



Industria y Comercio
SUPERINTENDENCIA

Delegatura de Propiedad Industrial
División de Nuevas Creaciones

ICT
SOLICITUD

PATENTE DE INVENCION

04-85343

21. EXPEDIENTE No.

54. TITULO

TRIPÉPTIDOS HETEROCICLICOS INHIBIDORES DE LA

HEPATITIS C

51. CLASIFICACION INTERNACIONAL

71. SOLICITANTE

BOEHRINGER INGELHEIM INTERNATIONAL GMBH

DOMICILIO

Ingelheim am Rhein, República Federal de Alemania.

74. APODERADO

EMILIO FERRERO

Código No. 1092

22. BOGOTA, D.C.,

JS

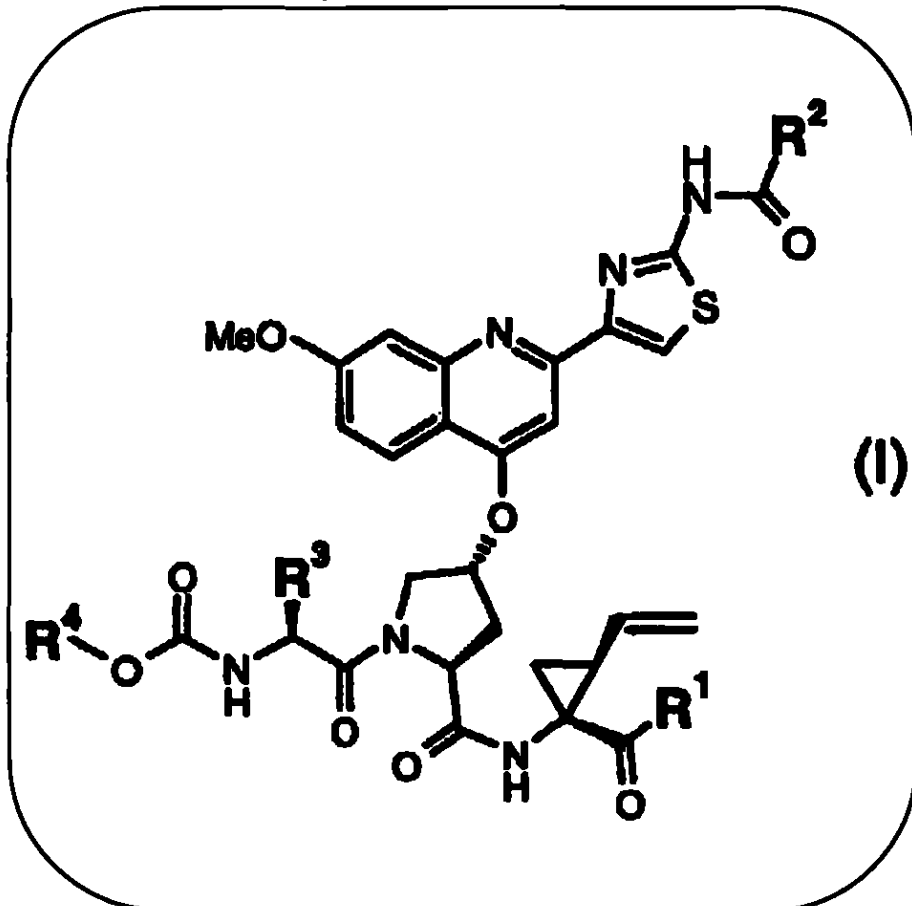
10

Anexos:

- ☒ Comprobante de pago de la tasa de presentación de la solicitud por \$ 422.000.00.
- ☐ Comprobante de pago por reivindicación de prioridad.
- ☒ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☒ Poderes, si fuere el caso.
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones.
- ☒ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Copia del examen preliminar efectuado por la IPEA.
- ☒ Traducción del examen preliminar efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ De ser el caso, certificado de depósito del material biológico.
- ☒ Arte final 12x12
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- ☐ Otros

11

FIGURA CARACTERÍSTICA



12

Solicito la concesión de la patente,

NOMBRE:

EMILIO FERRERO

FIRMA:

C.C. 438.019 de Usaquén T.P. 31.929

PRP

For patents, utility models, industrial designs,³
trademarks, commercial names, trade emblems,
slogans.

Para patentes, modelos de utilidad, diseños
industriales, marcas de fábrica, nombres
comerciales, enseñas, lemas comerciales.

REPRESENTACION Y PODER

REPRESENTATION AND
POWER OF ATTORNEY

I (we), the undersigned/Yo (nosotros), abajo firmado (s)
BOEHRINGER INGELHEIM INTERNATIONAL GMBH

of/domiciliado (s) en **D-55216 Ingelheim am Rhein,**
GERMANY

por el presente nombramos a GERMAN CAVELIER, tpa. 832; GONZALO PERDOMO, tpa. 831; EMILIO FERRERO, tpa. 31929; y LUZ CLEMENCIA DE PAEZ, tpa. 23555; domiciliados en la Carrera 4ª N° 72-35, Bogotá, Colombia, en obediencia a lo dispuesto en el parágrafo 1º del Artículo 543 del Código de Comercio, el primero como principal y los demás como suplentes, en su orden, como mis (nuestros) representantes en todos los asuntos de propiedad industrial con facultad para recibir notificaciones y nombrar apoderados judiciales o extrajudiciales. El primer representante suplente reemplazará al principal en caso de ausencia o impedimento temporal o definitivo de aquél. La misma regla se aplicará cuando el segundo representante suplente haya de reemplazar al primero, o el tercero al segundo. En tales casos, el representante principal, o el primero, o segundo suplente, en su caso, o ambos, declararán su intención de ejercer la representación mediante declaración autenticada por Notario Público en Colombia, o por Notario u otro Funcionario Público, o Cónsul de Colombia, en el extranjero; y si se trata de impedimento, con el certificado respectivo. Si alguno de los nombrados faltare definitivamente, el siguiente tomará su lugar. Cuando separe la ausencia o impedimento del representante principal, o de su primero o segundo suplentes, en su caso, podrán ejercer la representación, en su orden, uno u otros según el caso, asumiéndola por el solo hecho de ejercerla, cesando en ese momento el ejercicio de la representación por el primero, segundo u tercer suplente en su caso.

Además, confiero (conferimos) poder a los abogados arriba nombrados, para obtener la protección de mi (nuestra) propiedad industrial y contra la competencia desleal, a cuyo efecto autorizo (amos) a los dichos apoderados para que me (nos) representen, ante cualesquiera entidades o personas, ejerzan o no jurisdicción, especialmente ante las del orden administrativo, contencioso-administrativo y judicial, así como los tribunales de arbitramento, en todo lo concerniente a los siguientes asuntos: a) Obtención de patentes de invención, modelos de utilidad, diseños industriales; el registro de marcas de fábrica, colectivas, defensivas, de servicio, lemas comerciales, nombres comerciales, enseñas, variedades vegetales y denominaciones de origen; su renovación, traspaso, cambio de nombre o domicilio, cancelación voluntaria; y el registro de licencias de uso de esos derechos; b) Las acciones de competencia desleal, protección del consumidor, oposiciones u observaciones, cancelación administrativa y judicial, nulidad, medidas cautelares, concesión de licencias, la tutela, la protección de secretos industriales, y en general los juicios civiles, penales, administrativos o contencioso-administrativos relacionados con estos derechos; acciones y procesos que promovamos o se nos promuevan. Y para que en todos los asuntos arriba determinados, puedan sustituir este mandato, transigir, conciliar, admitir los hechos del proceso, desistirse, cancelar, recibir, renunciar, y ratificar los actos de agentes oficiosos.

In due compliance with the terms of paragraph 1st. of article 543 of the Commercial Code, do hereby appoint GERMAN CAVELIER, tpa. 832; GONZALO PERDOMO, tpa. 831; EMILIO FERRERO, tpa. 31929; and LUZ CLEMENCIA DE PAEZ, tpa. 23555; domiciled at Carrera 4ª N° 72-35, Bogotá, Colombia, the first as principal and the others as alternates, in the order of their appointment, as our representatives for all industrial property matters with faculty to receive notifications and to appoint attorneys in and out of Court. The first alternate shall replace the principal in case of his absence or of temporal or definitive impediment. The same rule will apply when the second alternate representative is to replace the first one, or when the third is to replace the first or second alternates in their turn, or both of them, second. In such cases, the principal representative, or the first or second alternates in their turn, or both of them, shall state their intention not to act as representatives through a declaration given before a Notary Public in Colombia, or before a Notary or another Public Official or a Colombian Consul abroad; and in case of impediment, the appropriate certificate shall suffice. If any of the appointees were to be definitely absent, the following alternate shall take his place. When the absence or impediment of the principal representative, or of the first or second alternate in their turn ceases, the representation can be taken again in their sequence, by the principal, the first or second alternate depending on case, and the mere fact or its exercise will suffice. The exercise of the representation by the first, second or third alternates in their turn shall end at such time.

In addition, we grant a Power of Attorney in favor of the above-named attorneys-at-law so that they may obtain the protection of my (our) industrial property and against the unfair competition, for which I (we) authorize the said attorneys to represent me (us) before any entity or persons, with or without jurisdiction, especially before administrative, administrative contentions, judicial and arbitral tribunals, in all matters concerning the following: a) Obtainment of patents, utility models and industrial designs; the registration of trademarks, collective, defensive and service marks, slogans, commercial names, trade emblems, vegetal varieties and denominations of origin; its renewal, assignment, change of name or domicile, voluntary cancellation; and the registration of licenses of use of the above rights; b) Actions of unfair competition, protection to the consumer, oppositions or observations, administrative or Court cancellation, nullity, infringement, granting of licenses, the action for writ mandamus, the protection of trade secrets, and in general the civil, penal, and administrative suits relating to those rights; actions and suits instituted by me (us) or against me (us). And that in all the above matters they may substitute this Power of Attorney, compromise, conciliate, admit the facts of the case, desist, cancel, receive, resign, and ratify acts done by representatives without a power of attorney.

Ingelheim am Rhein, 12.12.1995

Given and signed this/Dado y firmado hoy

BOEHRINGER INGELHEIM

in/en

INTERNATIONAL GMBH

Dr. Dieter Laudien

Signature

Dr. Gerhard Huber

Firma

LEGALIZATION

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

LEGALIZATION

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



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

Vorsichtige, vor mir gefertigte Namensunterschriften der Herren:

1. Dr. Dieter L a u d i e n, Dipl.-Chemiker, wohnhaft in 55127 Mainz,.

2. Dr. Gerhard H u b e r, Rechtsanwalt, wohnhaft in 55411 Bingen.

- mir bekannt -

beglaube ich hiermit.

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

Ingelheim am Rhein, den 12. Dezember 1995/hün

Notariatsverweser
anstelle des Notars
Prof. Dr. Weirich in Ingelheim



- 910 E 1 -



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

Kostenberechnung:
Gebühr für Legalisation
(Wert: DM 500,-)
JVKostO. v. 20.7.1967
BGBl. I S. 881
DM
13. DEZ 1995
Mainz, den



Mainz, den dreizehnten Dezember neunzehnhundertfünfundneunzig
Der Präsident des Landgerichts

Hans Paul Tüttenberg
(Dr. Hans Paul Tüttenberg)



CONSULADO GENERAL DE COLOMBIA

MUNICH

Nr. 169

El Cónsul General de Colombia

CERTIFICA

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

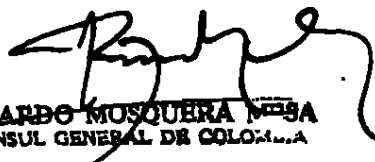
Que la firma BOEHRINGER INGELHEIM INTERNATIONAL GmbH, domiciliada en 55216 Ingelheim am Rhein, Registro Mercantil Nr. HR B 1063 del Juzgado Municipal de Hingen está constituida y ejerce su objeto social de conformidad con las leyes de la República Federal de Alemania.

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

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

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

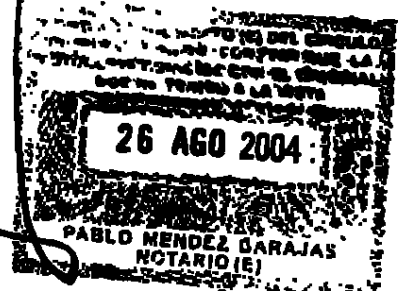
Munich, 18 de Diciembre de 1995


RICARDO MOSQUERA MESA
CONSUL GENERAL DE COLOMBIA



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

PAGADO





MINISTERIO DE RELACIONES
EXTERIORES
R. Ospina
NO SE ASUME
RESPONSABILIDAD DEL TEXTO
MAY 11 - 11:44
OFICINA DE LEGALIZACIONES
AV. CALI DE BOGOTA D.C.

1-044.51334 QZ F

6

TRADUCCION OFICIAL No. 5133

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

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

Legalización

Protocolo notarial No. 2659/95 V

Certifico la autenticidad de las firmas anteceder
mi por los señores:

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

a quienes conozco en persona.

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

Ingelheim am Rhein, 12 de diciembre de 1993/hún

Fdo.: firma ilegible

(Hilo y obiea roja en relieve:)

El notario suplente

El notario suplente - Ingelheim

del notario de Ingelheim

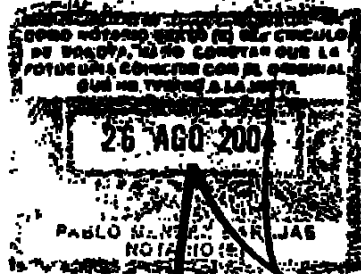
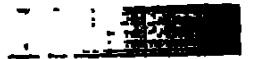
Prof. Dr. Weirich

(dos sellos redondos en tinta del Tribunal Regional

910 E 1

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

Mainz, trece de diciembre de mil novecientos
noventa y cinco



El Presidente del Tribunal Regional

(E) Fdo.: firma ilegible .

(Dr. Hanns Paul Tüttenberg)

Costas

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

EN ESPAÑOL: La legalización de las firmas antecedentes por el
Consulado de Colombia en Munich bajo el No. 169 del 18 de
diciembre de 1995, y por el Ministerio de Relaciones Exteriores
de Colombia bajo el No. 051606 del 4 de enero de 1996.

Pagados derechos y Fondo Rotatorio US\$ 105.000

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

Santafé de Bogotá, 6 de enero de 1996.

26 AGO 2004
NOTARIO
NO ARIBO EL

[Firma manuscrita]

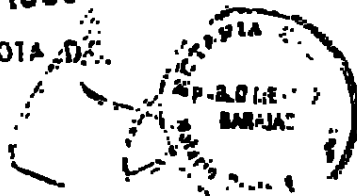
COMO NOTARIO SEXTO ENCARGADO DE SANTAFE
DE BOGOTA HAGO CONSTAR QUE LA FIRMA DE

[Handwritten signature]

C. G. _____
COINCIDE CON LA REGISTRADA POR EL (1123)
EN ESTA NOTARIA

09 ENE 1956

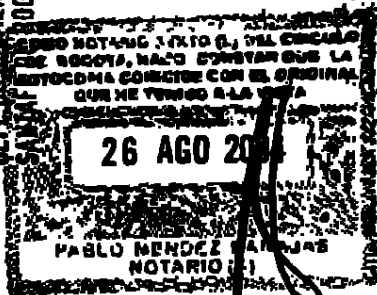
SANTAFE DE BOGOTA D.C.



NO SE ASUME
RESPONSABILIDAD DEL TEXTO

1496 ENE 10 A 11:33

JEFE DE REGISTRACIONES
SANTAFÉ DE BOGOTÁ D.C.



MINISTERIO DE RELACIONES
EXTERIORES

[Handwritten signature]

[Handwritten signature]

CUYA FIRMA
RECURRENTE
LAS FUNCIONES

TELEFAX (57) (1) 222-9850
TELEX: 45479 CAVE CO
12317407375 CAVE UC

CAVELIER

ABOGADOS

EDIFICIO SISKI

CARRERA 4ª, No. 72-35

BOGOTA 8, COLOMBIA

CORREO ELECTRONICO MUNDIAL: CAVE

TELEFONO (57) (1) 2120100

CABLES: CAVELEYES

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

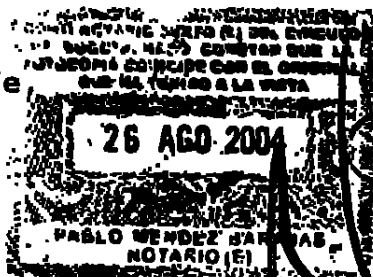
Poderdante: BOEHRINGER INGELHEIM INTERNATIONAL GMBH

Poder conferido el: 12-12-95.

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

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

Señor Jefe



GERMAN CAVELIER

T.P.A. No. 832

C.C. No. 2.877.924 de Bogotá

Acepto:

EMILIO FERRERO

T.P.A. No. 31.929

C.C. No. 438.019 de Usaquén

2e-780-2

NOTARIA SEXTA DE BOGOTÁ
RECONOCIMIENTO Y PRESENTACION PERSONAL

Bogotá, D. C. 11 ENE 1996

Ante mí, PABLO MENDEZ BARAJAS S.

NOTARIO SEXTO ENCARGADO DEL CÍRCULO DE BOGOTÁ.

Comparació(eron)

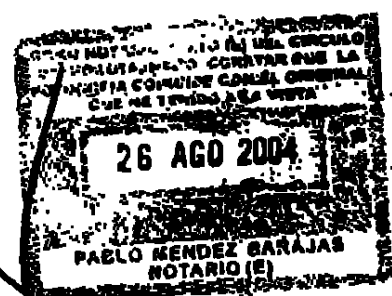
[Firma manuscrita]

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

y la(s) Libreta(s) Militar(es)

domiciliada(s) en Bogotá y declararon que la(s) firma(s) que
aparecen(en) en el presente documento es(son) la(s) suya(s) y
que el contenido del mismo es cierto.
En constancia se firma esta Diligencia.

[Firma manuscrita]



(19) World Intellectual Property Organization
International Bureau



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

PCT

(10) International Publication Number
WO 03/064416 A1

(51) International Patent Classification:
A61K 31/47, A61P 31/00, C07K 4/00

C07D 417/14

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

(21) International Application Number: PCT/CA03/00091

(22) International Filing Date: 24 January 2003 (24.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2,369,970 1 February 2002 (01.02.2002) CA

(71) Applicant (for all designated States except US):
BOEHRINGER INGELHEIM INTERNATIONAL
GMBH [DE/DE]; Binger Strasse 173, D-55216 Ingelheim
(DE).

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

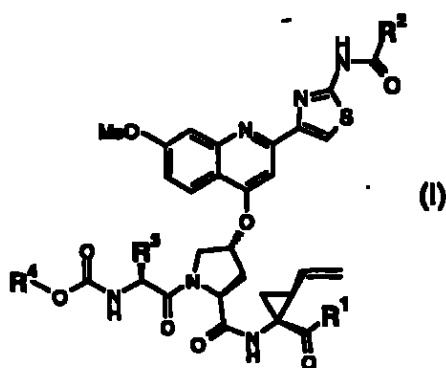
(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,
SK, TR), OAPI patent (BF, BJ, CF, CG,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

(72) Inventors; and
(75) Inventors/Applicants (for US only): LLINAS-BRUNET,
Montse [CA/CA]; 2100 Cunard Street, Laval, Québec
H7S 2G5 (CA). BAILEY, Murray, D. [CA/CA]; 2100
Cunard Street, Laval, Québec H7S 2G5 (CA). GHIRO,
Elise [CA/CA]; 2100 Cunard Street, Laval, Québec H7S
2G5 (CA).

Published:
— with international search report

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

(54) Title: HETEROCYCLIC TRIPEPTIDES AS HEPATITIS C INHIBITORS



(57) Abstract: Compounds of formula (I): wherein R¹ is hydroxyl or sulfon-
amide derivative; R² is t-butyl or -CH₂-C(CH₃)₃ or -CH₂-cyclopentyl; R³ is t-butyl
or cyclohexyl and R⁴ is cyclobutyl, cyclopentyl or cyclohexyl; or a pharmaceuti-
cally acceptable salt thereof, are described as useful as inhibitor of the HCV NS3
protease.

**HETEROCYCLIC TRIPEPTIDES AS HEPATITIS C INHIBITORS****FIELD OF THE INVENTION**

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

10 BACKGROUND OF THE INVENTION

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

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

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

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

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

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

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

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

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

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

- GB 2,337,262; JP10288151; JP 11126861; JP 11292840; JP 2001-103993;
US 6,159,938; US 6,187,905; WO 97/43310; WO 98/17679; WO 98/22496;
WO 98/46597; WO 98/46630; WO 99/38888; WO 99/50230; WO 99/64442;
15 WO 99/07733; WO 99/07734; WO 00/08543; WO 00/08558; WO 00/20400;
WO 00/59929; WO 00/31129; WO 01/02424; WO 01/07407; WO 01/16357;
WO 01/32691; WO 01/40262; WO 01/58929; WO 01/64678; WO 01/74768;
WO 01/77113; WO 01/81325; WO 02/08187; WO 02/08198; WO 02/08244;
WO 02/08251; WO 02/08258; WO 02/18369; WO 02/60926 and WO 02/79234.

20

One advantage of the present invention is that it provides tripeptide compounds that are inhibitory to the NS3 protease, an enzyme essential for the replication of the hepatitis C virus.

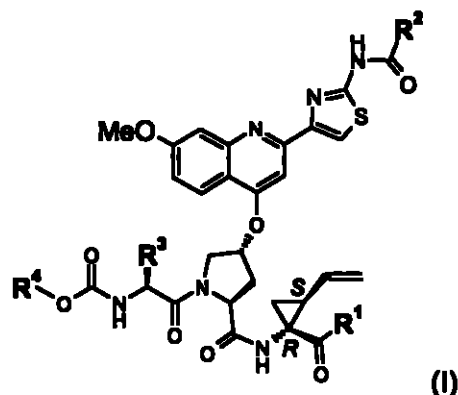
- 25 A further advantage of one aspect of the present invention resides in the fact that the compounds specifically inhibit the NS3 protease and do not show significant inhibitory activity against other serine proteases such as human leukocyte elastase (HLE), porcine pancreatic elastase (PPE), or bovine pancreatic chymotrypsin, or cysteine proteases such as human liver cathepsin B (Cat B). Furthermore, the
30 compounds are active in cell culture and have good pharmacokinetic profile *in vivo*.

A further advantage of the present invention is that it provides compounds that are orally bioavailable in mammals.

/4

SUMMARY OF THE INVENTION

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



(I)

- 5 wherein R¹ is hydroxy or NHSO₂R^{1A} wherein R^{1A} is (C₁₋₈)alkyl, (C₃₋₇)cycloalkyl or {(C₁₋₈)alkyl-(C₃₋₇)cycloalkyl}, which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, O-(C₁₋₈)alkyl, amido, amino or phenyl, or R^{1A} is C₆ or C₁₀ aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C₁₋₈)alkyl, O-(C₁₋₈)alkyl, amido, amino or phenyl; R² is t-butyl, -CH₂-C(CH₃)₃, or -CH₂-cyclopentyl; R³ is t-butyl or cyclohexyl and R⁴ is cyclobutyl, cyclopentyl, or cyclohexyl; or a pharmaceutically acceptable salt thereof.
- 10

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

15

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

20

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

25

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

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

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

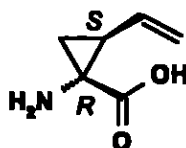
15

As used herein, the following definitions apply unless otherwise noted:

With reference to the instances where (*R*) or (*S*) is used to designate the absolute configuration of a substituent or asymmetric centre of a compound of formula I, the designation is done in the context of the whole compound and not in the context of the substituent or asymmetric centre alone.

