the above-mentioned figure from D1 a series of fluoromethyl derivatives (having a C-terminal Indolyl ring) are tested for their activity as caspase inhibitors.

D2 teaches the equivalence of terminal pyrrole- and Imidazole-based heterocycles in a structurally very closely related group caspase inhibitors.

Considering the fact that D1 discloses compounds having a fluoromethyl group on the N-terminal amino acid, D2 discloses compounds having the same Imidazol derivative as the present invention as C-terminal amino acid, as caspase inhibitors, the person skilled in the art would have arrived at the compounds of the present application with the expectation that they would possess the above-mentioned activity.

Consequently, in the absence of evidence of unexpected properties, the subject-matter of the present application cannot be regarded as involving an inventive step.

However, an inventive step could be acknowledged if evidence is provided showing unexpected effects of the compounds of the present application compared to the structurally closest compounds of the prior art.

4. Industrial applicability (Article 33(4) PCT).

For the assessment of the present claims 7-16 on the question whether they are industrial applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO,

INTERNATIONAL PRELIMINARY

International application No. PCT/US01/47688

EXAMINATION REPORT - SEPARATE SHEET

for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

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INDUSTRIA Y COMERCIO
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Tramite : 011 PCT-PATENT 379 FASE INCI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 03 - 41,005
FECHA : JUNIO 11 DE 2003

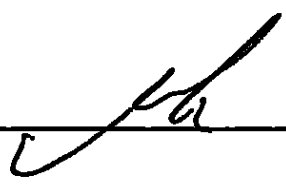
***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
PARRA RODRIGUEZ & CAVELIE	CONSIGNACION	BANCO POPULAR	050-00110-6	13228896	11/06/2003	1,989,000.00

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

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	400,000.00
		TOTAL :	\$ 400,000.00

SON: CUATROCIENTOS MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

128 126

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL
TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCION	()	()
MODELO DE UTILIDAD	()	()
- Indicación de que se solicita la concesión de una patente.	()	()
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	()	()
- Descripción de la invención.	()	()
- Dibujos de ser estos pertinentes.	()	()
- Comprobante de Pago de las tasas establecidas.	()	()

COMPLETA	()	INCOMPLETA	()
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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 y fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	()	()	
- Comprobante de pago de las tasas establecidas	()	()	
COMPLETA	()	INCOMPLETA	()

ESQUEMA DE TRAZADO	()		
- Indicación de que solicita el registro de un Esquema de trazado.	()	()	
- Datos de identificación del solicitante o de la persona que presenta La solicitud.	()	()	
- Representación gráfica del esquema de trazado	()	()	
- Comprobante de pago de las tasas establecidas.	()	()	
COMPLETA	()	INCOMPLETA	()

Firma Responsable:



Fecha: SUPERINDUSTRIA Y COMERCIO
Radicación: 03250947 00000000 Folios: 127
Fecha (GND): 2003-06-16 14:32:38
Trámite: 011 PCT-PATENT 379 FASE INICI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES



225
127

2020
Bogotá, D.C.,

Doctor (a):
HELENA CAMARGO WILLIAMSON
Apoderado (a)
Vertex Pharmaceuticals Incorporated

Oficio N° 09097

ASUNTO:	Radicación N°	03 050947
	Solicitud internacional	PCT/US01/47688
	Fecha inicio fase nacional	21/08/03
	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 enuncados. 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 OCT. 2003.

Oct. 24/03. Pp. 801-3 op. 4's.