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

As used herein the term "(1*R*, 2*S*)-vinyl-ACCA" refers to a compound of formula:



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

The term "(C₁₋₄)alkyl" as used herein, either alone or in combination with another

substituent, means acyclic, straight or branched chain alkyl substituents containing from 1 to 6 carbon atoms and includes, for example, methyl, ethyl, propyl, butyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl and hexyl. Similarly, the term "(C₁₋₈)alkyl" means acyclic, straight or branched chain alkyl
5 containing 1 to 8 carbon atoms, e.g. octyl.

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

10

The term "{(C₁₋₆)alkyl-(C₃₋₇)cycloalkyl}" as used herein means a cycloalkyl radical containing from 3 to 7 carbon atoms directly linked to an alkylene radical containing 1 to 6 carbon atoms; for example, cyclopropylmethyl, cyclopentylethyl, cyclohexylmethyl, cyclohexylethyl and cycloheptylpropyl. In the instance where R^{4A} is a {(C₁₋₆)alkyl-(C₃₋₆)cycloalkyl}, this group is attached to the SO₂ group via the (C₁₋₆)alkyl (i.e. the alkylene portion).
15

The term "C₆ or C₁₀ aryl" as used herein, either alone or in combination with another radical, means either an aromatic monocyclic group containing 6 carbon atoms or an aromatic bicyclic group containing 10 carbon atoms. For example, aryl includes phenyl, 1-naphthyl or 2-naphthyl.
20

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

The term "halo" as used herein means a halogen substituent selected from bromo, chloro, fluoro or iodo.
30

The term "pharmaceutically acceptable salt" means a salt of a compound of formula (I) which is, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation,

17

allergic response, and the like, commensurate with a reasonable benefit/risk ratio, generally water or oil-soluble or dispersible, and effective for their intended use. The term includes pharmaceutically-acceptable acid addition salts and pharmaceutically-acceptable base addition salts. Lists of suitable salts are found in, e.g., S.M. Birge et al., J. Pharm. Sci., 1977, 66, pp. 1-19, which is hereby incorporated by reference in its entirety.

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

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



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

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

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

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

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

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

10

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

15

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

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

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

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

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

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

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

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

2001-3 (ICN), Bam 205 (Novelos), XTL-001 (XTL), Imino-Sugars (Nonyl-DNJ) (Synergy), HepBzyme; and immunomodulator products such as: interferon alpha 2b, HE2000 (Hollis-Eden), Theradigm (Epimmune), EHT899 (Enzo Biochem), Thymosin alpha-1 (Zadaxin®), HBV DNA vaccine (PowderJect), HBV DNA vaccine
 5 (Jefferson Center), HBV antigen (OraGen), BayHep B® (Bayer), Nabi-HB® (Nabi) and Anti-hepatitis B (Cangene); and HBV vaccine products such as the following: Engerix B, Recombivax HB, GenHevac B, Hepacare, Bio-Hep B, TwinRix, Comvax, Hexavac.

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

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

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

- 20
- antiviral agents: ribavirin and amantadine;
 - Immunomodulatory agents: class I Interferons, class II Interferons and pegylated Interferons;
 - inhibitor of another target in the HCV life cycle that inhibits a target selected from: NS3 helicase, HCV NS2/3 protease or internal ribosome entry site (IRES);
- 25
- HIV inhibitors: nucleosidic Inhibitors, non-nucleosidic Inhibitors, protease Inhibitors, fusion Inhibitors and integrase Inhibitors; or
 - HBV Inhibitors: agents that inhibit HBV viral DNA polymerase or is an HBV vaccine.

30 As discussed above, combination therapy is contemplated wherein a compound of formula (1), or a pharmaceutically acceptable salt thereof, is co-administered with at least one additional agent selected from: an antiviral agent, an immunomodulatory agent, another Inhibitor of HCV NS3 protease, an Inhibitor of HCV polymerase, an Inhibitor of another target in the HCV life cycle, an HIV Inhibitor, an HAV Inhibitor

and an HBV inhibitor. Examples of such agents are provided in the Definitions section above. These additional agents may be combined with the compounds of this invention to create a single pharmaceutical dosage form. Alternatively these additional agents may be separately administered to the patient as part of a multiple dosage form, for example, using a kit. Such additional agents may be administered to the patient prior to, concurrently with, or following the administration of wherein a compound of formula (1), or a pharmaceutically acceptable salt thereof.

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

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

Preferred embodiments

Preferred are compounds of formula 1 as defined above wherein R^1 is hydroxy, $NHSO_2Me$, $NHSO_2$ -cyclopropyl, or $NHSO_2Ph$. More preferably, R^1 is $NHSO_2Me$ or hydroxy. Most preferably, R^1 is hydroxy.

Preferred are compounds of formula 1 as defined above wherein R^2 is t-butyl or $CH_2C(CH_3)_3$. More preferably, R^2 is $CH_2C(CH_3)_3$.

Preferably R^3 is t-butyl.

Preferably, compounds of formula 1 as defined above wherein R^4 is cyclopentyl or cyclohexyl. More preferably R^4 is cyclopentyl.

More preferably, a compound of formula 1 as defined above wherein R^1 is hydroxy, R^2 is $CH_2C(CH_3)_3$, R^3 is t-butyl and R^4 is cyclopentyl.

More preferably, a compound of formula 1 wherein R^1 is hydroxy, R^2 and R^3 each is

t-butyl and R⁴ is cyclopentyl.

More preferably, a compound of formula 1 wherein R¹ is hydroxy, R² is CH₂-C(CH₃)₃, R³ is cyclohexyl and R⁴ is cyclopentyl.

5

More preferably, a compound of formula 1 wherein R¹ is hydroxy, R² is CH₂-C(CH₃)₃, and R³ and R⁴ each is cyclohexyl.

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

More preferably, a compound of formula 1 wherein R¹ is hydroxy, R² is CH₂-C(CH₃)₃, R³ is t-butyl and R⁴ is cyclobutyl.

15 More preferably, a compound of formula 1 wherein R¹ is NHSO₂Me, R² is CH₂-C(CH₃)₃, R³ is t-butyl and R⁴ is cyclopentyl.

More preferably, a compound of formula 1 wherein R¹ is NHSO₂Ph, R² is CH₂-C(CH₃)₃, R³ is t-butyl and R⁴ is cyclopentyl.

20

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

25

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

30 According to another alternate embodiment, the pharmaceutical composition of this invention may additionally comprise an inhibitor of HCV polymerase.

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

entry site (IRES).

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

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

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

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

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

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

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

When these compounds or their pharmaceutically acceptable salts are formulated together with a pharmaceutically acceptable carrier, the resulting composition may be administered *in vivo* to mammals, such as man, to inhibit HCV NS3 protease or

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

10

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

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

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

25

A compound of formula 1 set forth herein may also be used as a laboratory reagent. A compound of this invention may also be used to treat or prevent viral contamination of materials and therefore reduce the risk of viral infection of laboratory or medical personnel or patients who come in contact with such materials (e.g. blood, tissue, surgical instruments and garments, laboratory instruments and garments, and blood collection apparatuses and materials).

A compound of formula 1 set forth herein may also be used as a research reagent. A compound of formula 1 may also be used as positive control to validate surrogate

cell-based assays or *in vitro* or *in vivo* viral replication assays.

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

5

EXAMPLES

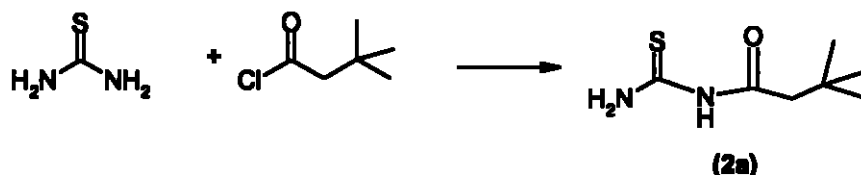
Temperatures are given in degrees Celsius. Solution percentages express a weight to volume relationship, and solution ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were
10 recorded on a Bruker 400 MHz spectrometer; the chemical shifts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

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

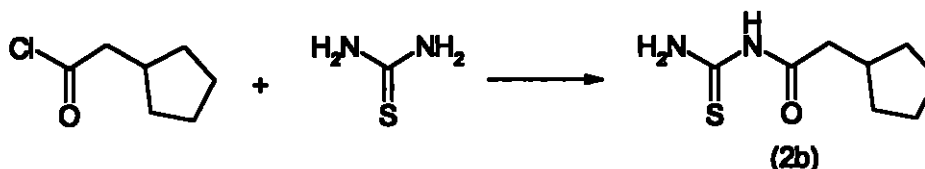
25

Synthesis of compounds of formula (I):

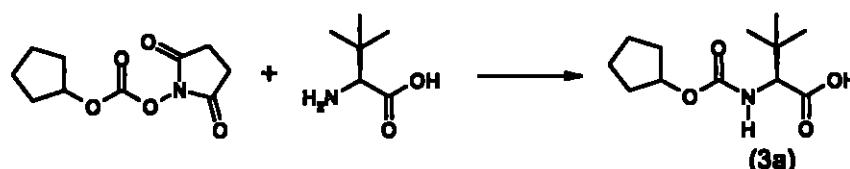
In general, the compounds of formula 1, and intermediates therefore, are prepared by known methods using reaction conditions which are known to be suitable for the reactants. Several such methods are disclosed in WO 00/09543, WO 00/09558 and
30 US Pat. No. 6,323,180, incorporated herein by reference.

Preparation of Thioureas**Preparation of Thiourea 2a**

- Thiourea (5.0 gm, 66mmol) was dissolved in toluene (50mL) and *tert*-butylacetyl chloride (8.88 gm, 66mmol) was added. The mixture was heated at reflux for 14h to give a yellow solution. The mixture was concentrated to dryness, and the residue partitioned between EtOAc and sat. NaHCO₃. The yellow organic phase was dried over MgSO₄, filtered and concentrated to give a yellow solid. The solid was dissolved into a minimum amount of EtOAc and triturated with hexane to give 2a as a white solid (8.52 g; 75%). M.S. (electrospray): 173 (M-H)⁻ 175 (M+H)⁺. Reverse Phase HPLC Homogeneity (0.06 % TFA ; CH₃CN : H₂O) : 99 %.

Preparation of Thiourea 2b

- Using the procedure described above and using commercially available cyclopentyl acetyl chloride instead of *tert*-butylacetyl chloride yielded thiourea 2b.

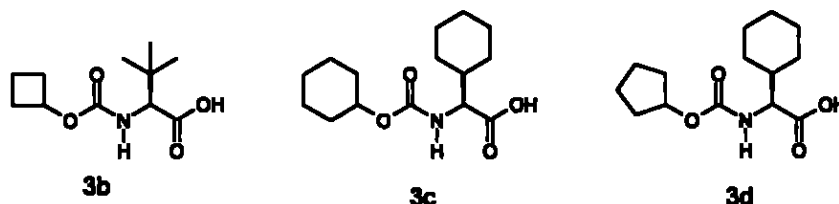
Synthesis of Intermediates 3**Preparation of carbamate 3a**

- Tetrahydrofuran (350mL) was added to a flask containing carbonic acid cyclopentyl ester 2,5-dioxo-pyrrolidine-1-yl ester (9.00g; 39.6mmol) and *tert*-leucine (6.24g; 47.5mmol) resulting in a suspension. Distilled water (100mL) was added with vigorous stirring. A small amount of solid remained undissolved. Triethylamine (16.6mL; 119mmol) was then added resulting in a homogenous solution which was stirred at R.T.. After 2.5h, the THF was evaporated and the aqueous residue diluted

with water (100mL) and the reaction rendered basic by the addition of 1 N NaOH (25mL - final pH >10). The solution was washed with EtOAc (2 x 200mL) and the aqueous phase then acidified with 1 N HCl (ca. 70mL - final pH <2). The turbid solution was extracted with EtOAc (200 + 150mL). The extract was dried (MgSO₄) and evaporated to give compound 3a as a white solid (8.68 g).

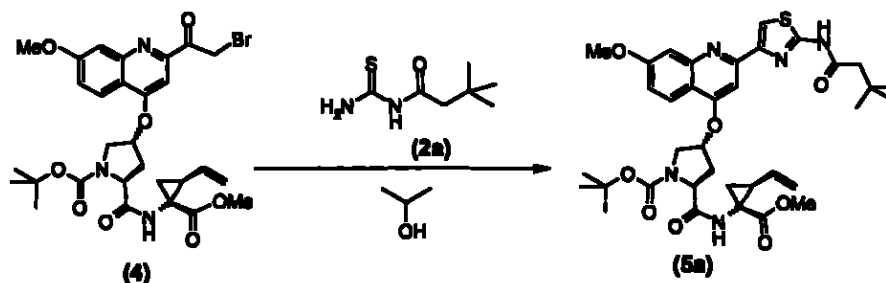
Preparation of carbamates 3b, 3c, and 3d

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



Preparation of Intermediate 5

15 Preparation of intermediate 5a:



α -Bromoketone 4 (3.61g ; 5.71mmol) was combined with thiourea 2a (1.09g ; 6.28mmol) in isopropanol (140mL) and the yellow solution was placed into a pre-heated oil bath of 70°C for 1.5h. The solution was cooled to R.T. and evaporated to dryness. The residue was dissolved in EtOAc. The EtOAc solution was washed with saturated NaHCO₃ (2x), water (2x) and brine (1x), dried (MgSO₄), and evaporated to give the product as an orange-brown foam. Flash column chromatography in 7:3 hexane : EtOAc removed the less polar impurities and 6:4 hexane : EtOAc provided pure product as a light yellow solid (3.05g ; 76%).

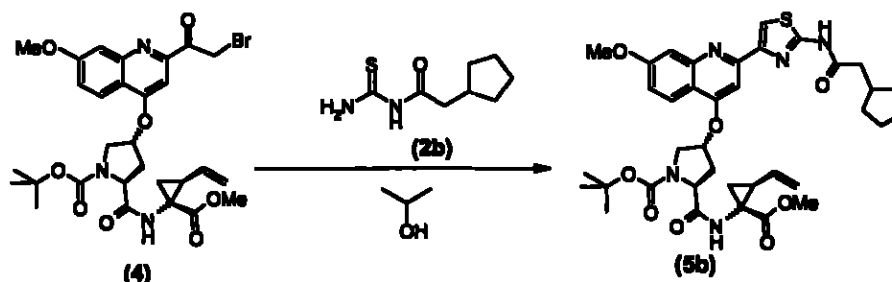
20 M.S.(electrospray) : 706.3 (M-H)⁻ 708.4 (M+H)⁺ . Reverse Phase HPLC

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

Preparation of Intermediate 5b:

Using the procedure described above and using thiourea 2b instead of thiourea 2a,

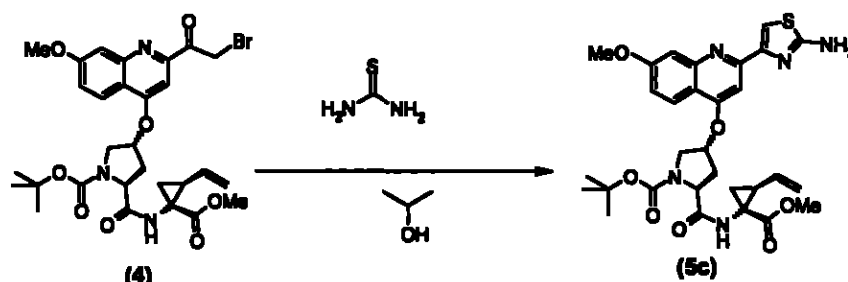
5 the corresponding Intermediate 5b is obtained:



Preparation of Intermediate 5c:

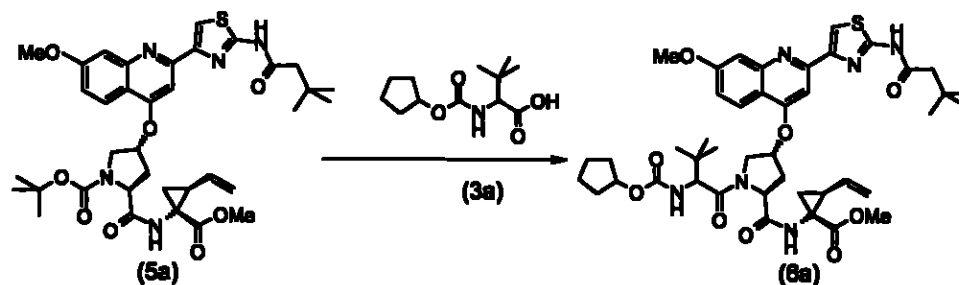
Using the procedure described above and using commercially available thiourea

10 instead of thiourea 2a, the corresponding Intermediate 5c is obtained:



EXAMPLE 1

Synthesis of Compound 100



15

Step 1: Preparation of Intermediate 6a

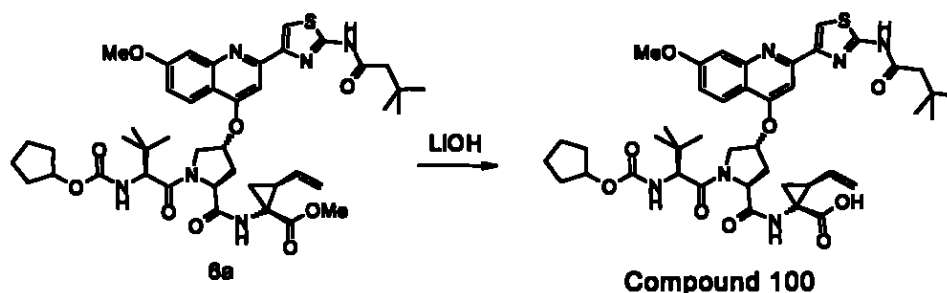
Boc-Dipeptide 5a (3.05g ; 4.31mmol) was dissolved in 4N HCl/dioxane (22mL).

After stirring at R.T. for 30 min., the HCl salt precipitated. MeOH (2mL) was added to dissolve the precipitate. After 2h, the reaction mixture was evaporated to

dryness. The resulting HCl salt was dissolved in DCM (22mL) and DIEA (3.0mL ; 17.24mmol); carbamate **3a** (1.15g ; 4.47mmol) and HATU (1.72g ; 4.516mmol) were added. The solution was stirred at R.T. for 6h. The mixture was then diluted with EtOAc and the solution washed with saturated NaHCO₃ (2x), water (2x) and brine (1x), dried (MgSO₄), filtered and evaporated to obtain compound **6a** as a yellow solid. Flash column chromatography eluting first with hexane : EtOAc 7:3 and then 6:4 afforded pure Me-ester **6a** as a white foam (3.25g ; 90%). M.S. (electrospray) : 831.4 (M-H)⁻ 833.4 (M+H)⁺ 855.4 (M+Na)⁺. Reverse Phase HPLC Homogeneity (0.06 % TFA ; CH₃CN : H₂O) : 98 %.

10

Step 2: Hydrolysis of Ester



Methyl ester **6a** (3.24mg; 3.89mmol) was dissolved in THF (40mL) and MeOH (20mL), and an aqueous solution of LiOH (1.63mg ; 38.9mmol in 25mL) was added. The yellow reaction mixture was stirred for 5.5h and then concentrated to provide an off-white suspension. The suspension was dissolved in EtOAc and brine, prepared with deionized water. The pH of the resulting solution was adjusted to 6 by the addition of 1N HCl. The layers were separated and the aqueous layer further extracted with EtOAc (2x). The combined EtOAc extracts were washed with deionized water (2x), deionized water prepared brine (1x), dried (MgSO₄), filtered and evaporated to obtain compound **100** as a pale yellow-white solid (3.02g; 95% yield).

Conversion to Na Salt

The neutral compound **100** (1.22g; 1.49mmol) was dissolved in MeOH (30mL) and 1 equivalent 0.01N NaOH (14.85mL) was added – no product precipitation. The clear yellow solution was concentrated, diluted with deionized water, frozen and lyophilized to obtain the product (Na salt) as a yellow-white amorphous solid (1.24g; 99 % yield)

Na Salt : MW : 840.98 $C_{42}H_{53}N_9O_9SNa$

M.S.(electrospray) : 817.3 (M-H)⁻ 819.4 (M+H)⁺ 841.4 (M+Na)⁺ . Reverse Phase HPLC Homogeneity (0.06 % TFA ; CH₃CN : H₂O) : 98 %.

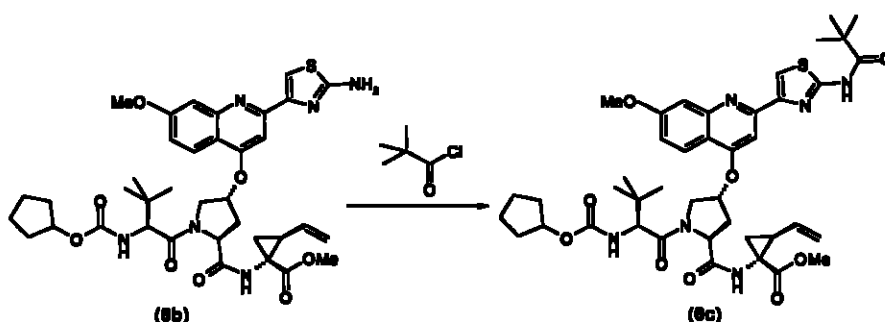
¹H NMR (400 MHz,DMSO-d₆): ca, 6:1 mbxture of rotamers ; δ 8.11-7.65 (m, 4H) ,

5 7.33 (bs, 1H) , 7.18-6.97 (m, 2H), 6.36-6.08 (m, 1H), 5.55-5.33 (m, 1H), 4.98 (d, J = 18.0 Hz, 1H), 4.85 (bs, 1H), 4.80 (d, J = 10.4 Hz, 1H), 4.50-4.41 (m, 1H), 4.22-4.02 (m, 2H), 3.92 (s, 3H), 2.72-2.45 (m, 1H), 2.50 (under DMSO, s, 2H), 2.40-2.26 (m, 1H), 1.89-1.43 (m, 9H), 1.37-1.30 (m, 1H), 1.30-1.12 (m, 1H), 1.03 & 0.90 (2x s, 9H), 0.98 & 0.94 (2x s, 9H).

10

EXAMPLE 2

Synthesis of Compound 101



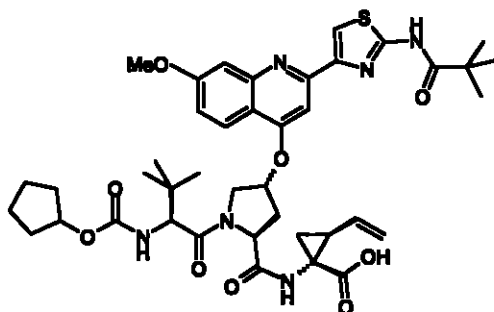
15 By following the same procedure as described in the first step of example 1, and using Boc-dipeptide 5c instead of 5a, compound 6b was obtained.

Compound 6b (70mg, 0.095mmol) was dissolved in 2mL of DCM and successively treated with DIEA (0.045mL, 0.26mmol) and pivaloyl chloride (0.015mL, 0.12mmol). After stirring for 1h at 40° an additional pivaloyl chloride (0.015mL, 0.12mmol) was added and stirring was continued for an additional 2h. After the solution was concentrated, the residue was dissolved in EtOAc. The solution was washed with a saturated solution of NaHCO₃ and brine, dried (MgSO₄) and concentrated to afford 82mg of crude compound 6c which was used without purification.

20 Methyl ester derivative 6c was hydrolyzed as in step 2 of example 1 and purified by preparative HPLC using a YMC Combi-Prep. ODS-AQ column, 50 x 20mm. ID, S - 5micron, 120 Å, and a linear gradient program from 2 to 100% AcCN /water (0.06% TFA).

Fractions were analyzed by analytical HPLC, and the pure fractions were combined,

concentrated, frozen and lyophilized to yield compound 101 as the trifluoroacetate salt:



Compound 101

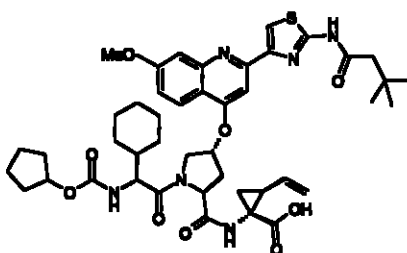
¹H NMR (400 MHz, DMSO-d₆): ca, 85:15 mixture of rotamers, major isomer

- 5 description: δ 8.56 (s, 1H), 8.40-8.22 (m, 1H), 8.17 (d, J = 8.9 Hz, 1H), 7.67 (bs, 1H), 7.52 (bs, 1H), 7.24-7.15 (m, 1H), 7.03 (d, J = 8.3 Hz, 1H), 5.78-5.67 (m, 1H), 5.61-5.53 (m, 1H), 5.19 (dd, J = 17.2, 1.6 Hz, 1H), 5.09-5.03 (m, 1H), 4.63-4.55 (m, 1H), 4.49-4.39 (m, 2H), 4.11-3.92 (m, 2H), 3.95 (s, 3H), 2.62-2.53 (m, 1H), 2.33-2.25 (m, 1H), 2.06-1.98 (m, 1H), 1.72-1.25 (m, 10H), 1.30 (s, 9H), 0.97 (s, 9H).
- 10 M.S.(electrospray) : 803.3 (M-H)⁻ 805.3 (M+H)⁺ . Reverse Phase HPLC Homogeneity (0.06 % TFA; CH₃CN : H₂O) : 97 %

EXAMPLE 3

Preparation of compound 102

- 15 By following the procedure described in example 1 and using carbamate 3d instead of 3a and using preparative HPLC to purify the final compound as described in example 2, compound 102 was obtained as the trifluoroacetate salt:



Compound 102

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

- 20 description: δ 12.37 (s, 1H), 8.54 (s, 1H), 8.40-8.06 (m, 2H), 7.67-7.40 (m, 2H), 7.26 (d, J = 8.0 Hz, 1H), 7.26-7.13 (m, 1H), 5.77-5.65 (m, 1H), 5.62-5.49 (m, 1H), 5.23-5.16 (m, 1H), 5.10-5.03 (m, 1H), 4.57-4.37 (m, 3H), 4.03-3.88 (m, 2H), 3.94 (s, 3H), 2.64-2.54 (m, 1H), 2.41 (s, 2H), 2.37-2.22 (m, 1H), 2.04-1.95 (m, 1H), 1.79-1.21 (m,

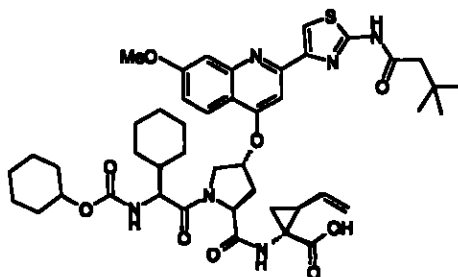
16H), 1.17-0.85 (m, 5H), 1.04 (s, 9H).

M.S.(electrospray) : 843.5 (M-H)⁻ 845.4 (M+H)⁺ . Reverse Phase HPLC

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

5 EXAMPLE 4

Preparation of compound 103



Compound 103

By following the procedure described in example 1 but using carbamate 3c instead of 3a and using preparative HPLC to purify the final compound as described in

10 example 2, compound 103 was obtained as the trifluoroacetate salt:

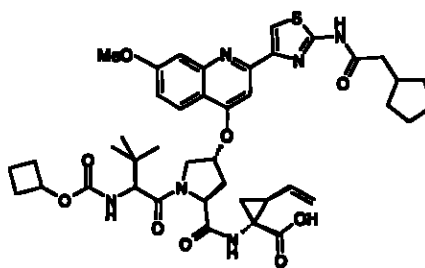
¹H NMR (400 MHz, DMSO-d₆): ca, 85:15 mixture of rotamers, major isomer description; δ 12.35 (s, 1H), 8.52 (m, 1H), 8.43-8.06 (m, 2H), 7.67-7.41 (m, 2H), 7.26 (d, J = 7.8 Hz, 1H), 7.24-7.11 (m, 1H), 5.77-5.65 (m, 1H), 5.62-5.48 (m, 1H), 5.23-5.15 (m, 1H), 5.10-5.03 (m, 1H), 4.52-4.38 (m, 2H), 4.15-4.05 (m, 1H), 4.03-3.89 (m, 15 2H), 3.94 (s, 3H), 2.63-2.52 (m, 1H), 2.41 (s, 2H), 2.35-2.23 (m, 1H), 2.05-1.96 (m, 1H), 1.81-1.41 (m, 11H), 1.38-0.86 (m, 12H), 1.04 (s, 9H).

M.S.(electrospray) : 857.5 (M-H)⁻ 859.4 (M+H)⁺ . Reverse Phase HPLC

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

20 EXAMPLE 5

Preparation of compound 104



Compound 104

By following the procedure described in example 1 but using carbamate 3b instead

of **3a** and Boc-dipeptide **5b** instead of **5a** and using preparative HPLC to purify the final compound as described in example 2, compound **104** was obtained as the trifluoroacetate salt:

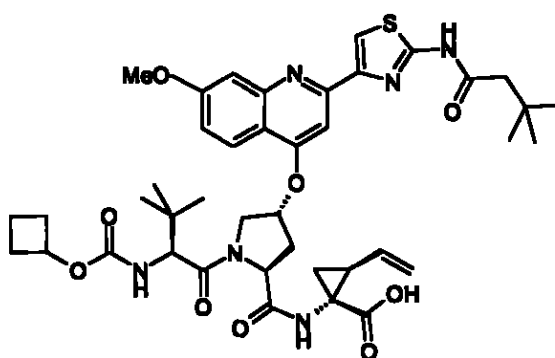
¹H NMR (400 MHz, DMSO-d₆): ca, 3:1 mixture of rotamers ; 88.02 (s, 1H) , 7.90 (s, 1H) , 7.84 (d, J = 7.0 Hz, 1H) , 7.70 (s, 1H) , 7.33 (d, J = 2.2 Hz, 1H), 7.14 (dd, J = 2.5, 8.2 Hz, 1H), 7.09 (dd, J = 2.2, 9.2 Hz, 1H), 6.29-6.08 (m, 1H), 5.54-5.32 (m, 1H), 4.99 (d, J = 15.9 Hz, 1H), 4.80 (d, J = 10.0 Hz, 1H), 4.76-4.64 (m, 1H), 4.46 (dd, J = 6.7, 13.9 Hz, 1H), 4.19-4.08 (m, 3H), 3.92 (s, 3H), 2.70-2.61 (m, 2H), 2.37-2.09 (m, 4H), 2.03-1.82 (m, 1H), 1.85 (bs, 2H), 1.77-1.44 (m, 8H), 1.35-1.29 (m, 1H), 1.29-1.15 (m, 4H), 0.98 & 0.90 (2x s, 9H).

M.S.(electrospray) : 815.3 (M-H)⁻ 817.3 (M+H)⁺ . Reverse Phase HPLC

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

EXAMPLE 6

15 Preparation of compound 105



Compound 105

By following the procedure described in example 1 but using carbamate **3b** instead of **3a** and using preparative HPLC to purify the final compound as described in example 2, compound **105** was obtained as the trifluoroacetate salt:

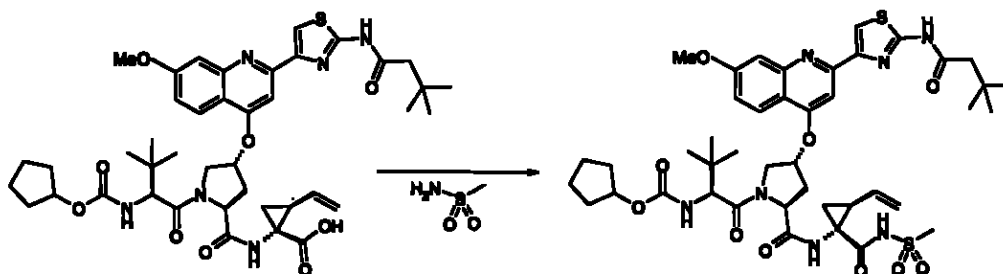
¹H NMR (400 MHz, DMSO-d₆): ca, 7:1 mixture of rotamers ; 88.58 (s, 1H) , 8.19 (d, J = 8.0 Hz, 1H) , 7.76-7.50 (m, 2H), 7.35-7.20 (m, 1H), 7.19 (d, J = 8.0 Hz, 1H), 5.78-5.67 (m, 1H), 5.65-5.50 (m, 1H), 5.19 (d, J = 17.0 Hz, 1H), 5.07 (d, J = 10.2 Hz, 1H), 4.51-4.42 (m, 2H), 4.42-4.31 (m, 1H), 4.02 (d, J = 7.4 Hz, 1H), 4.02-3.93 (m, 1H), 3.97 (s, 3H), 2.63-2.52 (m, 1H), 2.42 (s, 2H), 2.35-2.25 (m, 1H), 2.07-1.95 (m, 3H), 1.90-1.76 (m, 1H), 1.70-1.41 (m, 3H), 1.30-1.23 (m, 1H), 1.04 (s, 9H), 0.96 & 0.87 (2xs, 9H),

M.S.(electrospray) : 803.4 (M-H)⁻ 805.4 (M+H)⁺ . Reverse Phase HPLC

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

EXAMPLE 7

Preparation of compound 106

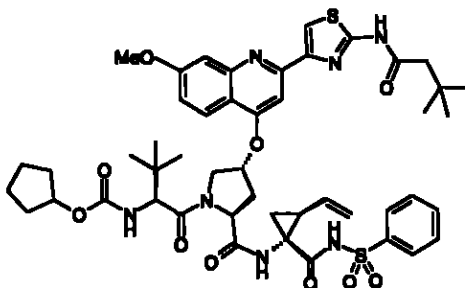


Compound 106

- 5 Compound 100 of example 1 (29.8mg, 0.036mmol) was combined with HATU (1.2 equiv., 19.7mg, 0.052mmol) and dissolved in anhydrous DMF (4mL). The solution was stirred at R.T. and DIPEA (5 equiv., 31.4μL, 0.18mmol) was added dropwise over ca. 1 min.. The mixture was stirred for 20min. at R.T. and analyzed by
- 10 analytical HPLC for the formation of the activated ester. A solution of methanesulfonamide (5.8equiv., 19.7mg, 0.207mmol), DMAP (5.4equiv., 23.5mg, 0.193mmol) and DBU (4.8equiv., 25.8μL, 0.172mmol) was added in DMF (1mL). The reaction mixture was stirred 16h at R.T. before being concentrated *in vacuo*. The residue was reconstituted in DMSO and purified by preparative HPLC.
- 15 Lyophilization gave the final product (23mg, 71.3%) as an off-white amorphous solid.
- ¹H-NMR (400MHz, DMSO-d₆), δ12.35 (s, 1H), 10.53 (s, 1H), 8.87 (s, 1H), 8.40-8.20 (m, 1H), 8.17 (d, *J* = 8.8 Hz, 1H), 7.61 (bs, 1H), 7.51 (bs, 1H), 5.67-5.55 (m, 2H), 5.23-5.18 (m, 1H), 5.10 (d, *J* = 12 Hz, 1H), 4.88-4.57 (m, 1H), 4.50 (bd, *J* = 12 Hz, 1H), 4.46-4.37 (m, 1H), 4.07 (d, *J* = 8.0 Hz, 1H), 3.95 (s, 3H), 3.17 (s, 3H), 2.78-2.58 (m, 1H), 2.42 (s, 2H), 2.29-2.19 (m, 1H), 2.19-2.09 (m, 1H), 1.71 (dd, *J* = 7.8, 7.6 Hz, 1H), 1.67-1.55 (m, 4H), 1.55-1.37 (m, 4H), 1.04 (s, 9H), 0.98 (s, 9H), 0.97-0.87 (m, 2H).
- MS (electrospray): 896.5 (M + H)⁺, and 894.5 (M - H)⁻.
- 25 RP-HPLC: Rt = 6.7 minutes (homogeneity = 100%).

EXAMPLE 8

Preparation of compound 107



Compound 107

Using the same procedure described in Example 7 and using benzenesulfonamide
5 instead of methylsulfonamide gave compound 107 as a pale yellow amorphous solid
in 54% yield.

¹H NMR (DMSO-d₆) δ 12.39 (s, 1H), 10.89 (s, 1H), 8.83 (s, 1H), 8.40-8.22 (m, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.90 (s, 1H), 7.88 (s, 1H), 7.67-7.63 (m, 1H), 7.63-7.54 (m, 3H), 7.54-7.45 (m, 1H), 7.30-7.15 (m, 1H), 7.10 (d, *J* = 7.8 Hz, 1H), 5.82-5.51 (m, 1H), 5.38-5.26 (m, 1H), 5.16-5.08 (m, 1H), 4.93 (d, *J* = 10.4 Hz, 1H), 4.70-4.58 (m, 1H), 4.57-4.49 (m, 1H), 4.48-4.39 (m, 1H), 4.09 (d, *J* = 7.8 Hz, 1H), 3.95 (s, 3H), 2.69-2.59 (m, 1H), 2.41 (s, 2H), 2.28-2.16 (m, 1H), 2.14-2.04 (m, 1H), 1.72-1.52 (m, 4H), 1.51-1.37 (m, 4H), 1.29-1.22 (m, 1H), 1.03 (s, 9H), 1.00 (s, 9H), 0.99-0.92 (m, 1H).

15 MS (electrospray); 958.5 (M + H)⁺, and 956.5 (M - H)⁻.

RP-HPLC: Rt = 7.2 minutes (homogeneity = 95%).

EXAMPLE 9

NS3-NS4A protease assay

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

EXAMPLE 10

Cell Based HCV RNA Replication Assay

25 Cell Culture

Huh7 cells that stably maintain a subgenomic HCV replicon were established as previously described (Lohman et al., 1999. *Science* 285: 110-113) and designated as the S22.3 cell-line. S22.3 cells are maintained in Dulbecco's Modified Earle

Medium (DMEM) supplemented with 10% FBS and 1mg/mL neomycin (Standard Medium). During the assay, DMEM medium supplemented with 10% FBS, containing 0.5% DMSO and lacking neomycin was used (Assay Medium). 16 hours prior to compound addition, S22.3 cells are trypsinized and diluted to 50 000 cells/ml in Standard Medium. 200µL (10 000 cells) are distributed into each well of a 96-well plate. The plate was then incubated at 37° with 5% CO₂ until the next day.

Reagents and Materials:

Product	Company	Catalog #	Storage
DMEM	Wisent Inc.	10013CV	4°C
DMSO	Sigma	D-2850	RT
Dulbecco's PBS	Gibco-BRL	14190-136	RT
Fetal Bovine Serum	Bio-Whittaker	14-901F	-20°C/4°C
Neomycin (G418)	Gibco-BRL	10131-027	-20°C/4°C
Trypsin-EDTA	Gibco-BRL	25300-054	-20°C/4°C
96-well plates	Costar	3997	RT
PVDF 0.22µm Filter Unit	Millipore	SLGV025LS	RT
Deep-Well Titer Plate Polypropylene	Beckman	267007	RT

10 Preparation of Test Compound

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

Application of test compound to cells

Cell culture medium was aspirated from the 96-well plate containing the S22.3 cells.

20 175µL of assay medium with the appropriate dilution of test compound was transferred from each well of the compound plate to the corresponding well of the cell culture plate (row H was used as the "No inhibition control"). The cell culture plate was incubated at 37° with 5% CO₂ for 72h.

Extraction of Total Cellular RNA

Following the 72h incubation period, the total cellular RNA was extracted from the S22.3 cells of the 96-well plate using the RNeasy 96 kit (Qiagen®, RNeasy Handbook, 1999.). Briefly, assay medium was completely removed from cells and 100 µL of RLT buffer (Qiagen®) containing 143 mM β-mercaptoethanol was added to each well of the 96-well cell-culture plate. The microplate was gently shaken for 20 sec. 100 µL of 70% ethanol was then added to each microplate well, and mixed by pipetting. The lysate was removed and applied to the wells of a RNeasy 96 (Qiagen®) plate that was placed on top of a Qiagen® Square-Well Block. The RNeasy 96 plate was sealed with tape and the Square-Well Block with the RNeasy 96 plate was loaded into the holder and placed in a rotor bucket of a 4K15C centrifuge. The sample was centrifuged at 6000 rpm (~5600 x g) for 4 min at room temperature. The tape was removed from the plate and 0.8 ml of Buffer RW1 (Qiagen® RNeasy 96 kit) was added to each well of the RNeasy 96 plate. The RNeasy 96 plate was sealed with a new piece of tape and centrifuged at 6000 rpm for 4 min at room temperature. The RNeasy 96 plate was placed on top of another clean Square-Well Block, the tape removed and 0.8 ml of Buffer RPE (Qiagen® RNeasy 96 kit) was added to each well of the RNeasy 96 plate. The RNeasy 96 plate was sealed with a new piece of tape and centrifuged at 6000 rpm for 4 min at room temperature. The tape was removed and another 0.8 ml of Buffer RPE (Qiagen® RNeasy 96 kit) was added to each well of the RNeasy 96 plate. The RNeasy 96 plate was sealed with a new piece of tape and centrifuged at 6000 rpm for 10 min at room temperature. Tape was removed, the RNeasy 96 plate was placed on top of a rack containing 1.2-mL collection microtubes. The RNA was eluted by adding 50 µL of RNase-free water to each well, sealing plate with a new piece of tape and incubated for 1 min at room temperature. The plate was then centrifuged at 6000 rpm for 4 min at room temperature. The elution step was repeated with a second volume of 50 µl RNase-free water. The microtubes with total cellular RNA are stored at -70°.

Quantification of Total Cellular RNA

RNA was quantified on the STORM® system (Molecular Dynamics®) using the RiboGreen® RNA Quantification Kit (Molecular Probes®). Briefly, the RiboGreen

reagent was diluted 200-fold in TE (10mM Tris-HCl pH =7.5, 1mM EDTA).
Generally, 50µL of reagent was diluted in 10mL TE. A Standard Curve of ribosomal RNA was diluted in TE to 2µg/mL and pre-determined amounts (100, 50, 40, 20, 10, 5, 2 and 0µL) of the ribosomal RNA solution are then transferred in a new 96-well plate (COSTAR # 3997) and the volume was completed to 100µL with TE.
5 Generally, column 1 of the 96-well plate was used for the standard curve and the other wells are used for the RNA samples to be quantified. 10µL of each RNA sample that was to be quantified, was transferred to the corresponding well of the 96-well plate and 90µL of TE was added. One volume (100µL) of diluted RiboGreen reagent was added to each well of the 96-well plate and incubated for 2 to 5 minutes at room temperature, protected from light (a 10 µL RNA sample in a 200 µL final volume generates a 20 X dilution). The fluorescence intensity of each well was measured on the STORM® system (Molecular Dynamics®). A standard curve was created on the basis of the known quantities of the ribosomal RNA and the resulting
10 fluorescent intensities. The RNA concentration in the experimental samples was determined from the standard curve and corrected for the 20X dilution.
15

Reagents and Materials:

Product	Company	Catalog #	Storage
DEPC	Sigma	D5758	4°C
EDTA	Sigma	E5134	RT
Trizma-Base	Sigma	T8524	RT
Trizma-HCl	Sigma	T7149	RT
Collection Tube Strips	Qiagen	19562	RT
Ribogreen RNA Quantitation Kit	Molecular Probe	R11490	-20°C
Rneasy 96 Kit	Qiagen	74183	RT
Square-Well Blocks	Qiagen	19573	RT

20 Real-Time RT-PCR
The Real-Time RT-PCR was performed on the ABI Prism 7700 Sequence Detection System using the TaqMan EZ RT-PCR Kit from (Perkin-Elmer Applied Biosystems®). RT-PCR was optimized for the quantification of the 5' IRES of HCV RNA by using the Taqman technology (Roche Molecular Diagnostics Systems)
25 similar to the technique previously described (Martell et al., 1999. J. Clin. Microbiol.

37: 327-332). The system exploits the 5'-3' nucleolytic activity of AmpliTaq DNA polymerase. Briefly, the method utilizes a dual-labeled fluorogenic hybridization probe (PUTR Probe) that specifically anneals to the template between the PCR primers (primers 8125 and 7028). The 5' end of the probe contains a fluorescent reporter (6-carboxyfluorescein [FAM]) and the 3' end contains a fluorescent quencher (6-carboxytetramethylrhodamine [TAMRA]). The FAM reporter's emission spectrum was suppressed by the quencher on the intact hybridization probe. Nuclease degradation of the hybridization probe releases the reporter, resulting in an increase in fluorescence emission. The ABI Prism 7700 sequence detector measures the increase in fluorescence emission continuously during the PCR amplification such that the amplified product was directly proportion to the signal. The amplification plot was analysed early in the reaction at a point that represents the logarithmic phase of product accumulation. A point representing a defined detection threshold of the increase in the fluorescent signal associated with the exponential growth of the PCR product for the sequence detector was defined as the cycle threshold (C_T). C_T values are inversely proportional to the quantity of input HCV RNA; such that under identical PCR conditions, the larger the starting concentration of HCV RNA, the lower the C_T . A standard curve was created automatically by the ABI Prism 7700 detection system by plotting the C_T against each standard dilution of known HCV RNA concentration.

Reference samples for the standard curve are included on each RT-PCR plate. HCV Replicon RNA was synthesized (by T7 transcription) *in vitro*, purified and quantified by OD₂₆₀. Considering that 1 µg of this RNA = 2.15×10^{11} RNA copies, dilutions are made in order to have 10^8 , 10^7 , 10^6 , 10^5 , 10^4 , 10^3 or 10^2 genomic RNA copies / 5 µL. Total cellular Huh-7 RNA was also incorporated with each dilution (50 ng / 5 µL). 5 µL of each reference standard (HCV Replicon + Huh-7 RNA) was combined with 45 µL of Reagent Mix, and used in the Real-Time RT-PCR reaction.

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

42

Reagents and Materials:

Product	COMPANY	Catalog #	Storage
TaqMan EZ RT-PCR Kit	PE Applied Biosystems	N808-0238	-20°C
MicroAmp Optical Caps	PE Applied Biosystems	N801-0935	RT
MicroAmp Optical 96-Well Reaction Plate	PE Applied Biosystems	N801-0580	RT

Reagent Mix preparation:

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

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

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

Note: Those primers amplify a region of 258-nt present within the 5' untranslated region of HCV.

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

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

- 10 **Thermal Cycling Conditions:**
- 50°C 2 min
 - 60°C 30 min
 - 95°C 5 min
- 15

95°C	15 sec	} for 2 cycles
60°C	1 min	
- | | | |
|------|--------|-----------------|
| 90°C | 15 sec | } for 40 cycles |
| 60°C | 1 min | |

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

25 interpolate an RNA copy number based on the standard curve.

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

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

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

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

5

When the compounds of this invention were evaluated in the preceding enzymatic and cell based assays, the compounds were found to be highly active. More specifically, the compounds had IC_{50} 's below 0.1 μ M in the NS3-NS4A protease assay, and EC_{50} 's below 0.5 μ M in the cell based HCV RNA replication assay.

10

EXAMPLE 11

Specificity assays

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

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

EXAMPLE 12

20 Pharmacokinetic properties

The present compounds also show good pharmacokinetic properties such as detectable plasma levels in the rat at 1 hour and 2h after an oral dose of 4 or 5mg/kg.

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

Materials and Methods:

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

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

as basis for pooling 3-4 compounds into one "cassette".

2. Oral vehicle and compound preparation:

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

3. Dosing and plasma sampling:

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

15 **4. Compound extraction and analysis:**

From each cassette, plasma samples at 1 and 2h, blank plasma, blank plasma
spiked with all the compounds at 0.5 μ M of each, are extracted by the solid phase
extraction method. Samples were analyzed by HPLC and HPLC/MS for comparison
purpose. Plasma concentrations are estimated based on the single concentration of
20 0.5 μ M standard.

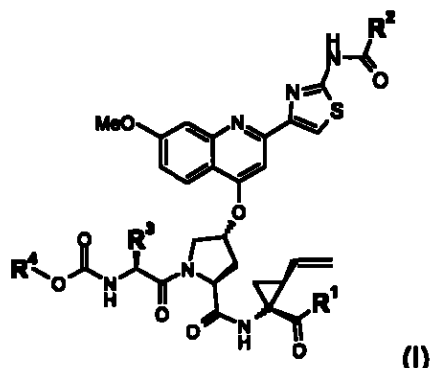
Results

When assayed in the preceding screen the compounds of examples 1 to 8 of this
invention were found to present in significant levels in the plasma at the 1 hour and
25 2 hour intervals following oral administration, averaging blood plasma levels of
0.83 μ M and 0.75 μ M respectively.

This demonstration of *in vivo* oral absorption for the tripeptide compounds of this
invention is noteworthy, in view of the poor oral absorption generally attributed to this
30 class of peptides. The ready oral absorption renders the compounds useful for
treating of HCV infection.

CLAIMS

1. A compound of formula (I):



(I)

- wherein R¹ is hydroxy or NHSO₂R^{1A} wherein R^{1A} is (C₁₋₈)alkyl, (C₃₋₇)cycloalkyl or {(C₁₋₈)alkyl-(C₃₋₇)cycloalkyl}, which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, O-(C₁₋₈)alkyl, amido, amino or phenyl, or R^{1A} is C₆ or C₁₀ aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C₁₋₈)alkyl, O-(C₁₋₈)alkyl, amido, amino or phenyl; R² is t-butyl, -CH₂-C(CH₃)₃, or -CH₂-cyclopentyl; R³ is t-butyl or cyclohexyl and R⁴ is cyclobutyl, cyclopentyl, or cyclohexyl; or a pharmaceutically acceptable salt thereof.
2. The compound of formula I according to claim 1, wherein R¹ is hydroxy, NHSO₂Me, NHSO₂-cyclopropyl, or NHSO₂Ph.
 3. The compound of formula I according to claim 2, wherein R¹ is NHSO₂Me or hydroxy.
 4. The compound of formula I according to claim 3, wherein R¹ is hydroxy.
 5. The compound of formula I according to any one of claims 1 to 4, wherein R² is t-butyl or CH₂-C(CH₃)₃.
 6. The compound of formula I according to claim 5, wherein R₂ is CH₂-C(CH₃)₃.
 7. The compound of formula I according to any one of claims 1 to 6, wherein R³ is t-butyl.
 8. The compound of formula I according to any one of claims 1 to 7, wherein R⁴ is cyclopentyl or cyclohexyl.
 9. The compound of formula I according to claim 8, wherein R⁴ is cyclopentyl.
 10. The compound of formula I as defined in claim 1, wherein R¹ is hydroxy, R² is CH₂-C(CH₃)₃, R³ is t-butyl and R⁴ is cyclopentyl.
 11. The compound of formula I as defined in claim 1, wherein R¹ is hydroxy, R² and R³ each is t-butyl and R⁴ is cyclopentyl.

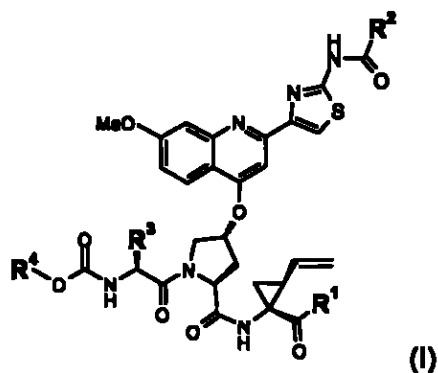
12. The compound of formula I as defined in claim 1, wherein R¹ is hydroxy, R² is CH₂-C(CH₃)₃, R³ is cyclohexyl and R⁴ is cyclopentyl.
13. The compound of formula I as defined in claim 1, wherein R¹ is hydroxy, R² is CH₂-C(CH₃)₃, and R³ and R⁴ each is cyclohexyl.
14. The compound of formula I as defined in claim 1, wherein R¹ is hydroxy, R² is cyclopentylmethyl, R³ is t-butyl, and R⁴ is cyclobutyl.
15. The compound of formula I as defined in claim 1, wherein R¹ is hydroxy, R² is CH₂-C(CH₃)₃, R³ is t-butyl and R⁴ is cyclobutyl.
16. The compound of formula I as defined in claim 1, wherein R¹ is NHSO₂Me, R² is CH₂-C(CH₃)₃, R³ is t-butyl and R⁴ is cyclopentyl.
17. The compound of formula I as defined in claim 1, wherein R¹ is NHSO₂Ph, R² is CH₂-C(CH₃)₃, R³ is t-butyl and R⁴ is cyclopentyl.
18. A pharmaceutical composition comprising an anti-hepatitis C virally effective amount of a compound of formula I according to any one of claims 1 to 17, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.
19. The pharmaceutical composition according to claim 18, further comprising a therapeutically effective amount of one or more other anti-HCV agent.
20. The pharmaceutical composition according to claim 19, wherein said other anti-HCV agent is selected from: α-interferon or pegylated α-interferon.
21. The pharmaceutical composition according to claim 19 or 20, wherein said other anti-HCV agent is ribavirin.
22. The pharmaceutical composition according to claim 19, 20 or 21, wherein said other anti-HCV agent is selected from inhibitors of: helicase, NS2/3 protease and internal ribosome entry site (IRES).
23. A method for the treatment or prevention of a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of a compound of formula I according to any one of claims 1 to 17, or a pharmaceutically acceptable salt thereof.
24. A method for the treatment or prevention of a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of the composition according any one of claims 18 to 22.
25. A method for the treatment or prevention of a hepatitis C viral infection in a mammal by administering thereto an anti-hepatitis C virally effective amount of a compound

of formula I according to any one of claims 1 to 17, or a therapeutically acceptable salt thereof in combination with one or more other anti-HCV agent.

26. The method according to claim 25, wherein said other anti-HCV agent is selected from: α -interferon or pegylated α -interferon.
27. The method according to claim 25 or 26, wherein said other anti-HCV agent is ribavirin.
28. The method according to claim 25, 26 or 27, wherein said other anti-HCV agent is selected from inhibitors of: helicase, NS2/3 protease and internal ribosome entry site (IRES).
29. Use of a compound of formula I according to any one of claims 1 to 17 for the manufacture of a medicament for the treatment or prevention of hepatitis C viral infection.
30. The pharmaceutical composition according to claim 19, 20 or 21, wherein said other anti-HCV agent is an inhibitor of HCV polymerase.
31. The method according to claim 25, 26 or 27, wherein said other anti-HCV agent is an inhibitor of HCV polymerase.

ABSTRACT

Compounds of formula (I):



5

wherein R¹ is hydroxyl or sulfonamide derivative; R² is t-butyl or -CH₂-C(CH₃)₃ or -CH₂-cyclopentyl; R³ is t-butyl or cyclohexyl and R⁴ is cyclobutyl, cyclopentyl or cyclohexyl; or a pharmaceutically acceptable salt thereof, are described as useful as
10 inhibitor of the HCV NS3 protease.

SEQUENCE LISTING

<110> BOEHRINGER INGELHEIM INTERNATIONAL GmbH

<120> HEPATITIS C INHIBITOR TRI-PEPTIDES

<130> 13/107

<150> 2,369,970

<151> 2002-02-01

<160> 3

<170> FastSEQ for Windows Version 4.0

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<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Forward primer

<400> 1

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30

<210> 2

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse primer

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30

<210> 3

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> PUTR probe

<400> 3

tggctctgcgg aaccggtgag tacacc

26

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To: BERNIER, Louise G. Boehringer Ingelheim (Canada) Ltd. 2100 Cunard Street Laval, Québec H7S 2G5 Canada		PCT NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE (PCT Rule 20.5(c))	
		Date of mailing (day/month/year)	31 January 2003 (31.01.2003)

Applicant's or agent's file reference 13/107pct		IMPORTANT NOTIFICATION	
International application No. PCT/CA03/00030	International filing date (day/month/year) 14 January 2003 (14.01.2003)	Priority date (day/month/year) 01 February 2002 (01.02.2002)	
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GmbH ET AL			
Title of the invention HEPATTIS C INHIBITOR TRI-PEPTIDES			

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

[X] was transmitted to the International Bureau on 31 January 2003 (31.01.2003).

[] has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:

[] because the necessary national security clearance has not yet been obtained.

[] because (reason to be specified):

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

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

From the RECEIVING OFFICE

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

PCT

NOTIFICATION CONCERNING PAYMENT OF PRESCRIBED FEES

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

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

Applicant's or agent's file reference
13/107pct

PAYMENT DUE See item 3 for time limits

International Application No.
PCT/CA03/00030

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

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

Applicant BOEHRINGER INGELHEIM INTERNATIONAL GmbH ET AL

1. The applicant is hereby notified that this receiving Office has received:
☐ the payment of all the prescribed fees, and ☒ an overpayment, which will be refunded in due course.
☐ no or insufficient payment of the prescribed fees and the applicant is hereby invited to pay the balance due, as summarized under item 2, within the time limit(s) indicated under item 3.
2. Fees and payment calculation:

<u>\$ 200.00</u> -	<u>\$ 2,917.00</u> =	<u>\$2,717.00</u>
Total fees payable	Amount paid	Balance to be refunded.

☐ The details of the calculation are given in the Annex.
3. Time limit(s) for payment and amount(s) payable (Rules 14.1, 15.4 and 16.1(f)):
☐ within ONE MONTH from the date of receipt of the international application (for the transmittal fee (if any), the search fee, the basic fee and the designation fee). The amount payable for each fee is the amount applicable on the date of receipt of the international application.
☐ within ONE YEAR from the priority date (only for the designation fee and only if this time limit expires later than the above time limit).
 - If the designation fee is paid within one month from the date of receipt of the international application, the amount payable is the amount applicable on that date of receipt.
 - If the designation fee is paid within one year from the priority date but later than one month from the date of receipt of the international application, the amount payable is the amount applicable on the date of payment. The receiving Office should be consulted for the applicable amount.
☐ within 16 MONTHS from the priority date (only for the fee for priority document). The applicant's attention is drawn to the fact the request made by the applicant under Rule 17.1(b) will be considered not to have been made unless the fee is paid within that time limit.
4. Additional observations (if necessary):
☐ The search copy will not be transmitted to the International Searching Authority until the search fee is paid (therefore the start of the international search will be delayed) (Rule 23.1(a) and (b)).

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Authorized Officer

Carole Millaire
 Telephone no. (819) 994-6587

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PATENT COOPERATION TREATY

From the RECEIVING OFFICE

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

Applicant's or agent's file reference 13/107pct	REPLY DUE See paragraph 1 below
International Application No. PCT/CA03/00030	International filing date <i>(day/month/year)</i> 14 January 2003 (14.01.2003)
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GmbH ET AL	

1. ☐ REPLY DUE within ____ months/days from the above date of mailing ☐ NO REPLY DUE , however, see below _____ ☒ IMPORTANT COMMUNICATION ☐ INFORMATION ONLY	
2. COMMUNICATION: RE: PRIORITY DOCUMENT CA (2,369,970) 01-02-2002 PLEASE BE ADVISED THAT THE PRIORITY DOCUMENT IN THE ABOVE INTERNATIONAL APPLICATION HAS NOT YET BEEN RECEIVED. THE DOCUMENT SHOULD BE SENT TO THE WORLD INTELLECTUAL PROPERTY ORGANIZATION (WIPO), PCT DIVISION, 34, CHEMIN DES COLOMBETTES, 1211 GENEVA 20, SWITZERLAND BEFORE THE END OF THE 16th MONTH FROM THE PRIORITY DATE. WITHIN THE SAME TIME LIMIT, THE DOCUMENT CAN ALSO BE SENT TO THIS OFFICE FOR TRANSMITTAL TO WIPO. IF FURTHER INFORMATION IS REQUIRED, PLEASE FEEL FREE TO COMMUNICATE WITH THIS OFFICE.	

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

13/107pct

Original (for SUBMISSION) - printed on 19.12.2002 01:23:19 PM

0	For receiving Office use only	
0-1	International Application No.	PCT/CA 03 / 00030
0-2	International Filing Date	14 JANUARY 2003 14.01.03
0-3	Name of receiving Office and "PCT International Application"	RO/CA
0-4	Form - PCT/ROH01 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.92 (updated 01.06.2002)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	Canadian Patent Office (RO/CA)
0-7	Applicant's or agent's file reference	13/107pct
I	Title of invention	HEPATITIS C INHIBITOR TRI-PEPTIDES
II	Applicant	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	BOEHRINGER INGELHEIM INTERNATIONAL GmbH
II-5	Address:	Binger Strasse 173 D- 55216 Ingelheim Germany
II-6	State of nationality	DE
II-7	State of residence	DE
II-8	Telephone No.	011 49 6132 77 2326
II-9	Facsimile No.	011 49 6132 77 3000
III-1	Applicant and/or inventor	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	LLINAS-BRUNET, Montse
III-1-5	Address:	2100 Cunard Street Laval, Québec H7S 2G5 Canada
III-1-6	State of nationality	CA
III-1-7	State of residence	CA

55

2/5

PCT REQUEST

13/107pat

Original (for SUBMISSION) - printed on 19.12.2002 01:23:19 PM

III-2	Applicant and/or inventor	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	BAILEY, Murray, D.
III-2-5	Address:	2100 Cunard Street Laval, Québec H7S 2G5 Canada
III-2-6	State of nationality	CA
III-2-7	State of residence	CA
III-3	Applicant and/or inventor	
III-3-1	This person is:	applicant and inventor
III-3-2	Applicant for	US only
III-3-4	Name (LAST, First)	GHIRO, Elise
III-3-5	Address:	2100 Cunard Street Laval, Québec H7S 2G5 Canada
III-3-6	State of nationality	CA
III-3-7	State of residence	CA
IV-1	Agent or common representative; or address for correspondence The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	BERNIER, Louise, G.
IV-1-2	Address:	Boehringer Ingelheim (Canada) Ltd. 2100 Cunard Street Laval, Québec H7S 2G5 Canada
IV-1-3	Telephone No.	(450) 682-4640
IV-1-4	Facsimile No.	(450) 682-8434
IV-1-5	e-mail	lbernier@lav.boehringer-ingelheim.ca

PCT REQUEST

13/107pct

Original (for SUBMISSION) - printed on 19.12.2002 01:23:19 PM

V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GE GM KE LS MW NE SD SL SZ TE UG ZM ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KE MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE BG CH LI CY CZ DE DK EE ES FI FR GB GR IE IT LU MC NL PT SE SK TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BE CA CH LI CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MY NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
V-3	Precautionary Designation Statement In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-3 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-4	Exclusion(s) from precautionary designations	NONE
VI-1	Priority claim of earlier national application	
VI-1-1	Filing date	01 February 2002 (01.02.2002)
VI-1-2	Number	2,369,970
VI-1-3	Country	CA
VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)

PROCA

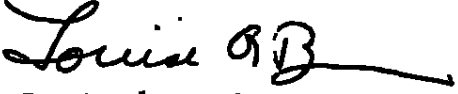
CANCELLED BY RO/CA

4/5

PCT REQUEST

19/107pct

Original (for SUBMISSION) - printed on 19.12.2002 01:23:19 PM

VIII	Declarations	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	
IX	Check list	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	5	-
IX-2	Description (excluding sequence listing part)	35	-
IX-3	Claims	3	-
IX-4	Abstract	1	EEABST00.TXT
IX-5	Drawings	0	-
IX-7a	Sub-total number of sheets	44	
IX-6	Sequence listing part of description	1	-
IX-7	TOTAL	45	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-13	Priority document(s)	Item(s) VI-1 ✓	-
IX-16	Sequence listing in computer readable form:		
IX-16 (f)	copy submitted for the purposes of international search under Rule 18ter only (and not as part of the international application)	-	1 Diskette
IX-17	PCT-EASY diskette	-	Diskette
IX-19	Figure of the drawings which should accompany the abstract	—	
IX-20	Language of filing of the international application	English	
X-1	Signature of applicant, agent or common representative		
X-1-1	Name (LAST, First)	BERNIER, Louise, G.	

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	14 JANUARY 2003 14.01.03
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	✓
10-3	Corrected date of actual receipt due to later, but timely received papers or drawings completing the purported international application	

PCT REQUEST

13/107pct

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10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-8	Transmitted of search copy delayed until search fee is paid	✓

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	
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PCT (ANNEX - FEE CALCULATION SHEET)

13/107pct

Original (for SUBMISSION) - printed on 19.12.2002 01:23:19 PM

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

0	For receiving Office use only		
0-1	International Application No.	PCT/CA 03 / 00030	
0-2	Date stamp of the receiving Office	14 JANUARY 2003 14.01.03	
0-4	Form - PCT/RO/101 (Annex)		
0-4-1	PCT Fee Calculation Sheet Prepared using	PCT-EASY Version 2.92 (updated 01.06.2002)	
0-9	Applicant's or agent's file reference	13/107pct	
2	Applicant	BOEHRINGER INGELHEIM INTERNATIONAL GmbH, et al.	
12	Calculation of prescribed fees	fee amount/multiplier	Total amounts (CAD)
12-1	Transmittal fee 7	⇒	200 4
12-2-1	Search fee 8	⇒	1,350 1440
12-2-2	International search to be carried out by	EP	
12-3	International fee		
	Basic fee (first 30 sheets) b1	639	
12-4	Remaining sheets	15	
12-5	Additional amount (X)	15	
12-6	Total additional amount b2	225	
12-7	b1 + b2 = B	864	
12-8	Designation fees		
	Number of designations contained in international application	94	
12-9	Number of designation fees payable (maximum 5)	5	
12-10	Amount of designation fee (X)	138	
12-11	Total designation fees D	690	
12-12	PCT-EASY fee reduction R	-197	
12-13	Total international fee (B+D-R) I	⇒	1,357 4
12-17	TOTAL FEES PAYABLE (T+B+I+P)	⇒	2,917 2997
12-19	Mode of payment	authorization to charge deposit account	
12-20	Deposit account instructions		
	The receiving Office:	Canadian Patent Office (RO/CA)	
12-20-1	Authorization to charge the total fees indicated above.	✓	
12-20-2	Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.	✓	
12-20-3	Authorization to charge the fee for priority document.	✓	
12-21	Deposit account No.	cc1127	
12-22	Date	19 December 2002 (19.12.2002)	

PCT (ANNEX - FEE CALCULATION SHEET)

13/107pct

Original (for SUBMISSION) - printed on 19.12.2002 01:23:19 PM

12-23	Name and signature	BERNIER, Louise, G. <i>Louise G.B.</i>
-------	--------------------	---

VALIDATION LOG AND REMARKS

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

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To: BOEHRINGER INGELHEIM (CANADA) LTD. 2100 Cunard Street Laval, Quebec Canada H7S 2G5 Attention to BERNIER, Louise	PCT NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE (PCT Rule 20.5(c))
	Date of mailing (day/month/year) 07 February 2003 (07-02-2003)

Applicant's or agent's file reference 037107 PCT-2		IMPORTANT NOTIFICATION	
International application No. PCT/CA03/00091	International filing date (day/month/year) 24 January 2003 (24-01-2003)	Priority date (day/month/year) 01 February 2002 (01-02-2002)	
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH ET AL			
Title of the invention HEPATITIS C INHIBITOR TRI-PEPTIDES			

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.	
2. The applicant is further notified that the record copy of the international application:	
☒ [X] was transmitted to the International Bureau on <u>31 January 2003 (31-01-2003)</u> .	
☐ [] has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:	
☐ [] because the necessary national security clearance has not yet been obtained.	
☐ [] because (reason to be specified):	
* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).	
Name and mailing address of the Receiving Office Commissioner of Patents Canadian Receiving Office Box PCT, Ottawa/Hull K1A 0C9 Facsimile No. (819) 953-9538	Authorized Officer Chantal Hebert (819) 997-6295

PATENT COOPERATION TREATY

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From the RECEIVING OFFICE

To:
BOEHRINGER INGELHEIM (CANADA)
LTD.
2100 Cunard Street
Laval, Quebec
Canada H7S 2G5
Attention to BERNIER, Louise

PCT

**INVITATION TO CORRECT DEFECTS IN
THE INTERNATIONAL APPLICATION**

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

deadline March 7/03

Date of mailing (day/month/year) **07 February 2003 (07-02-2003)**

Applicant's or agent's file reference
13/107 PCT-2

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

International application No.
PCT/CA03/00091

International filing date **24 January 2003 (24-01-2003)**
(day/month/year)

Applicant **BOEHRINGER INGELHEIM INTERNATIONAL GMBH ET AL**

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

☒ Annex A

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

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

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

☐ Annex A

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

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

Additional observations (if necessary):

HOW TO CORRECT THE DEFECTS?

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

ATTENTION

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

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

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

Authorized Officer

Chantal Hebert (819) 997-6295

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

1. As to signature* of the international application (Rules 4.15 and 90.4), the request:
 - a. ☐ is not signed.
 - b. ☐ is not signed by all the applicants.
 - c. ☐ is not accompanied by the statement referred to in the check list in Box No. VIII of the request explaining the lack of the signature of an applicant for the designation of the United States of America.
 - d. ☒ is signed by what appears to be an agent/common representative but:
 - ☐ the international application is not accompanied by a power of attorney appointing him. *
 - ☐ the power of attorney accompanying the international application was not signed by all the applicants.
 - e. ☒ other (specify): I'm returning to you the power of attorney received at time of filing because it was not signed.

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

2. As to indications concerning the applicant, the request (Rules 4.4 and 4.5):
 - a. ☐ does not properly indicate the applicant's name (specify):
 - b. ☐ does not indicate the applicant's address.
 - c. ☐ does not properly indicate the applicant's address (specify):
 - d. ☐ does not indicate the applicant's nationality.
 - e. ☐ does not indicate the applicant's residence.
 - f. ☐ other (specify):

3. As to the language of certain elements of the international application, other than the description and claims (Rules 12.1(c) and 26.3ter(a) and (c)):
 - a. ☐ the request is not in a language of publication accepted by this receiving Office; (the) language(s) accepted by this receiving Office is/are:
 - b. ☐ the text matter of the drawings is not in the language in which the international application is to be published, which is:
 - c. ☐ the abstract is not in the language in which the international application is to be published, which is:

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

5. As to the abstract (Rule 8):
 - ☐ the international application does not contain an abstract.



Boehringer
Ingelheim

Via the Regional Office of Montreal

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

c/o Chantal Hébert

Boehringer Ingelheim
(Canada) Ltd.

Research and Development
Patents

February 13, 2003

**Re.: Invitation to correct defects in the International Application
PCT/CA03/00091 filed January 24, 2003 entitled:
"Hepatitis C Inhibitor Tri-Peptides"
Our case: 13/107 pct2**

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

Dear Ms. Hébert:

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

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

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

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

Respectfully submitted,

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

Enclosures: Duly signed Power of Attorney

/ra

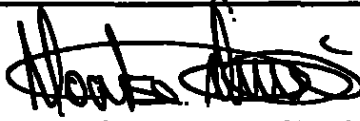
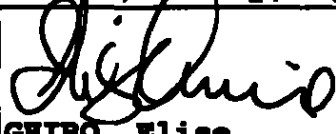
65

PCT POWER OF ATTORNEY

1/2

13/107 PCT-2

Printed on 22.01.2003 04:09:35 PM

0-1	PCT Power of Attorney (for an International application filed under the Patent Cooperation Treaty) (PCT Rule 90.4)	
0-1-1	Prepared using	PCT-EASY Version 2.92 (updated 01.06.2002)
1	The undersigned applicant(s)	BOEHRINGER INGELHEIM INTERNATIONAL GmbH; LLINAS-BRUNET, Montse; BAILEY, Murray, D.; GHIRO, Elise
1-1-1	hereby appoints (appoint) the following person	BERNIER, Louise, G. Boehringer Ingelheim (Canada) Ltd. 2100 Cunard Street Laval, Québec H7S 2G5 Canada
1-2	as	agent
1-3	to represent the undersigned before	all the competent International Authorities
1-4	In connection with the international application identified below:	
1-4-1	Title of the invention	HEPATITIS C INHIBITOR TRI-PEPTIDES
1-4-2	Applicant's or agent's file reference	13/107 PCT-2
1-4-3	International application number (if already available)	
1-4-4	filed with the following Office as receiving Office	Canadian Patent Office (RO/CA)
1-5	and to make or receive payments on behalf of the undersigned.	
2-1	Signature of applicant	 
2-1-1	Name	BOEHRINGER INGELHEIM INTERNATIONAL GmbH
2-1-2	Name of signatory	Heinz Hamann / Heinz-Gerd Klaes
2-1-3	Capacity	Director Corp.Div.Patent/Head of Patent
2-2	Signature of applicant	
2-2-1	Name	LLINAS-BRUNET, Montse
2-3	Signature of applicant	
2-3-1	Name	BAILEY, Murray, D
2-4	Signature of applicant	
2-4-1	Name	GHIRO, Elise
3	Date	22 January 2003 (22.01.2003)

PATENT COOPERATION TREATY

66

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

From the INTERNATIONAL BUREAU

To:

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

Date of mailing (day/month/year) 03 March 2003 (03.03.03)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 13/107 PCT-2	
International application No. PCT/CA03/00091	International filing date (day/month/year) 24 January 2003 (24.01.03)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 01 February 2002 (01.02.02)
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al	

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

<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
01 Febr 2002 (01.02.02)	2,389,970	CA	17 Febr 2003 (17.02.03)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338.89.95	Authorized officer T. Vernhes (Fax 338-8995) Telephone No. (41-22) 338 8133
--	---

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

IPEA/ _____

PCT

CHAPTER II

DEMAND

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

For International Preliminary Examining Authority use only	
Identification of IPEA	Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION	
Applicant's or agent's file reference 13/107 pct-2	
International application No. PCT/CA03/00091	International filing date (day/month/year) 24/01/2003
(Earliest) Priority date (day/month/year) 01/02/2002	
Title of invention HEPATITIS C INHIBITOR TRI-PEPTIDES	
Box No. II APPLICANT(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
BOEHRINGER INGELHEIM INTERNATIONAL GMBH	
Binger Strasse 173	
D-55216 Ingelheim	
GERMANY	
Telephone No. 011 49 6132 77 2326	
Facsimile No. 011 49 6132 77 3000	
Teleprinter No.	
Applicant's registration No. with the Office	
State (that is, country) of nationality: DE	State (that is, country) of residence: DE
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
LLINAS-BRUNET, Montse	
2100 Cunard Street	
Laval, Québec H7S 2G5	
CANADA	
State (that is, country) of nationality: CA	State (that is, country) of residence: CA
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
BAILEY, Murray, D.	
2100 Cunard Street	
Laval, Québec H7S 2G5	
CANADA	
State (that is, country) of nationality: CA	State (that is, country) of residence: CA
☒ Further applicants are indicated on a continuation sheet.	

Sheet No. 2.

International application No.
PCT/CA03/00091

Continuation of Box No. II APPLICANT(S)

*If none of the following sub-boxes is used, this sheet should not be included in the demand.*Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GHIRO, Elise
2100 Cunard Street
Laval, Québec H7S 2G5
CANADA

State *(that is, country)* of nationality:

CA

State *(that is, country)* of residence:

CA

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

Further applicants are indicated on another continuation sheet.

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

The following person is ☒ agent ☐ common representative
and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.
☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.
☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)</i> BERNIER, Louise, G. c/o Boehringer Ingelheim (Canada) Ltd. 2100 Cunard Street Laval, Québec H7S 2G5 CANADA	Telephone No. (450) 682-4640
	Facsimile No. (450) 682-8434
	Teleprinter No.
	Agent's registration No. with the Office

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

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

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

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

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

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

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

Language for the purposes of international preliminary examination: English.....

☒	which is the language in which the international application was filed.
☐	which is the language of a translation furnished for the purposes of international search.
☐	which is the language of publication of the international application.
☐	which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*
excluding the following States which the applicant wishes not to elect:

Box No. VI CHECK LIST

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

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

For International Preliminary Examining Authority use only

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

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

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

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

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

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

Louise G. BERNIER
Agent for Applicant

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

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

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

☐ The applicant has been informed accordingly.

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

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

For International Bureau use only

Demand received from IPEA on:

PCT

FEE CALCULATION SHEET

Annex to the Demand

International application No. PCT/CA03/00091		For International Preliminary Examining Authority use only	
Applicant's or agent's file reference 13/107 pct-2		Date stamp of the IPEA	
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH			
CALCULATION OF PRESCRIBED FEES			
1. Preliminary examination fee		1530.00 EURO	P
2. Handling fee (<i>Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.</i>)		159.00 EURO	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box		1689.00 EURO	
		TOTAL	
MODE OF PAYMENT			
☐ authorization to charge deposit account with the IPEA (see below)	☐ cash		
☐ cheque	☐ revenue stamps		
☐ postal money order	☐ coupons		
☒ bank draft	☐ other (<i>specify</i>):		
AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT (<i>This mode of payment may not be available at all IPEAs</i>)			
☐ Authorization to charge the total fees indicated above.		IPEA/	
☐ (<i>This check-box may be marked only if the conditions for deposit accounts of the IPEA so permit</i>) Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.		Deposit Account No.:	
		Date:	
		Name:	
		Signature:	

PATENT COOPERATION TREATY

AUG 28 2003

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITYAgent
PCT

To:

BERNIER, Louise G.
BOEHRINGER INGELHEIM (CANADA) LTD.
2100 Cunard Street
Laval Quebec H7S 2G5
CANADA
SCHUPO updated
Scanned / sent to GmbH
Scanned / sent to BIPI (with encl.)

ENP due: _____

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

22-08-2003

Applicant's or agent's file reference

13/107 PCT-2

IMPORTANT NOTIFICATION

International application No.

PCT/CA 03/ 00091

International filing date (day/month/year)

24/01/2003

Priority date (day/month/year)

01/02/2002

Applicant

BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al.

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

11/08/2003

2. This date of receipt is:

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

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

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

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

Name and mailing address of the IPBA/

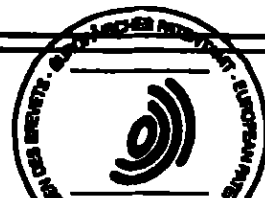


European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer

FERRO VASCONCELOS M

Tel. no. 7005



PATENT COOPERATION TREATY

Corrected Version / Version Corrigée
PCT

From the INTERNATIONAL BUREAU

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

(PCT Rule 47.1(c), first sentence)

To:

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

PATENT DEPARTMENT

Date of mailing (day/month/year) 07 August 2003 (07.08.03)		Scanned / sent to GmbH Scanned / sent to BURL (with encl.)		007 9 3 28/03	
Applicant's or agent's file reference 13/107 PCT-2		ENP due: _____		Agent: _____ IMPORTANT NOTICE	
International application No. PCT/CA03/00091		International filing date (day/month/year) 24 January 2003 (24.01.03)		Priority date (day/month/year) 01 February 2002 (01.02.02)	
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH					

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 30, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

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

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

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

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

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

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 07 August 2003 (07.08.03) under No. 03/064416

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

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

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

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

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

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

Authorized officer

Judith Zahra

PATENT COOPERATION TREATY

PATENT DEPARTMENT

SEP 19 2003

PCT

From the INTERNATIONAL BUREAU

Agent:

INFORMATION CONCERNING ELECTED
OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:

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

Date of mailing (day/month/year) 08 September 2003 (08.09.03)		
Applicant's or agent's file reference 13/107 PCT-2		
IMPORTANT INFORMATION		
International application No. PCT/CA03/00091	International filing date (day/month/year) 24 January 2003 (24.01.03)	Priority date (day/month/year) 01 February 2002 (01.02.02)
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al		

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

EP : AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,
SK, TR
National : BG, CA, CN, DE, GB, IL, JP, 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, SE, 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, AU, AZ, BA, BB, BR, BY, BZ, CH, CO, CR, CU, CZ, DK, DM, DZ, EC,
EE, ES, FI, GD, GE, GH, GM, HR, HU, ID, IN, IS, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MW, MX, MZ, NZ, OM, PH, PT, SD, SE, SG, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VG, VN,
YU, ZA, ZM, ZW

3. The applicant is reminded that he must enter the "national phase" before the expiration of 30 months from the priority date before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 36(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(a) and Rule 74.1).

Some offices have fixed time limits expiring later than the above-mentioned time limits. 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.

ESHUPG-updated
Scanned / sent to GmbH
Scanned / sent to BIPF (with email)
ENP due Aug 1, 2004

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338 89 85	Authorized officer: Catherine TOLU (Fax 338-8995) Telephone No. (41-22) 338 88 66
--	---

PATENT COOPERATION TREATY

PATENT DEPARTMENT

From the INTERNATIONAL SEARCHING AUTHORITY

MAR 04 2003
PCT
Agent

To:

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

COMMUNICATION IN CASES FOR WHICH
NO OTHER FORM IS APPLICABLE

Date of mailing
(day/month/year)

28/02/2003

Applicant's or agent's file reference

IS/107 PCT-2

REPLY DUE

See paragraph 1 below

International application No.

PCT/CA 03/ 00091

International filing date
(day/month/year)

24/01/2003

Applicant

BOEHRINGER INGELHEIM INTERNATIONAL GMBH

1. ☐ REPLY DUE within _____ 30/31 days from the above date of mailing

☒ NO REPLY DUE

2. COMMUNICATION:

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

Although the time limit for entering the national phase before designated offices under Article 22(1) PCT and elected offices under Article 39(1) PCT has, with effect from 1 April 2002 (see PCT Gazette 44/2001 Section IV) been set at 30 months from the priority date (before the EPO the time limit is 31 months from the priority date - see Rule 107 EPC as amended with effect from 2 January 2002 - OJ EPO 8-9/2001, 373) not all PCT contracting states have yet made the necessary changes to their national laws and will for the time being continue to require entry to the national phase at 20/21 months from the priority date if a demand has not been filed before the end of 19 months from the priority date - see PCT Gazette/PCT Newsletter available on the WIPO internet site at <http://www.wipo.int/pat/en/index.html> for an up to date list of the applicable time limits.

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

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

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

Finally, applicants are reminded that as of 3 January 2002 a rationalised PCT II procedure may apply, see OJ EPO 11/2001, 639 and that the EPO as ISA will not carry out international search on an application which relates to no more than a method of doing business, see OJ EPO 10/2001, 482. Applicants should also bear in mind the restriction of the EPO's competence as ISA and IPEA in certain technical fields in respect of certain international applications, see OJ EPO 1/2002, 52 and PCT Newsletter 1/2002 for further details.

Name and mailing address of the International Searching Authority



European Patent Office, P.B. 6818 Patentlaan 2
NL-6220 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

ISA/EP



P.B.5018 - Patentamt 2
2250 HV Rüschlik (ZH)
T (070) 3 40 30 40
FAX (070) 3 40 30 16

Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

PATENT DEPARTMENT

Generalktion 1

Directorate General 1

Direction générale 1

JUN 15 2004

Agent: _____

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



epoline® Customer Services

Tel.: +31 (0)70 340 45 00

~~ENP updated~~
~~sent / sent to GmbH~~
~~sent / sent to BIP (with encl)~~
ENP due: August 1/2004

Date
14.06.04

Reference	Application No./Patent No. 03700745.7 - 2117 PCT/CA0300091	13:67
Applicant/Proprietor Boehringer Ingelheim International GmbH		

Entry into the European phase before the European Patent Office

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

1. The above-mentioned international patent application has been given European application No. 03700745.7.
2. Applicants without a residence or their principal place of business in an EPC contracting state may themselves initiate European processing of their international applications, provided they do so before expiry of the 31st month from the priority date (see also point 6 below).

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

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

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

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



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5. To enter the European phase before the EPO, the following acts must be performed.
(N.B.: Failure validly to do so will entail loss of rights or other adverse legal consequences.)
- 5.1 If the EPO is acting as designated or elected Office (Arts. 22(1)(3) and 39(1) PCT respectively), applicants must, within 31 months from the date of filing or (where applicable) the earliest priority date:
- a) Supply a translation of the international application into an EPO official language, if the International Bureau did not publish the application in such a language (Art. 22(1) PCT and Rule 107(1)(a) EPC).
If the translation is not filed in time, the international application is deemed withdrawn before the EPO (Rule 108(1) EPC).
This loss of rights is deemed not to have occurred if the translation is then filed within a two-month grace period as from notification of an EPO communication, provided a surcharge is paid at the same time (Rule 108(3) EPC).
 - b) Pay the national basic fee (EUR 125,00) and, where a supplementary European search report has to be drawn up, the search fee (EUR 690,00 ; Rule 107(1)(c) and (e) EPC).
 - c) If the time limit under Article 79(2) EPC expires before the 31-month time limit, pay the designation fee (EUR 75,00) for each contracting state designated (Rule 107(1)(d) EPC).
 - d) If the time limit under Article 94(2) EPC expires before the 31-month time limit, file the written request for examination and pay the examination fee (EUR 1430,00 ; Rule 107(1)(f) EPC).
 - e) Pay the third-year renewal fee (EUR 380,00) if it falls due before expiry of the 31-month time limit (Rule 107(1)(g) EPC).
- If the fees under (b) to (d) above are not paid in time, or the written request for examination is not filed in time, the international application is deemed withdrawn before the EPO, or the contracting-state designation(s) in question is (are) deemed withdrawn (Rule 108(1) and (2) EPC). However, the fees may still be validly paid within a two-month grace period as from notification of an EPO communication, provided the necessary surcharges are paid at the same time (Rule 108(3) EPC). For the renewal fee under (e) above, the grace period is six months from the fee's due date (Article 86(2) EPC).
- 5.2 If the application documents on which the European grant procedure is to be based comprise more than ten claims, a claims fee is payable within the 31-month time limit under Rule 107(1) EPC for the eleventh and each subsequent claim (Rule 110(1) EPC). The fee can however still be paid within a one-month grace period as from notification of an EPO communication pointing out the failure to pay (Rule 110(2) EPC).
6. If the applicant had a representative during the application's international phase, the present notes will be sent to the representative, asking him to inform the applicant accordingly.

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



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

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

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

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

RECEIVING SECTION



PATENT COOPERATION TREATY

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From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

To:
BOEHRINGER INGELHEIM (CANADA) LTD.
Attn. Bernier, Louise G.
2100 Cunard Street
Laval Quebec H7S 2G5
CANADA
SCHUPO updated, 2-
Scanned / sent to GmbH
Scanned / sent to BIPI (with encl.)
ENP due: _____

Amendments (PCT Rule 17) PATENT DEPARTMENT
under Art. 19 : MAY 15 2003
JULY 14 / 2003

Date of mailing (day/month/year)	Agent: _____ 14/05/2003
-------------------------------------	----------------------------

Applicant's or agent's file reference
13/107 PCT-2

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/CA 03/ 00091

International filing date
(day/month/year) 24/01/2003

Applicant
BOEHRINGER INGELHEIM INTERNATIONAL GMBH

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 46):

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 Offices.

☐ 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 80bis.1 and 80bis.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 18 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 Patentlaan 2
NL-2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx. 31 851 spo nl,
Fax (+31-70) 340-3018

Authorized officer

Eva Boháčová

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 13/107 PCT-2	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/CA 03/ 00091	International filing date (day/month/year) 24/01/2003	(Earliest) Priority Date (day/month/year) 01/02/2002
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH		

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

This International Search Report consists of a total of 3 sheets.

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

1. Basis of the report

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

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

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

☐ contained in the international application in written form.

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

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

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

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

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

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

4. With regard to the title,

☐ the text is approved as submitted by the applicant.

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

HETEROCYCLIC TRIPEPTIDES AS HEPATITIS C INHIBITORS

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.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/00091

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D417/14 A61K31/47 A61P31/00 C07K4/00

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 A61K C07K

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

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

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 09543 A (BOEHRINGER INGELHEIM CA LTD ;GOUDREAU NATHALIE (CA); GHIRO ELISE ()) 24 February 2000 (2000-02-24) cited in the application p. 90, cpd. 35p; p. 91, cpd. 333 claim 1	1-31
A	WO 00 59929 A (BOEHRINGER INGELHEIM CA LTD ;GOUDREAU NATHALIE (CA); GHIRO ELISE ()) 12 October 2000 (2000-10-12) cited in the application the whole document	1-31

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

8 May 2003

Date of mailing of the international search report

14/05/2003

Name and mailing address of the ISA

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Authorized officer

Fritz, M

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 03/00091

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0009543	A	24-02-2000	AU	5273199 A	06-03-2000
			BG	105232 A	30-11-2001
			BR	9913646 A	05-06-2001
			CA	2338946 A1	24-02-2000
			WO	0009543 A2	24-02-2000
			CN	1323316 T	21-11-2001
			CZ	20010516 A3	15-08-2001
			EE	200100081 A	15-08-2002
			EP	1105413 A2	13-06-2001
			HR	20010102 A1	28-02-2002
			HU	0105144 A2	29-04-2002
			JP	2002522554 T	23-07-2002
			NO	20010683 A	02-04-2001
			PL	346626 A1	25-02-2002
			SK	2062001 A3	08-10-2001
			TR	200100432 T2	21-09-2001
			TR	200200129 T2	21-06-2002
			US	6534523 B1	18-03-2003
			US	6323180 B1	27-11-2001
			US	6268207 B1	31-07-2001
			US	6329379 B1	11-12-2001
			US	6329417 B1	11-12-2001
			US	2002016442 A1	07-02-2002
			US	2002037998 A1	28-03-2002
WO 0059929	A	12-10-2000	AU	3548000 A	23-10-2000
			BG	105970 A	31-05-2002
			BR	0009599 A	15-01-2002
			WO	0059929 A1	12-10-2000
			CA	2367127 A1	12-10-2000
			CN	1346365 T	24-04-2002
			CZ	20013467 A3	12-06-2002
			EE	200100516 A	16-12-2002
			EP	1169339 A1	09-01-2002
			HR	20010720 A1	31-12-2002
			JP	2002542160 T	10-12-2002
			NO	20014857 A	31-10-2001
			PL	350855 A1	10-02-2003
			SK	14072001 A3	05-03-2002
			TR	200102878 T2	21-01-2002

PATENT COOPERATION TREATY

PATENT DEPARTMENT
SEP 08 2003

Agent: _____

PCT

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

SCHUBO updated 7
Scanned / sent to GmbH
Scanned / sent to GPT (intensity)
ENP due:

Date of mailing
(day/month/year)

02/09/2003

Applicant's or agent's file reference

13/107 PCT-2

IMPORTANT NOTIFICATION

International application No.

PCT/CA 03/ 00091

International filing date (day/month/year)

24/01/2003

Priority date (day/month/year)

01/02/2002

Applicant

BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al.

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

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/D01).

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

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

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

Name and mailing address of the IPEA/



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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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

Applicant's or agent's file reference 13/107 PCT-2	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/CA 03/ 00091	International filing date (day/month/year) 24/01/2003	Priority date (day/month/year) 01/02/2002
International Patent Classification (IPC) or national classification and IPC C07D417/14		
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al.		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2.	This REPORT consists of a total of <u> 2 </u> sheets, including this cover sheet. ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consists of a total of <u> </u> 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 11/08/2003	Date of completion of this report 28/08/2003
Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer TZSCHOPPE D A Tel. (+49-89) 2399 2828



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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No.PCT/ CA 03/ 00091

I. Basis of the report

The basis of this international preliminary examination is the application as originally filed.

V. Reasoned statement under Rule 66.2(a)(II) with regard to novelty, inventive step or industrial applicability

In light of the documents cited in the international search report, it is considered that the invention as defined in the claims meets the criteria mentioned in Article 33 (1) PCT, i.e. it appears to be novel, to involve an inventive step and to be industrially applicable.

Traducción

36

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

Referencia del expediente del agente o solicitante 13/107 PCT-2	PARA ACTUACIONES ADICIONALES	Ver notificación de Transmisión del Reporte de Examen Preliminar (Formato PCT/IPEA410)
Solicitud Internacional No. PCT/CA03/00081	Fecha de presentación Internacional (día/mes/año) 24/01/2003	Fecha de prioridad (día/mes/año) 01/02/2002
Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC C07D 417/14		
Solicitante BOEHRINGER INGELHEIM INTERNATIONAL GMBH et al.		

1. Este informe de examen preliminar internacional ha sido preparado por esta Administración encargada del Examen Preliminar Internacional y se envía al solicitante en concordancia con el artículo 36.

2. Este INFORME consta de una total de 2 hojas, incluyendo la portada

☐ Este informe también se acompaña de ANEXOS, esto es documentos de la descripción, reivindicaciones y/o dibujos que han sido corregidos y que son la base de este informe y/o documentos que contienen rectificaciones realizadas ante esta Administración (Ver Regla 70.16 y Sección 607 de las Instrucciones Administrativas según el PCT)

Estos anexos constan de un total de ____ hojas.

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

I ☒ Fundamentos del Informe

II ☐ Prioridad

III ☐ No se establece concepto relativo a la novedad, nivel inventivo y aplicación industrial

IV ☐ Carencia de unidad de la invención

V ☒ Declaración razonada según el artículo 35(2), en relación con la novedad, nivel inventivo o aplicación industrial; citaciones y explicaciones que soportan dicha declaración

VI ☐ Ciertos documentos citados

VII ☐ Ciertos defectos de la solicitud internacional

VIII ☐ Ciertas observaciones respecto a la solicitud internacional

Fecha de presentación de la demanda 11/08/2003	Fecha de terminación de este reporte 28/08/2003
Nombre y dirección de correo de la autoridad encargada del examen preliminar  Oficina Europea de Patentes D-80298 Munich Tel. +49 89 2399 - 0 Tlx 523858 epmu d Fax: +49 89 2399 - 4465	Funcionario Autorizado Tzschoppe D A Teléfono No. (+49-89) 2399 2828

Formato PCT/IPEA409 (portada) (julio 1998)

I. Base del Informe

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

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

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

COLO 09255-841-008-7
PRP/WPCL9255/ID-248

TRIPÉPTIDOS INHIBIDORES DE LA HEPATITIS C

CAMPO DE LA INVENCION

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

FUNDAMENTO DE LA INVENCION

El virus de la hepatitis C (VHC) es el principal
agente etiológico de hepatitis no-A no-B post-transfusión
15 y extrahospitalaria en el mundo. Se estima que más de 200
millones de personas en el mundo están infectadas por el
virus. Un alto porcentaje de portadores llega a
infectarse de manera crónica y muchos progresan hacia una
enfermedad hepática crónica, denominada hepatitis C
20 crónica. Este grupo es por lo tanto de alto riesgo para
enfermedades graves del hígado tales como cirrosis
hepática, carcinoma hepatocelular y enfermedades
hepáticas terminales que conducen a la muerte.

El mecanismo por el cual el VHC establece una
25 persistencia viral y causa una alta tasa de enfermedades
hepáticas crónicas no ha sido elucidado a fondo. No se
conoce cómo interacciona el VHC con el sistema
inmunitario del anfitrión y cómo lo evade. Además,
todavía tiene que establecerse el papel de las respuestas
30 inmunitarias celulares y humorales en la protección
contra la infección y enfermedad por el VHC. Se ha
informado sobre las inmunoglobulinas para la profilaxis
de la hepatitis viral asociada a la transfusión, sin
embargo, el Centro para el Control de Enfermedades (CDC)

no recomienda actualmente el tratamiento con inmunoglobulinas para este propósito. La falta de una respuesta inmunitaria de protección eficaz impide el desarrollo de una vacuna o medidas de profilaxis post-exposición adecuadas, de este modo, en un plazo cercano, las esperanzas están fijadas firmemente en intervenciones antivirales.

Se han llevado a cabo varios estudios clínicos con el objetivo de identificar agentes farmacéuticos capaces de tratar eficazmente la infección por VHC en pacientes que sufren hepatitis C crónica. Estos estudios han implicado el uso de interferón alfa, solo y en combinación con otros agentes antivirales. Tales estudios han mostrado que un número sustancial de los participantes no responde a estas terapias, y de los que responden favorablemente, se encontró que una gran proporción sufría una recaída después de la terminación del tratamiento.

Hasta recientemente, el interferón (IFN) era la única terapia disponible de beneficio probado aprobada en el consultorio para pacientes con hepatitis C crónica. Sin embargo la tasa de respuesta sostenida es baja, y el tratamiento con interferón también induce efectos secundarios intensos (es decir, retinopatía, tiroiditis, pancreatitis aguda, depresión), que disminuyen la calidad de vida de los pacientes tratados. Recientemente, se ha aprobado el uso de interferón en combinación con ribavirina, para pacientes que no responden al IFN solo. Sin embargo, los efectos secundarios causados por el IFN no se mitigan con esta terapia de combinación. Las formas pegiladas de interferón, tales como PEG-Intron® y Pegasys® pueden tratar aparentemente de manera parcial estos efectos secundarios nocivos, pero los fármacos

antivirales continúan todavía como el camino de elección para un tratamiento oral del VHC.

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

El VHC es un virus de ARN de cadena positiva, con envoltura, de la familia de los *Flaviviridae*. El genoma de ARN monocatenario del VHC es de aproximadamente 9500
10 nucleótidos de longitud y tiene una única estructura de lectura abierta (ORF) que codifica una única gran poliproteína de aproximadamente 3000 aminoácidos. En células infectadas, esta poliproteína es escindida en múltiples sitios por proteasas celulares y virales, para
15 producir las proteínas estructurales y no estructurales (NS). En el caso del VHC, la generación de proteínas no estructurales maduras (NS2, NS3, NS4A, NS4B, NS5A y NS5B) se lleva a cabo por dos proteasas virales. La primera, todavía caracterizada pobremente, escinde en la unión
20 NS2-NS3 (en lo sucesivo se hará referencia a ella como proteasa NS2/3); la segunda es una serin-proteasa contenida en la región N-terminal de NS3 (proteasa NS3) y media todas las escisiones hacia abajo de NS3, tanto en *cis*, en el sitio de escisión NS3-NS4A, como en *trans*,
25 para los sitios restantes NS4A-NS4B, NS4B-NS5A, NS5A-NS5B. La proteína NS4A parece servir para múltiples funciones, actuando como un cofactor para la proteasa NS3 y asistiendo posiblemente a la localización en la membrana de NS3 y otros componentes de replicasa viral.
30 La formación del complejo de la proteasa NS3 con NS4A parece necesaria para los eventos de procesamiento, mejorando la eficiencia proteolítica en todos los sitios. La proteína NS3 también manifiesta actividades de nucleósido-trifosfatasa y ARN-helicasa. La NS5B es una

ARN-polimerasa dependiente de ARN que está implicada en la replicación del VHC.

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

Lo siguiente es una lista de solicitudes de patentes publicadas en los últimos años que describen análogos peptídicos inhibidores de la proteasa NS3 del VHC que son estructuralmente diferentes de los compuestos de la
10 presente invención:

GB 2.337.262; JP 10298151; JP 11126861; JP 11292840; JP 2001-103993; US 6.159.938; US 6.187.905; WO 97/43310; WO 98/17679; WO 98/22496; WO 98/46597; WO 98/46630; WO 99/38888; WO 99/50230; WO 99/64442; WO 99/07733; WO
15 99/07734; WO 00/09543; WO 00/09558; WO 00/20400; WO 00/59929; WO 00/31129; WO 01/02424; WO 01/07407; WO 01/16357; WO 01/32691; WO 01/40262; WO 01/58929; WO 01/64678; WO 01/74768; WO 01/77113; WO 01/81325; WO 02/08187; WO 02/08198; WO 02/08244; WO 02/08251; WO
20 02/08256; WO 02/18369; WO 02/60926 y WO 02/79234.

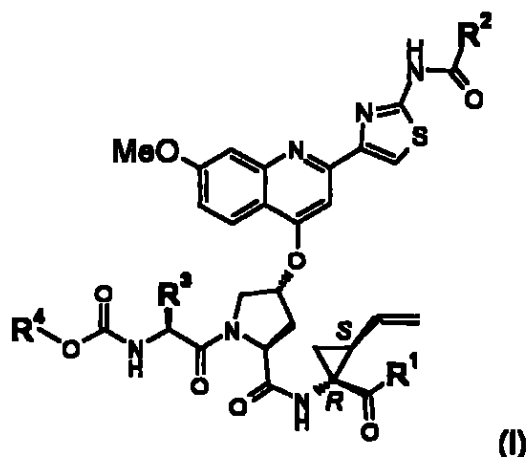
Una ventaja de la presente invención es que proporciona compuestos tripeptídicos que son inhibidores de la proteasa NS3, una enzima esencial para la replicación del virus de la hepatitis C.

Una ventaja adicional de un aspecto de la presente invención reside en el hecho que los compuestos inhiben específicamente la proteasa NS3 y no muestran una actividad inhibidora significativa frente a otras serin-proteasas, tales como la elastasa de leucocitos humanos
25 (HLE), elastasa pancreática porcina (PPE), o quimotripsina pancreática bovina, o cisteín-proteasas tales como la catepsina B de hígado humano (Cat B). Además, los compuestos son activos en cultivo celular y tienen un buen perfil farmacocinético in vivo.

Una ventaja adicional de la presente invención es que proporciona compuestos que son biodisponibles por vía oral en mamíferos.

5 SUMARIO DE LA INVENCION

Está incluido en el alcance de la invención un compuesto de fórmula (1):



en la que R¹ es hidroxilo o NHSO₂R^{1A} en el que R^{1A} es
 10 alquilo(C₁₋₆), cicloalquilo(C₃₋₇) o {alquilo(C₁₋₆)-
 cicloalquilo(C₃₋₇)}, que están todos ellos sustituidos
 opcionalmente desde 1 a 3 veces con halo, ciano, nitro,
 O-alquilo(C₁₋₆), amido, amino o fenilo, o R^{1A} es arilo C₆ o
 C₁₀ que está sustituido opcionalmente desde 1 a 3 veces
 15 con halo, ciano, nitro, alquilo(C₁₋₆), O-alquilo(C₁₋₆),
 amido, amino o fenilo; R² es t-butilo, -CH₂-C(CH₃)₃, o -
 CH₂-ciclopentilo; R³ es t-butilo o ciclohexilo y R⁴ es
 ciclobutilo, ciclopentilo, o ciclohexilo; o una sal
 farmacéuticamente aceptable del mismo.

20 Está incluida dentro del alcance de esta invención
 una composición farmacéutica que comprende una cantidad
 eficaz contra el virus de la hepatitis C de un compuesto
 de fórmula 1, o una sal terapéuticamente aceptable del
 mismo, mezclado con un vehículo o agente auxiliar
 25 farmacéuticamente aceptable.

Conforme a una realización, la composición farmacéutica de esta invención comprende además interferón (pegilado o no), o ribavirina, o uno o más de otros agentes anti-VHC, o cualquier combinación de los
5 anteriores.

Otro aspecto importante de la invención implica un método para tratar una infección viral de hepatitis C en un mamífero, administrando al mamífero una cantidad eficaz contra el virus de la hepatitis C de un compuesto
10 de fórmula 1, una sal terapéuticamente aceptable del mismo, o una composición como se ha descrito anteriormente, solo o en combinación con uno o más de: interferón (pegilado o no), o ribavirina, o uno o más de otros agentes anti-VHC, administrados juntos o
15 separadamente.

Otro aspecto importante de la invención implica un método para impedir una infección viral de hepatitis C en un mamífero, administrando al mamífero una cantidad eficaz contra el virus de la hepatitis C de un compuesto
20 de fórmula 1, una sal terapéuticamente aceptable del mismo, o una composición como se ha descrito anteriormente, solo o en combinación con uno o más de: interferón (pegilado o no), o ribavirina, o uno o más de otros agentes anti-VHC, todos ellos administrados juntos
25 o separadamente.

Está también dentro del alcance de esta invención el uso de un compuesto de fórmula 1, como se ha descrito aquí, para la fabricación de un medicamento para el tratamiento o prevención de una infección viral de
30 hepatitis C.

DESCRIPCION DETALLADA DE LAS REALIZACIONES PREFERIDAS

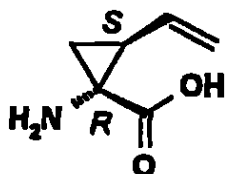
Definiciones

Como se usa aquí, se aplican las siguientes definiciones a menos que se notifique de otra manera:

Con referencia a los casos en los que se use (R) o (S) para designar la configuración absoluta de un
5 sustituyente o centro asimétrico de un compuesto de fórmula 1, la designación se hace en el contexto del compuesto en su conjunto y no en el contexto del sustituyente o centro asimétrico solo.

La designación "P1, P2, y P3", como se usa aquí,
10 hace referencia a la posición de los restos aminoácido comenzando desde el término C extremo de los análogos peptídicos y extendiéndose hacia el término N (es decir, P1 hace referencia a la posición 1 desde el término C, P2: segunda posición desde el término C, etc.) (véase
15 Berger A. y Schechter I., Transactions of the Royal Society London series B257, 249-264 (1970)).

Como se usa aquí, el término "(1R, 2S)-vinil-ACCA" hace referencia a un compuesto de fórmula:



20 es decir, ácido (1R, 2S) 1-amino-2-etenilciclopropilcarboxílico.

El término "alquilo(C₁₋₆)" como se usa aquí, solo o en combinación con otro sustituyente, quiere decir sustituyentes alquilo acíclicos, de cadena lineal o
25 ramificada, que contienen desde 1 hasta 6 átomos de carbono, e incluye por ejemplo, metilo, etilo, propilo, butilo, 1-metiletilo, 1-metilpropilo, 2-metilpropilo, 1,1-dimetiletilo y hexilo. De manera similar, el término "alquilo(C₁₋₆)" quiere decir un alquilo acíclico, de

95

cadena lineal o ramificada, que contiene desde 1 hasta 8 átomos de carbono, por ejemplo, octilo.

El término "cicloalquilo(C₃₋₇)" como se usa aquí, solo o en combinación con otro sustituyente, quiere decir
5 un sustituyente cicloalquilo que contiene desde 3 hasta 7 átomos de carbono, e incluye ciclopropilo, ciclobutilo, ciclopentilo, ciclohexilo y cicloheptilo.

El término "{alquilo(C₁₋₆)-cicloalquilo(C₃₋₇)}" como se usa aquí, quiere decir un radical cicloalquilo que
10 contiene desde 3 hasta 7 átomos de carbono unidos directamente a un radical alquilenos que contiene desde 1 hasta 6 átomos de carbono; por ejemplo, ciclopropilmetilo, ciclopentiletilo, ciclohexilmetilo, ciclohexiletilo y cicloheptilpropilo. En el caso en el
15 que R^{4A} sea {alquilo(C₁₋₆)-cicloalquilo(C₃₋₆)}, este grupo está unido al grupo SO₂ por el alquilo(C₁₋₆) (es decir, la parte de alquilenos).

La expresión "arilo C₆ o C₁₀" como se usa aquí, solo o en combinación con otro radical, quiere decir un grupo
20 monocíclico aromático que contiene 6 átomos de carbono o un grupo bicíclico aromático que contiene 10 átomos de carbono. Por ejemplo, arilo incluye fenilo, 1-naftilo o 2-naftilo.

El término "O-alquilo(C₁₋₆)" como se usa aquí, solo o
25 en combinación con otro radical, quiere decir el radical -O-alquilo(C₁₋₆) en el que el alquilo es como se ha definido anteriormente, conteniendo hasta seis átomos de carbono, e incluye metoxi, etoxi, propoxi, 1-metiletoksi, butoxi y 1,1-dimetiletoksi. El último radical es conocido
30 comúnmente como terc-butoxi.

El término "halo" como se usa aquí, quiere decir un sustituyente halógeno seleccionado de bromo, cloro, flúor o yodo.

La expresión "sal farmacéuticamente aceptable" quiere decir una sal de un compuesto de fórmula (1) que es, dentro del alcance de un juicio médico razonable, adecuada para usar en contacto con los tejidos de seres humanos y animales inferiores sin toxicidad, irritación, respuesta alérgica y similares, indebidas, proporcionada con una relación beneficio/riesgo razonable, generalmente soluble o que se puede dispersar en agua o aceite, y eficaz para su uso deseado. La expresión incluye sales de adición de un ácido farmacéuticamente aceptables y sales de adición de una base farmacéuticamente aceptables. Se encuentran listas de sales adecuadas en, por ejemplo, S.M. Birge et al., J. Pharm. Sci., 1977, 66, pp. 1-19, que se incorpora por la presente por referencia en su totalidad.

La expresión "sal de adición de un ácido farmacéuticamente aceptable" quiere decir las sales que retienen la eficacia biológica y las propiedades de las bases libres y que no son biológicamente o de otra manera indeseables, formadas con ácidos inorgánicos tales como ácido clorhídrico, ácido bromhídrico, ácido sulfúrico, ácido sulfámico, ácido nítrico, ácido fosfórico, y similares, y ácidos orgánicos tales como ácido acético, ácido trifluoroacético, ácido adípico, ácido ascórbico, ácido aspártico, ácido bencenosulfónico, ácido benzoico, ácido butírico, ácido canfórico, ácido canforsulfónico, ácido cinámico, ácido cítrico, ácido diglucónico, ácido etanosulfónico, ácido glutámico, ácido glicólico, ácido glicerofosfórico, ácido hemisulfico, ácido hexanoico, ácido fórmico, ácido fumárico, ácido 2-hidroxietanosulfónico (ácido isetiónico), ácido láctico, ácido hidroximaleico, ácido málico, ácido malónico, ácido mandélico, ácido mesitilensulfónico, ácido metanosulfónico, ácido naftalenosulfónico, ácido

nicotínico, ácido 2-naftalenosulfónico, ácido oxálico, ácido pamoico, ácido pectínico, ácido fenilacético, ácido 3-fenilpropiónico, ácido piválico, ácido propiónico, ácido pirúvico, ácido salicílico, ácido esteárico, ácido succínico, ácido sulfanílico, ácido tartárico, ácido p-toluensulfónico, ácido undecanoico, y similares.

La expresión "sal de adición de una base farmacéuticamente aceptable", quiere decir las sales que retienen la eficacia biológica y las propiedades de los ácidos libres y que no son indeseables biológicamente o de otra manera, formadas con bases inorgánicas tales como amoniaco, o hidróxido, carbonato, o bicarbonato de amonio o un de catión metálico tal como sodio, potasio, litio, calcio, magnesio, hierro, zinc, cobre, manganeso, aluminio, y similares. Son particularmente preferidas las sales de amonio, potasio, sodio, calcio y magnesio. Las sales derivadas de bases orgánicas no tóxicas farmacéuticamente aceptables incluyen sales de aminas primarias, secundarias y terciarias, compuestos de aminas cuaternarias, aminas sustituidas incluyendo aminas sustituidas que existen en la naturaleza, aminas cíclicas y resinas básicas de intercambio iónico, tales como metilamina, dimetilamina, trimetilamina, etilamina, dietilamina, trietilamina, isopropilamina, tripropilamina, tributilamina, etanolamina, dietanolamina, 2-dimetilaminoetanol, 2-dietilaminoetanol, dicitclohexilamina, lisina, arginina, histidina, cafeína, hidrabamina, colina, betaína, etilendiamina, glucosamina, metilglucamina, teobromina, purinas, piperazina, piperidina, N-etilpiperidina, compuestos de tetrametilamonio, compuestos de tetraetilamonio, piridina, N,N-dimetilanilina, , N-metilpiperidina, N-metilmorfolina, dicitclohexilamina, dibencilamina, N,N-dibencilfenetilamina, 1-efenamina, N,N'-dibenciletil-

endiamina, resinas de poliamina, y similares. Son particularmente preferidas las bases orgánicas no tóxicas isopropilamina, dietilamina, etanolamina, trimetilamina, diclohexilamina, colina y cafeína.

5 La expresión "agente antiviral" como se usa aquí quiere decir un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación de un virus en un mamífero. Esto incluye agentes que interfieran con los mecanismos del anfitrión o del virus
10 necesarios para la formación y/o replicación de un virus en un mamífero. Los agentes antivirales incluyen, por ejemplo, ribavirina, amantadina, VX-497 (merimepodib, Vertex Pharmaceuticals), VX-498 (Vertex Pharmaceuticals), levovirina, viramidina, Ceplene (maxamina), XTL-001 y
15 XTL-002 (XTL Biopharmaceuticals).

 La expresión "otro agente anti-VHC", como se usa aquí, quiere decir los agentes que son eficaces para disminuir o impedir la progresión de los síntomas relacionados con la enfermedad de la hepatitis C. Tales
20 agentes pueden seleccionarse de: agentes antivirales, agentes inmunomoduladores, inhibidores de la proteasa NS3 del VHC, inhibidores de polimerasa del VHC o inhibidores de otra diana en el ciclo biológico del VHC.

 La expresión "agente inmunomodulador", como se usa
25 aquí, quiere decir los agentes (compuestos o biológicos) que son eficaces para mejorar o potenciar la respuesta del sistema inmunitario en un mamífero. Los agentes inmunomoduladores incluyen, por ejemplo, interferones de clase I (tales como α -, β -, δ - y omega-interferones, tau-interferones, interferones de consenso y asialo-
30 interferones), interferones de clase II (tales como γ -interferones) e interferones pegilados.

 La expresión "inhibidor de la proteasa NS3 del VHC", como se usa aquí, quiere decir un agente (compuesto o

biológico) que es eficaz para inhibir la función de la proteasa NS3 del VHC en un mamífero. Los inhibidores de la proteasa NS3 del VHC incluyen, por ejemplo, los compuestos descritos en los documentos de patente WO 99/07733, WO 99/07734, WO 00/09558, WO 00/09543, WO 00/59929 o WO 02/060926, y el candidato pre-desarrollado por Vertex/Eli Lilly identificado como VX-950 o LY-570310. En particular, los compuestos n°s 2, 3, 5, 6, 8, 10, 11, 18, 19, 29, 30, 31, 32, 33, 37, 38, 55, 59, 71, 91, 103, 104, 105, 112, 113, 114, 115, 116, 120, 122, 123, 124, 125, 126 y 127, descritos en la tabla de las páginas 224-226 en el documento WO 02/060926, se pueden utilizar en combinación con los compuestos de la presente invención.

La expresión "inhibidor de polimerasa de HCV", tal como se utiliza en esta memoria, significa un agente (compuesto o biológico) que es eficaz para inhibir la función de una polimerasa de HCV en un mamífero. Esto incluye, por ejemplo, inhibidores de la polimerasa NS5B de HCV. Inhibidores de polimerasa de HCV incluyen no nucleósidos, por ejemplo los compuestos descritos en:

- solicitud de EE.UU. n° 10/198.680, que corresponde al documento PCT/CA02/01127, ambos presentados el 18 de julio de 2002 (Boehringer Ingelheim),
- solicitud de EE.UU. n° 10/198.384, que corresponde al documento PCT/CA02/01128, ambos presentados el 18 de julio de 2002 (Boehringer Ingelheim),
- solicitud de EE.UU. n° 10/198.259, que corresponde al documento PCT/CA02/01129, ambos presentados el 18 de julio de 2002 (Boehringer Ingelheim),
- documento WO 02/100846 A1 y documento WO 02/100851 A2 (ambos de Shire),
- documento WO 01/85172 A1 y documento WO 02/098424 A1 (ambos de GSK),
- documento WO 00/06529 y documento WO 02/06246 A1

(ambos de Merck),

- documento WO 01/47883 y documento WO 03/000254 (ambos de Japan Tobacco) y
- documento EP 1 256 628 A2 (Agouron).

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

- documento WO 01/90121 A2 (Idenix),
- documento WO 02/069903 A2 (Biocryst Pharmaceuticals

10 Inc.), y

- documento WO 02/057287 A2 y documento WO 02/057425 A2 (ambos de Merck/Isis).

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

 La expresión "inhibidor de otra diana en el ciclo biológico del VHC", como se usa aquí, quiere decir un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación del VHC en un mamífero de un
20 modo diferente a la inhibición de la función de la proteasa NS3 del VHC. Esto incluye agentes que interfieren con los mecanismos del anfitrión o del virus necesarios para la formación y/o replicación del VHC en un mamífero. Los inhibidores de otra diana en el ciclo
25 biológico del VHC incluyen, por ejemplo, agentes que inhiben una diana seleccionada de una helicasa, una proteasa NS2/3 y un inhibidor del sitio de entrada interno del ribosoma (IRES). Un ejemplo específico de inhibidores de otra diana en el ciclo biológico del VHC
30 incluyen ISIS-14803 (ISIS Pharmaceuticals).

 La expresión "inhibidor del VIH", como se usa aquí, quiere decir un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación del VIH en un mamífero. Esto incluye agentes que interfieren con

los mecanismos del anfitrión o del virus necesarios para la formación y/o replicación del VIH en un mamífero. Los inhibidores del VIH incluyen, por ejemplo, inhibidores nucleosídicos, inhibidores no nucleosídicos, inhibidores de proteasa, inhibidores de la fusión e inhibidores de integrasa.

La expresión "inhibidor del VHA", como se usa aquí, quiere decir un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación del VHA en un mamífero. Esto incluye agentes que interfieren con los mecanismos del anfitrión o del virus necesarios para la formación y/o replicación del VHA en un mamífero. Los inhibidores del VHA incluyen vacunas de la hepatitis A, por ejemplo, Havrix[®] (GlaxoSmithKline), VAQTA[®] (Merck) y Avaxim[®] (Aventis Pasteur).

La expresión "inhibidor del VHB", como se usa aquí, quiere decir un agente (compuesto o biológico) que es eficaz para inhibir la formación y/o replicación del VHB en un mamífero. Esto incluye agentes que interfieren con los mecanismos del anfitrión o del virus necesarios para la formación y/o replicación del VHB en un mamífero. Los inhibidores del VHB incluyen, por ejemplo, agentes que inhiben la polimerasa de ADN viral del VHB o vacunas de VHB. Ejemplos específicos de inhibidores del VHB incluyen lamivudina (Epivir-HBV[®]), Adefovir Dipivoxil, Entecavir, FTC (Coviracil[®]), DAPD (DXG), L-FMAU (Clevudine[®]), AM365 (Amrad), Ldt (Telbivudine), monoal-LdC (Valtorcitabine), ACH-126,443 (L-Fd4C) (Achillion), MCC478 (Eli Lilly), Racivir (RCV), nucleósidos Fluoro-L y D, Robustaflavone, ICN 2001-3 (ICN), Bam 205 (Novelos), XTL-001 (XTL), Imino-Sugars (Nonyl-DNJ) (Synergy), HepBzyme; y productos inmunomoduladores tales como: interferón alfa 2b, HE2000 (Hollis-Eden), Theradigm (Epimmune), EHT899 (Enzo Biochem), Thymosin alfa-1 (Zadaxin[®]), vacuna de ADN del

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VHB (PowerJect), vacuna de ADN del VHB (Jefferson Center), antígeno de VHB (OraGen), BayHep B[®] (Bayer), Nabi-HB[®] (Nabi) y Anti-hepatitis B (Cangene); y productos de vacuna de VHB tales como los siguientes: Engerix B,
 5 Recombivax HB, GenHevac B, Hepacare, Bio-Hep B, TwinRix, Comvax y Hexavac.

La expresión "interferón de clase I", como se usa aquí, quiere decir un interferón seleccionado de un grupo de interferones que se unen todos ellos a un receptor de
 10 tipo I. Esto incluye interferones de clase I producidos tanto de forma natural como sintética. Ejemplos de interferones de clase I incluyen α -, β -, omega-interferones, tau-interferones, interferones de consenso y asialo-interferones.

15 La expresión "interferón de clase II", como se usa aquí, quiere decir un interferón seleccionado de un grupo de interferones que se unen todos ellos a un receptor de tipo II. Ejemplos de interferones de clase II incluyen γ -interferones.

20 Se listan a continuación ejemplos preferidos específicos de algunos de estos agentes:

- agentes antivirales: ribavirina y amantadina;
- agentes inmunomoduladores: interferones de clase I, interferones de clase II e interferones pegilados;
- 25 • inhibidor de otra diana en el ciclo biológico del VHC, que inhibe una diana seleccionada de:
 helicasa NS3, proteasa NS2/3 del VHC o un sitio de entrada interno del ribosoma (IRES);
- inhibidores del VIH: inhibidores nucleosídicos,
 30 inhibidores no nucleosídicos, inhibidores de proteasa, inhibidores de la fusión e inhibidores de integrasa; o
- inhibidores del VHB: agentes que inhiben la polimerasa de ADN viral del VHB o una vacuna de VHB.

Como se ha discutido anteriormente, se contempla una terapia de combinación, en la que se co-administra un compuesto de fórmula (1), o una sal farmacéuticamente aceptable del mismo, con al menos un agente adicional seleccionado de: un agente antiviral, un agente inmunomodulador, otro inhibidor de la proteasa NS3 del VHC, un inhibidor de polimerasa del VHC, un inhibidor de otra diana en el ciclo biológico del VHC, un inhibidor del VIH, un inhibidor del VHA y un inhibidor del VHB. Se proporcionan ejemplos de tales agentes en la sección de definiciones anterior. Estos agentes adicionales pueden combinarse con los compuestos de esta invención para crear una forma de dosificación farmacéutica simple. Alternativamente, estos agentes adicionales pueden administrarse separadamente al paciente como parte de una forma de dosificación múltiple, por ejemplo, usando un equipo. Tales agentes adicionales pueden administrarse al paciente antes de, concurrentemente con, o a después de la administración de un compuesto de fórmula (1), o una sal farmacéuticamente aceptable del mismo.

Como se usa aquí, el término "tratamiento" quiere decir la administración de un compuesto o composición conforme a la presente invención para mitigar o eliminar los síntomas de la enfermedad de la hepatitis C y/o reducir la cantidad de virus en un paciente.

Como se usa aquí, el término "prevención" quiere decir la administración de un compuesto o composición conforme a la presente invención después de la exposición de la persona al virus, pero antes de la aparición de los síntomas de la enfermedad, y/o antes de la detección del virus en la sangre.

Realizaciones preferidas

Se prefieren los compuestos de fórmula 1, como se ha definido anteriormente, en la que R^1 es hidroxí, NHSO_2Me ,
5 NHSO_2 -ciclopropilo, o NHSO_2Ph . Más preferiblemente, R^1 es NHSO_2Me o hidroxí. Lo más preferiblemente, R^1 es hidroxí.

Se prefieren los compuestos de fórmula 1, como se ha definido anteriormente, en la que R^2 es t-butilo o $\text{CH}_2\text{-C}(\text{CH}_3)_3$. Más preferiblemente, R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$.

10 Preferiblemente, R^3 es t-butilo.

Preferiblemente, compuestos de fórmula 1 como se ha definido anteriormente, en la que R^4 es ciclopentilo o ciclohexilo. Más preferiblemente, R^4 es ciclopentilo.

Más preferiblemente, un compuesto de fórmula 1 como
15 se ha definido anteriormente, en la que R^1 es hidroxí, R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es t-butilo y R^4 es ciclopentilo.

Más preferiblemente, un compuesto de fórmula 1, en la que R^1 es hidroxí, R^2 y R^3 son cada uno t-butilo, y R^4 es ciclopentilo.

20 Más preferiblemente, un compuesto de fórmula 1, en la que R^1 es hidroxí, R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es ciclohexilo y R^4 es ciclopentilo.

Más preferiblemente, un compuesto de fórmula 1, en la que R^1 es hidroxí, R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, y R^3 y R^4 son cada
25 uno ciclohexilo.

Más preferiblemente, un compuesto de fórmula 1, en la que R^1 es hidroxí, R^2 es ciclopentilmetilo, R^3 es t-butilo y R^4 es ciclobutilo.

Más preferiblemente, un compuesto de fórmula 1, en
30 la que R^1 es hidroxí, R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es t-butilo y R^4 es ciclobutilo.

Más preferiblemente, un compuesto de fórmula 1, en la que R^1 es NHSO_2Me , R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es t-butilo y R^4 es ciclopentilo.

Más preferiblemente, un compuesto de fórmula 1, en la que R^1 es NHSO_2Ph , R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es t-butilo y R^4 es ciclopentilo.

Conforme a una realización alternativa, la
5 composición farmacéutica de esta invención puede comprender adicionalmente otro agente anti-VHC. Ejemplos de agentes anti-VHC incluyen α - (alfa), β - (beta), δ - (delta), γ - (gamma), tau- o ω - (omega) interferón, ribavirina y amantadina.

10 Conforme a otra realización alternativa, la composición farmacéutica de esta invención puede comprender adicionalmente otro inhibidor de la proteasa NS3 del VHC.

Conforme a otra realización alternativa, la
15 composición farmacéutica de esta invención puede comprender adicionalmente un inhibidor de la polimerasa del VHC.

Conforme a aún otra realización alternativa, la
20 composición farmacéutica de esta invención puede comprender adicionalmente un inhibidor de otras dianas en el ciclo biológico del VHC, que incluye pero no está limitado a, helicasa, proteasa NS2/3 o un sitio de entrada interno del ribosoma (IRES).

La composición farmacéutica de esta invención puede
25 administrarse por vía oral, parenteral o por medio de un depósito implantado. Se prefiere una administración oral o una administración por inyección. La composición farmacéutica de esta invención puede contener cualquier excipiente, adyuvante o vehículo farmacéuticamente
30 aceptable no tóxico convencional. En algunos casos, el pH de la formulación puede ajustarse con ácidos, bases o tampones farmacéuticamente aceptables, para mejorar la estabilidad del compuesto formulado o su forma de distribución. El término parenteral, como se usa aquí,

incluye una inyección subcutánea, intracutánea, intravenosa, intramuscular, intra-articular, intrasínovial, intrarraquídea, intratecal, y dentro de la lesión, o técnicas de infusión.

5 La composición farmacéutica puede estar en forma de una preparación estéril inyectable, por ejemplo, como una suspensión acuosa u oleaginosa estéril inyectable. Esta suspensión puede formularse conforme a técnicas conocidas en la técnica, usando agentes de dispersión o humectantes
10 adecuados (tales como, por ejemplo, Tween 80) y agentes de suspensión.

La composición farmacéutica de esta invención puede administrarse por vía oral en cualquier forma de dosificación aceptable por vía oral que incluye, pero no
15 está limitada a, cápsulas, comprimidos, y suspensiones y disoluciones acuosas. En el caso de comprimidos para uso oral, los excipientes usados comúnmente incluyen lactosa y almidón de maíz. También se añaden típicamente agentes lubricantes, tales como estearato de magnesio. Para una
20 administración oral en forma de cápsula, los diluyentes útiles incluyen lactosa y almidón de maíz seco. Cuando se administran por vía oral suspensiones acuosas, el ingrediente activo se combina con agentes emulsionantes y de suspensión. Si se desea, pueden añadirse ciertos
25 agentes edulcorantes y/o aromatizantes y/o colorantes.

Pueden encontrarse otros vehículos o excipientes adecuados para las formulaciones y composiciones citadas anteriormente en textos farmacéuticos clásicos, por ejemplo, en "Remington's Pharmaceutical Sciences", The
30 Science and Practice of Pharmacy, 19th Ed. Mack Publishing Company, Easton, Penn., (1995).

Son útiles niveles de dosificación de entre aproximadamente 0,01 y aproximadamente 100 mg/kg de peso corporal por día, preferiblemente entre aproximadamente

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0,1 y aproximadamente 50 mg/kg de peso corporal por día del compuesto inhibidor de proteasa descrito en la presente invención, en una monoterapia para la prevención y tratamiento de una enfermedad mediada por VHC.

5 Típicamente, la composición farmacéutica de esta invención se administrará desde aproximadamente 1 hasta 5 veces por día o, alternativamente, como una infusión continua. Puede usarse tal administración como una terapia prolongada o breve. La cantidad de ingrediente

10 activo que puede combinarse con los excipientes, para producir una forma de dosificación simple, variará dependiendo del anfitrión tratado y del modo particular de administración. Una preparación típica contendrá desde aproximadamente 5% hasta aproximadamente 95% de compuesto

15 activo (p/p). Preferiblemente, tales preparaciones contienen desde aproximadamente 20% hasta aproximadamente 80% de compuesto activo.

Como apreciará una persona experta, pueden requerirse dosis inferiores o superiores a las citadas

20 anteriormente. La dosificación específica y los regímenes de tratamiento para cualquier paciente particular dependerán de una variedad de factores, que incluyen la actividad del compuesto específico empleado, la edad, peso corporal, estado de salud general, sexo, dieta,

25 tiempo de administración, velocidad de excreción, combinación de fármacos, la gravedad y curso de la infección, la disposición del paciente a la infección y el criterio del médico que lo está tratando. Generalmente, el tratamiento se inicia con pequeñas

30 dosificaciones del péptido substancialmente inferiores a la dosis óptima. Después, la dosificación se aumenta en pequeños incrementos hasta que se alcanza el efecto óptimo de acuerdo con las circunstancias. En general, el compuesto se administra lo más deseablemente a un nivel

de concentración que proporcionará generalmente resultados eficaces contra el virus sin causar ningún efecto secundario dañino o nocivo.

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

Cuando estos compuestos o sus sales farmacéuticamente aceptables se formulan junto con un vehículo farmacéuticamente aceptable, la composición resultante puede administrarse *in vivo* a mamíferos, tales como un ser humano, para inhibir la proteasa NS3 del VHC o para tratar o impedir la infección por el virus VHC. Tal tratamiento puede lograrse también usando un compuesto de esta invención en combinación con agentes que incluyen, pero no están limitados a: α -, β -, δ -, ω -, tau- o γ -interferón, ribavirina, amantadina; otros inhibidores de la proteasa NS3 del VHC; inhibidores de la polimerasa del VHC, inhibidores de otras dianas en el ciclo biológico del VHC, que incluyen, pero no están limitados a, helicasa, proteasa NS2/3, o un sitio de entrada interno del ribosoma (IRES); o combinaciones de los mismos. Los agentes adicionales pueden combinarse con compuestos de esta invención para crear una forma de dosificación simple. Alternativamente, estos agentes adicionales pueden administrarse separadamente a un mamífero como parte de una forma de dosificación múltiple.

De acuerdo con esto, otra realización de esta invención proporciona un método para inhibir la actividad

de la proteasa NS3 del VHC en un mamífero administrando un compuesto de fórmula 1.

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

Si la composición farmacéutica comprende solamente un compuesto de esta invención como componente activo, tal método puede comprender adicionalmente la etapa de administrar a dicho mamífero un agente seleccionado de un agente inmunomodulador, un agente antiviral, un inhibidor de la proteasa NS3 del VHC, un inhibidor de la polimerasa del VHC o un inhibidor de otras dianas en el ciclo biológico del VHC, tal como helicasa, proteasa NS2/3 o IRES. Tal agente adicional puede administrarse al mamífero antes de, concurrentemente con, o después de la administración de la composición de esta invención.

Un compuesto de fórmula 1 definido en la presente invención puede usarse también como un reactivo de laboratorio. Un compuesto de esta invención puede usarse también para tratar o impedir la contaminación viral de materiales, y por lo tanto reducir el riesgo de infección viral de personal de laboratorio o médico, o de pacientes que entran en contacto con tales materiales (por ejemplo sangre, tejido, instrumentos y prendas quirúrgicas, instrumentos y prendas de laboratorio, y aparatos y materiales de recogida de sangre).

Un compuesto de fórmula 1 definido en la presente invención puede usarse también como un reactivo de investigación. Un compuesto de fórmula 1 puede usarse también como un testigo positivo para validar ensayos alternativos en células o ensayos de replicación viral *in vitro* o *in vivo*.

110

Se ilustran más detalles de la invención en los siguientes ejemplos, que se entiende que no son limitantes con respecto a las reivindicaciones adjuntas.

5

EJEMPLOS

Las temperaturas se dan en grados Celsius. Los tantos por ciento en las disoluciones expresan una relación peso a volumen, y las relaciones en las disoluciones expresan una relación volumen a volumen, a menos que se especifique de otra manera. Los espectros de resonancia magnética nuclear (RMN) fueron registrados en un espectrómetro Bruker 400 MHz; los desplazamientos químicos (δ) están descritos en partes por millón. Las cromatografías de desarrollo rápido se llevaron a cabo en gel de sílice (SiO_2) conforme a la técnica de cromatografía de desarrollo rápido de Still (W. C. Still et al., J. Org. Chem. (1978), 43, 2923).

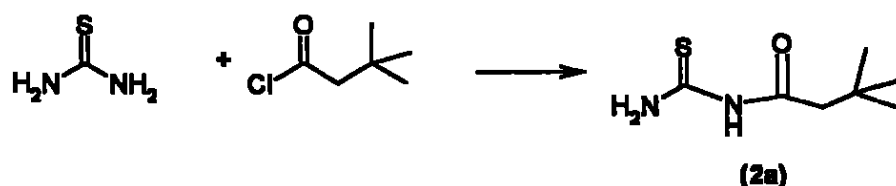
Las abreviaturas usadas en los ejemplos incluyen DBU: 1,8-diazabicyclo[5.5.0]undec-7-eno; DCM: cloruro de metileno; DIEA: dietilamina; DIPEA: diisopropiletilamina; DMF: N,N-dimetilformamida; DMAP: 4-(dimetilamino)piridina; AcOEt: acetato de etilo; HATU: [hexafluorofosfato de O-(7-azabenzotriazol-1-il)-1,1,3,3-tetrametiluronio]; HPLC: cromatografía líquida de alta resolución; EM: espectrometría de masas (MALDI-TOF: espectrometría de masas con ionización por desorción de láser asistida por matriz en tiempo de vuelo, FAB: bombardeo con átomos rápidos); Me: metilo; MeOH: metanol; Ph: fenilo; t.a.: temperatura ambiente (18°-22°); terc-butilo o t-butilo: 1,1-dimetiletilo; Tbg: terc-butilglicina: terc-leucina; TFA: ácido trifluoroacético; y THF: tetrahidrofurano.

Síntesis de compuestos de fórmula (1):

En general, los compuestos de fórmula 1, y sus productos intermedios, se preparan por métodos conocidos usando condiciones de reacción que se conoce que son adecuadas para los agentes reaccionantes. Se describen
 5 varios de tales métodos en los documentos de patente WO 00/09543, WO 00/09558 y en la patente de EE.UU. N° 6.323.180 incorporada por referencia en la presente invención.

10 Preparación de tioureas

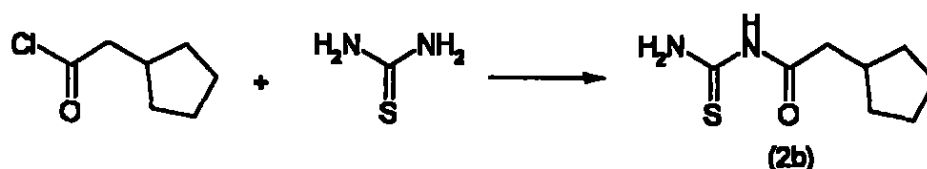
Preparación de la tiourea 2a



Se disolvió tiourea (5,0 g, 66 mmoles) en tolueno (50 ml), y se añadió cloruro de terc-butilacetilo (8,88
 15 g, 66 mmoles). La mezcla se calentó a reflujo durante 14 h, para dar una disolución amarilla. La mezcla se concentró hasta sequedad, y el residuo se dividió entre AcOEt y NaHCO₃ sat. La fase orgánica amarilla se secó sobre MgSO₄, se filtró y se concentró para dar un sólido
 20 amarillo. El sólido se disolvió en la mínima cantidad de AcOEt y se trituyó con hexano para dar 2a como un sólido blanco (8,52 g; 75%). E.M. (electropulverización): 173 (M-H)- 175 (M+H)+. Homogeneidad en HPLC de fase inversa (TFA al 0,06%; CH₃CN:H₂O): 99 %.

25

Preparación de la tiourea 2b

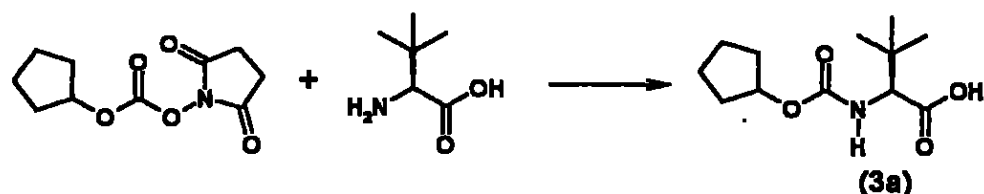


Usando el procedimiento descrito anteriormente, y usando cloruro de ciclopentilacetilo disponible comercialmente en lugar de cloruro de terc-butilacetilo, se obtuvo la tiourea 2b.

5

Síntesis de productos intermedios 3

Preparación del carbamato 3a



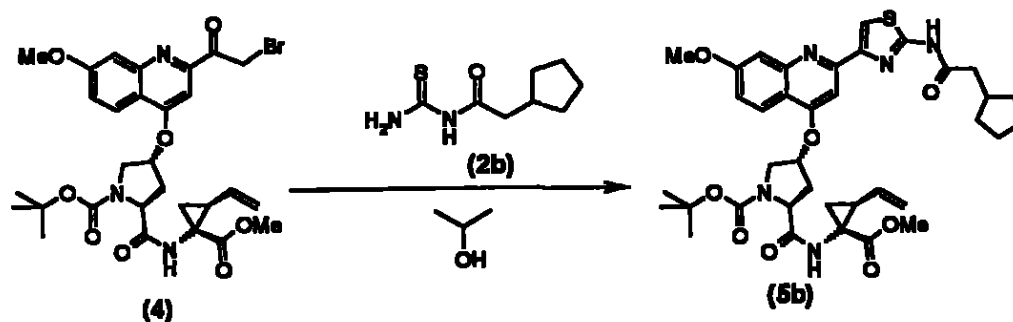
Se añadió tetrahidrofurano (350 ml) a un matraz que
 10 contenía éster de ciclopentilo-éster de 2,5-dioxo-
 pirrolidín-1-ilo del ácido carbónico (9,00 g; 39,6
 mmoles) y terc-leucina (6,24 g; 47,5 mmoles), dando como
 resultado una suspensión. Se añadió agua destilada (100
 ml) con agitación vigorosa. Permaneció sin disolverse una
 15 pequeña cantidad de sólido. Se añadió luego trietilamina
 (16,6 ml; 119 mmoles), dando como resultado una
 disolución homogénea, que se agitó a t.a. Después de 2,5
 h, el THF se evaporó y el residuo acuoso se diluyó con
 agua (100 ml), y la mezcla de reacción se volvió básica
 20 por adición de NaOH 1N (25 ml - pH final >10). La
 disolución se lavó con AcOEt (2x200 ml), y luego la fase
 acuosa se acidificó con HCl 1N (aproximadamente 70 ml -
 pH final <2). La disolución turbia se extrajo con AcOEt
 (200+150 ml). El extracto se secó (MgSO₄) y se evaporó
 25 para dar el compuesto 3a como un sólido blanco (8,68 g).

Preparación de los carbamatos 3b, 3c y 3d

Usando el procedimiento descrito anteriormente, y
 usando combinaciones apropiadas de terc-butil-glicina, o
 30 ciclohexil-glicina y éster de ciclobutilo, ciclopentilo,

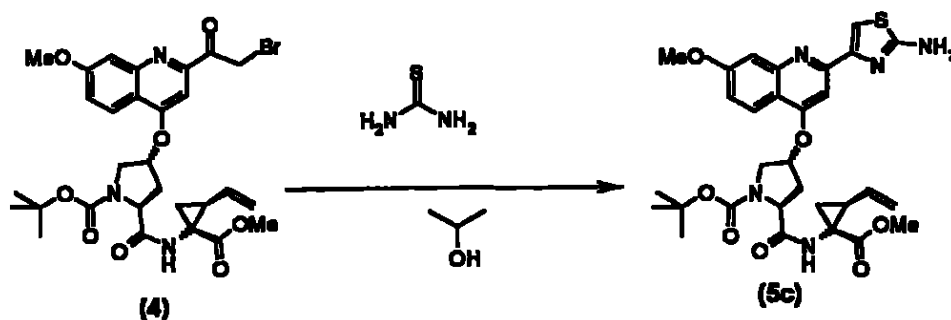
Preparación del producto intermedio 5b:

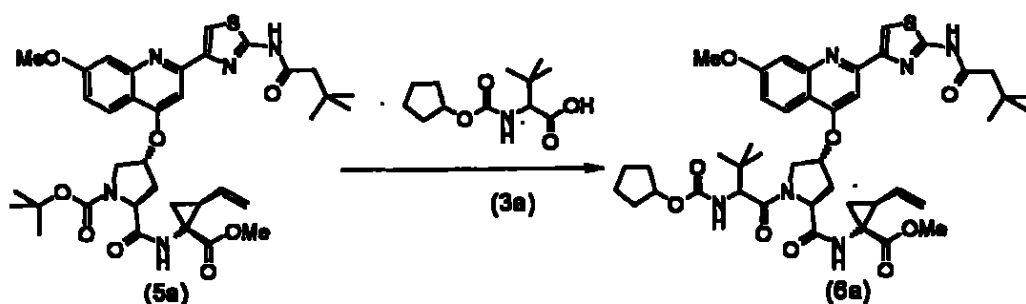
Usando el procedimiento descrito anteriormente, y usando tiourea 2b en lugar de tiourea 2a, se obtiene el producto intermedio 5b correspondiente:

Preparación del producto intermedio 5c:

Usando el procedimiento descrito anteriormente, y usando tiourea disponible comercialmente en lugar de tiourea 2a, se obtiene el producto intermedio 5c correspondiente:

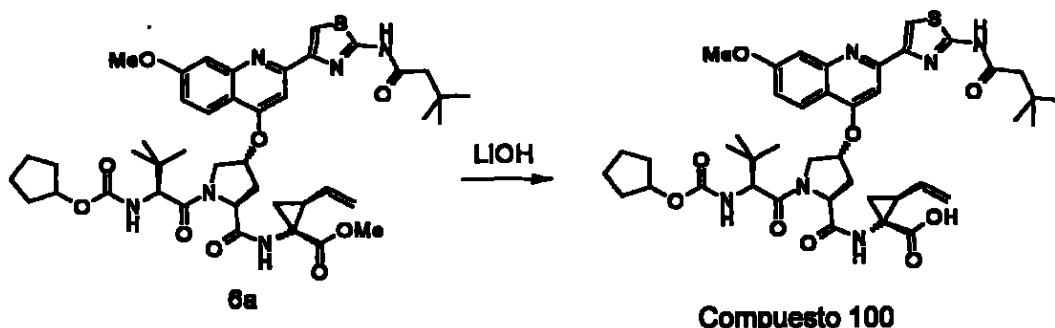
10

15 **EJEMPLO 1**Síntesis del compuesto 100



Etapas 1: Preparación del producto intermedio 6a

El Boc-dipéptido 5a (3,05 g; 4,31 mmoles) se disolvió en HCl 4N/dioxano (22 ml). Después de agitar a t.a. durante 30 min, precipitó la sal de HCl. Se añadió MeOH (2 ml) para disolver el precipitado. Después de 2 h, la mezcla de reacción se evaporó hasta sequedad. La sal de HCl resultante se disolvió en DCM (22 ml), y se añadieron DIEA (3,0 ml; 17,24 mmoles); carbamato 3a (1,15 g; 4,47 mmoles) y HATU (1,72 g; 4,516 mmoles). La disolución se agitó a t.a. durante 6 h. La mezcla de reacción se diluyó luego con AcOEt y la disolución se lavó con NaHCO₃ saturado (2x), agua (2x) y salmuera (1x), se secó (MgSO₄), se filtró y se evaporó para obtener el compuesto 6a como un sólido amarillo. Una cromatografía en columna de desarrollo rápido, eluyendo en primer lugar con hexano:AcOEt 7:3 y luego con 6:4 proporcionó el Me-éster 6a puro como una espuma blanca (3,25 g; 90%). E.M. (electropulverización): 831,4 (M-H)- 833,4 (M+H)+ 855,4 (M+Na)+. Homogeneidad en HPLC de fase inversa (TFA al 0,06%; CH₃CN:H₂O): 98%.

Etapa 2: Hidrólisis del éster

El éster de metilo 6a (3,24 mg; 3,89 mmoles) se
 5 disolvió en THF (40 ml) y MeOH (20 ml), y se añadió una
 disolución acuosa de LiOH (1,63 mg; 38,9 mmoles en 25
 ml). La mezcla de reacción amarilla se agitó durante 5,5
 h y luego se concentró, para proporcionar una suspensión
 blanquecina. La suspensión se disolvió en AcOEt y
 10 salmuera, preparada con agua desionizada. El pH de la
 disolución resultante se ajustó a 6 por adición de HCl
 1N. Se separaron las fases, y la capa acuosa se extrajo
 con más AcOEt (2x). Los extractos combinados de AcOEt se
 lavaron con agua desionizada (2x), salmuera preparada con
 15 agua desionizada (1x), se secaron (MgSO₄), se filtraron y
 se evaporaron, para obtener el compuesto 100 como un
 sólido de color blanco-amarillo pálido (3,02 g; 95% de
 rendimiento).

20 Conversión en la sal de Na:

El compuesto neutro 100 (1,22 g; 1,49 mmoles) se
 disolvió en MeOH (30 ml), y se añadió 1 equivalente de
 NaOH 0,01 N (14,85 ml) - no hubo precipitación de
 producto. La disolución amarilla transparente se
 25 concentró, se diluyó con agua desionizada, se congeló y
 se liofilizó para obtener el producto (sal de Na) como un
 sólido amorfo de color blanco-amarillo (1,24 g; 99% de
 rendimiento).

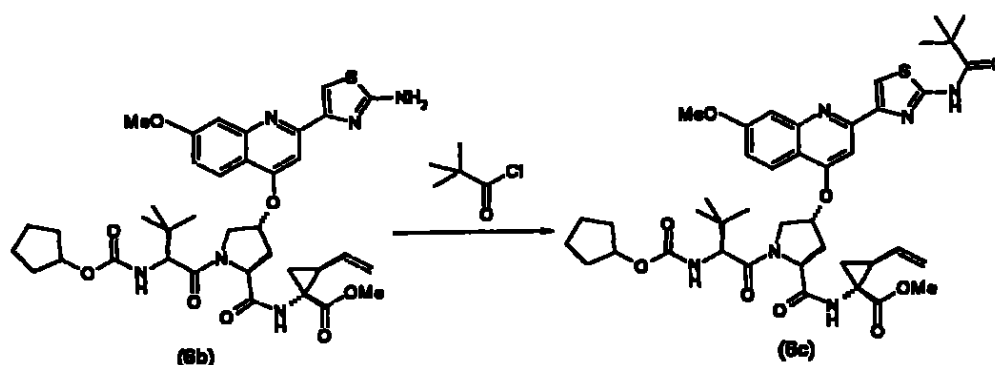
Sal de Na: PM: 840,98 $C_{42}H_{53}N_6O_9SNa$

E.M. (electropulverización): 817,3 (M-H) - 819,4 (M+H)+ 841,4 (M+Na)+. Homogeneidad en HPLC de fase inversa (TFA al 0,06%; $CH_3CN:H_2O$): 98%.

- 5 ^1H RMN (400 MHz, $DMSO-d_6$): aproximadamente, mezcla 6:1 de isómeros de rotación; δ 8,11-7,65 (m, 4H), 7,33 (sa, 1H), 7,18-6,97 (m, 2H), 6,36-6,08 (m, 1H), 5,55-5,33 (m, 1H), 4,98 (d, $J = 18,0$ Hz, 1H), 4,85 (sa, 1H), 4,80 (d, $J = 10,4$ Hz, 1H), 4,50-4,41 (m, 1H), 4,22-4,02 (m, 2H), 3,92
- 10 (s, 3H), 2,72-2,45 (m, 1H), 2,50 (bajo la señal de DMSO, s, 2H), 2,40-2,26 (m, 1H), 1,89-1,43 (m, 9H), 1,37-1,30 (m, 1H), 1,30-1,12 (m, 1H), 1,03 y 0,90 (2x s, 9H), 0,98 y 0,94 (2x s, 9H).

15 EJEMPLO 2

Síntesis del compuesto 101



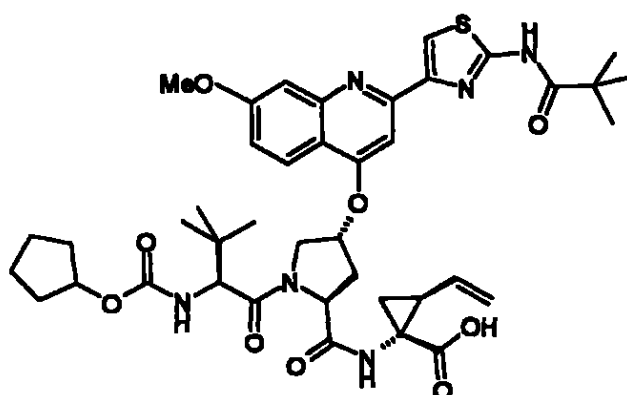
- 20 Siguiendo el mismo procedimiento como se describe en la primer etapa del ejemplo 1, y usando Boc-dipéptido 5c en lugar de 5a, se obtuvo el compuesto 6b.

- Se disolvió el compuesto 6b (70 mg, 0,095 mmoles) en 2 ml de DCM, y se trató sucesivamente con DIEA (0,045 ml, 0,26 mmoles) y cloruro de pivaloílo (0,015 ml, 0,12 mmoles). Después de agitar durante 1 h a 40°, se añadió
- 25 cloruro de pivaloílo adicional (0,15 ml, 0,12 mmoles,) y la agitación continuó durante 2 h más. Después de concentrar la disolución, el residuo se disolvió en

AcOEt. La disolución se lavó con una disolución saturada de NaHCO₃ y salmuera, se secó (MgSO₄) y se concentró para proporcionar 82 mg del compuesto 6c crudo, que se usó sin purificación.

5 El derivado éster de metilo 6c se hidrolizó como en la etapa 2 del ejemplo 1, y se purificó por HPLC preparativa usando una columna YMC Combi-Prep. ODS-AQ, 50x20 mm. ID, S-5 micrómetros, 120 Å, y un programa de gradiente lineal desde 2 hasta 100% de AcCN/agua (TFA al
10 0,06%).

Se analizaron las fracciones por HPLC analítica, y las fracciones puras se combinaron, se concentraron, se congelaron y se liofilizaron para dar lugar al compuesto 101 como la sal de trifluoroacetato:



Compuesto 101

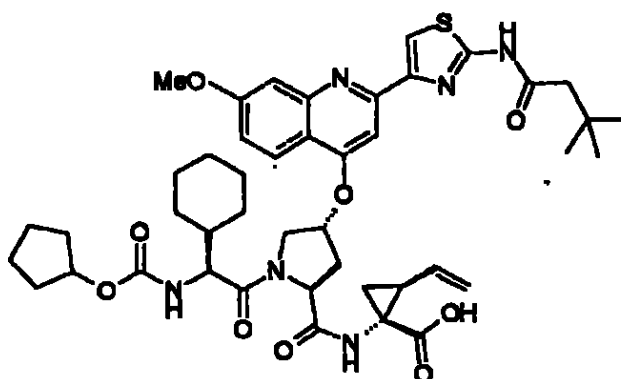
15 ¹H RMN (400 MHz, DMSO-d₆): aproximadamente, mezcla 85:15 de isómeros de rotación, descripción del isómero principal; δ 8,56 (s, 1H), 8,40-8,22 (m, 1H), 8,17 (d, J = 8,9 Hz, 1H), 7,67 (sa, 1H), 7,52 (sa, 1H), 7,24-7,15
20 (m, 1H), 7,03 (d, J = 8,3 Hz, 1H), 5,78-5,67 (m, 1H), 5,61-5,53 (m, 1H), 5,19 (dd, J = 17,2, 1,6 Hz, 1H), 5,09-5,03 (m, 1H), 4,63-4,55 (m, 1H), 4,49-4,39 (m, 2H), 4,11-3,92 (m, 2H), 3,95 (s, 3H), 2,62-2,53 (m, 1H), 2,33-2,25 (m, 1H), 2,06-1,98 (m, 1H), 1,72-1,25 (m, 10H), 1,30 (s,
25 9H), 0,97 (s, 9H).

E.M. (electropulverización): 803,3 (M-H)- 805,3 (M+H)+.
Homogeneidad en HPLC de fase inversa (TFA al 0,06%;
CH₃CN:H₂O): 97%.

5 EJEMPLO 3

Preparación del compuesto 102

Siguiendo el procedimiento descrito en el ejemplo 1,
y usando carbamato 3d en lugar de 3a, y usando HPLC
preparativa para purificar el producto final como se
10 describe en el ejemplo 2, se obtuvo el compuesto 102 como
la sal de trifluoroacetato:



Compuesto 102

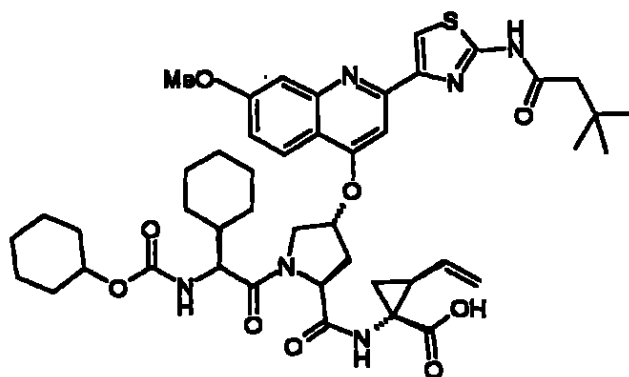
¹H RMN (400 MHz, DMSO-d₆): aproximadamente, mezcla 90:10
de isómeros de rotación, descripción del isómero
15 principal; δ 12,37 (s, 1H), 8,54 (s, 1H), 8,40-8,06 (m,
2H), 7,67-7,40 (m, 2H), 7,26 (d, J = 8,0 Hz, 1H), 7,26-
7,13 (m, 1H), 5,77-5,65 (m, 1H), 5,62-5,49 (m, 1H), 5,23-
5,16 (m, 1H), 5,10-5,03 (m, 1H), 4,57-4,37 (m, 3H), 4,03-
3,88 (m, 2H), 3,94 (s, 3H), 2,64-2,54 (m, 1H), 2,41 (s,
20 2H), 2,37-2,22 (m, 1H), 2,04-1,95 (m, 1H), 1,79-1,21 (m,
16H); 1,17-0,85 (m, 5H), 1,04 (s, 9H).

E.M. (electropulverización): 843,5 (M-H)- 845,4 (M+H)+.
Homogeneidad en HPLC de fase inversa (TFA al 0,06%;
CH₃CN:H₂O): 99%.

25

EJEMPLO 4

120

Preparación del compuesto 103

Compuesto 103

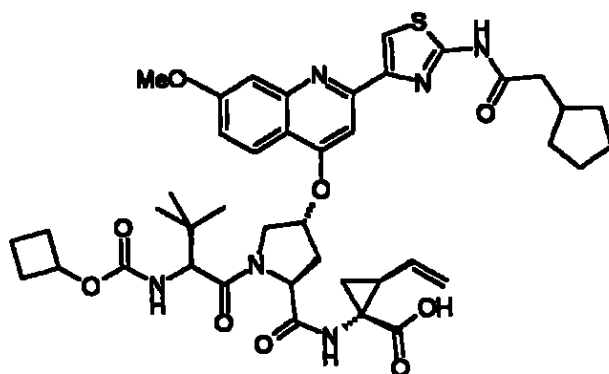
Siguiendo el procedimiento descrito en el ejemplo 1, pero usando carbamato 3c en lugar de 3a, y usando HPLC preparativa para purificar el producto final como se describe en el ejemplo 2, se obtuvo el compuesto 103 como la sal de trifluoroacetato:

¹H RMN (400 MHz, DMSO-d₆): aproximadamente, mezcla 85:15 de isómeros de rotación, descripción del isómero principal; δ 12,35 (s, 1H), 8,52 (m, 1H), 8,43-8,06 (m, 2H), 7,67-7,41 (m, 2H), 7,26 (d, J = 7,8 Hz, 1H), 7,24-7,11 (m, 1H), 5,77-5,65 (m, 1H), 5,62-5,48 (m, 1H), 5,23-5,15 (m, 1H), 5,10-5,03 (m, 1H), 4,52-4,38 (m, 2H), 4,15-4,05 (m, 1H), 4,03-3,89 (m, 2H), 3,94 (s, 3H), 2,63-2,52 (m, 1H), 2,41 (s, 2H), 2,35-2,23 (m, 1H), 2,05-1,96 (m, 1H), 1,81-1,41 (m, 11H), 1,38-0,86 (m, 12H), 1,04 (s, 9H).

E.M (electropulverización): 857,5 (M-H) - 859,4 (M+H)+.
Homogeneidad en HPLC de fase inversa (TFA al 0,06%; CH₃CN:H₂O): 99%.

EJEMPLO 5Preparación del compuesto 104

121



Compuesto 104

Siguiendo el procedimiento descrito en el ejemplo 1, pero usando carbamato 3b en lugar de 3a, y Boc-dipéptido 5b en lugar de 5a, y usando HPLC preparativa para purificar el compuesto final como se describe en el ejemplo 2, se obtuvo el compuesto 104 como la sal de trifluoroacetato:

^1H RMN (400 MHz, DMSO- d_6): aproximadamente, mezcla 3:1 de isómeros de rotación, δ 8,02 (s, 1H), 7,90 (s, 1H), 7,84 (d, $J = 7,0$ Hz, 1H), 7,70 (s, 1H), 7,33 (d, $J = 2,2$ Hz, 1H), 7,14 (dd, $J = 2,5, 8,2$ Hz, 1H), 7,09 (dd, $J = 2,2, 9,2$ Hz, 1H), 6,29-6,08 (m, 1H), 5,54-5,32 (m, 1H), 4,99 (d, $J = 15,9$ Hz, 1H), 4,80 (d, $J = 10,0$ Hz, 1H), 4,76-4,64 (m, 1H), 4,46 (dd, $J = 6,7, 13,9$ Hz, 1H), 4,19-4,08 (m, 3H), 3,92 (s, 3H), 2,70-2,61 (m, 2H), 2,37-2,09 (m, 4H), 2,03-1,82 (m, 1H), 1,85 (sa, 2H), 1,77-1,44 (m, 8H), 1,35-1,29 (m, 1H), 1,29-1,15 (m, 4H), 0,98 y 0,90 (2x s, 9H).

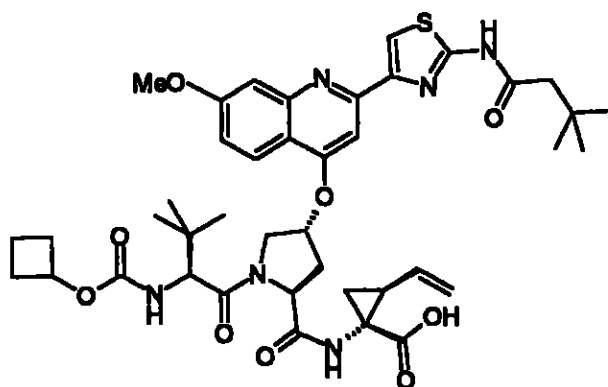
E.M. (electropulverización): 815,3 (M-H) - 817,3 (M+H)+.

Homogeneidad en HPLC de fase inversa (TFA al 0,06%; $\text{CH}_3\text{CN}:\text{H}_2\text{O}$): 98%.

EJEMPLO 6

Preparación del compuesto 105

122



Compuesto 105

Siguiendo el procedimiento descrito en el ejemplo 1, pero usando carbamato 3b en lugar de 3a, y usando HPLC preparativa para purificar el producto final como se describe en el ejemplo 2, se obtuvo el compuesto 105 como la sal de trifluoroacetato:

^1H RMN (400 MHz, DMSO- d_6): aproximadamente, mezcla 7:1 de isómeros de rotación, δ 8,58 (s, 1H), 8,19 (d, J = 8,0 Hz, 1H), 7,76-7,50 (m, 2H), 7,35-7,20 (m, 1H), 7,19 (d, J = 8,0 Hz, 1H), 5,78-5,67 (m, 1H), 5,65-5,50 (m, 1H), 5,19 (d, J = 17,0 Hz, 1H), 5,07 (d, J = 10,2 Hz, 1H), 4,51-4,42 (m, 2H), 4,42-4,31 (m, 1H), 4,02 (d, J = 7,4 Hz, 1H), 4,02-3,93 (m, 1H), 3,97 (s, 3H), 2,63-2,52 (m, 1H), 2,42 (s, 2H), 2,35-2,25 (m, 1H), 2,07-1,95 (m, 3H), 1,90-1,76 (m, 1H), 1,70-1,41 (m, 3H), 1,30-1,23 (m, 1H), 1,04 (s, 9H), 0,96 y 0,87 (2xs, 9H).

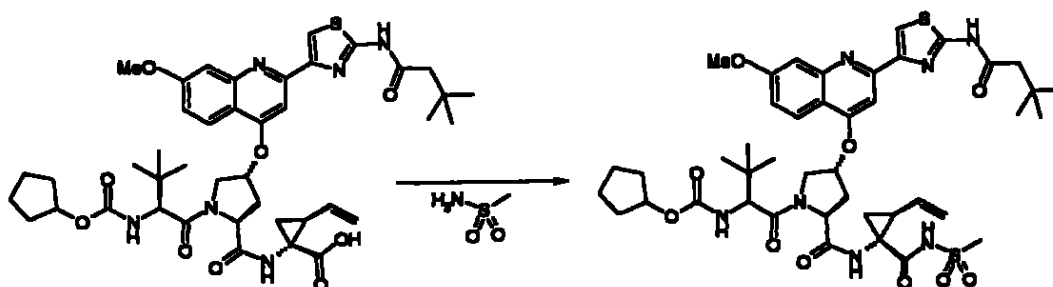
E.M. (electropulverización): 803,4 (M-H) - 805,4 (M+H)+.

Homogeneidad en HPLC de fase inversa (TFA al 0,06%; $\text{CH}_3\text{CN}:\text{H}_2\text{O}$): 98%.

20

EJEMPLO 7

Preparación del compuesto 106



Compuesto 106

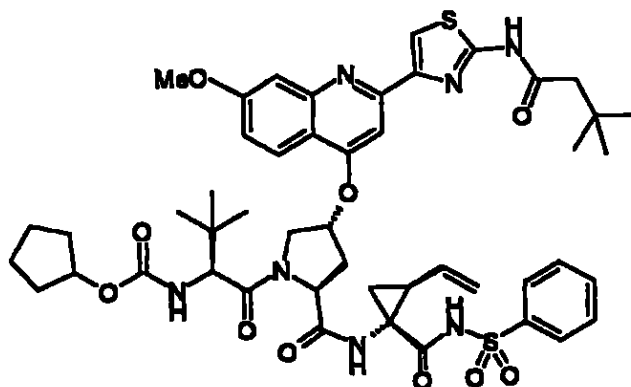
El compuesto 100 del ejemplo 1 (29,8 mg, 0,036 mmoles) se combinó con HATU (1,2 eq., 19,7 mg, 0,052 mmoles), y se disolvió en DMF anhidra (4 ml). La disolución se agitó a t.a. y se añadió DIPEA (5 eq., 31,4 μ l, 0,18 mmoles) gota a gota durante aproximadamente 1 min. La mezcla se agitó durante 20 min a t.a. y se analizó por HPLC analítica para seguir la formación del éster activado. Se añadió una disolución de metanosulfonamida (5,8 eq., 19,7 mg, 0,207 mmoles), DMAP (5,4 eq., 23,5 mg, 0,193 mmoles) y DBU (4,8 eq., 25,8 μ l, 0,172 mmoles) en DMF (1 ml). La mezcla de reacción se agitó 16 h a t.a. antes de concentrarse a vacío. El residuo se re-disolvió en DMSO y se purificó por HPLC preparativa. La liofilización dio el producto final (23 mg, 71,3%) como un sólido amorfo blanquecino.

^1H RMN (400 MHz, $\text{DMSO}-d_6$): δ 12,35 (s, 1H), 10,53 (s, 1H), 8,87 (s, 1H), 8,40-8,20 (m, 1H), 8,17 (d, $J = 8,8$ Hz, 1H), 7,61 (sa, 1H), 7,51 (sa, 1H), 5,67-5,55 (m, 2H), 5,23-5,18 (m, 1H), 5,10 (d, $J = 12$ Hz, 1H), 4,68-4,57 (m, 1H), 4,50 (da, $J = 12$ Hz, 1H), 4,46-4,37 (m, 1H), 4,07 (d, $J = 8,0$ Hz, 1H), 3,95 (s, 3H), 3,17 (s, 3H), 2,78-2,58 (m, 1H), 2,42 (s, 2H), 2,29-2,19 (m, 1H), 2,19-2,09 (m, 1H), 1,71 (dd, $J = 7,8, 7,6$ Hz, 1H); 1,67-1,55 (m, 4H), 1,55-1,37 (m, 4H), 1,04 (s, 9H), 0,98 (s, 9H), 0,97-0,87 (m, 2H).

E.M. (electropulverización): 896,5 (M+H)+ y 894,5 (M-H)-. 124
HPLC de fase inversa: Rt = 6,7 minutos (homogeneidad = 100%).

5 EJEMPLO 8

Preparación del compuesto 107



Compuesto 107

Usando el mismo procedimiento descrito en el ejemplo 7, y usando bencenosulfonamida en lugar de metilsulfonamida, se obtuvo el compuesto 107 como un sólido amorfo de color amarillo pálido, con 54% de rendimiento.

¹H RMN (DMSO-d₆): δ 12,39 (s, 1H), 10,89 (s, 1H), 8,83 (s, 1H), 8,40-8,22 (m, 1H), 8,18 (d, J = 8,4 Hz, 1H), 7,90 (s, 1H), 7,88 (s, 1H), 7,67-7,63 (m, 1H), 7,63-7,54 (m, 3H), 7,54-7,45 (m, 1H), 7,30-7,15 (m, 1H), 7,10 (d, J = 7,8 Hz, 1H), 5,62-5,51 (m, 1H), 5,38-5,26 (m, 1H), 5,16-5,08 (m, 1H), 4,93 (d, J = 10,4 Hz, 1H), 4,70-4,58 (m, 1H), 4,57-4,49 (m, 1H), 4,48-4,39 (m, 1H), 4,09 (d, J = 7,8 Hz, 1H), 3,95 (s, 3H), 2,69-2,59 (m, 1H), 2,41 (s, 2H), 2,28-2,16 (m, 1H), 2,14-2,04 (m, 1H), 1,72-1,52 (m, 4H), 1,51-1,37 (m, 4H), 1,29-1,22 (m, 1H) 1,03 (s, 9H), 1,00 (s, 9H), 0,99-0,92 (m, 1H).

E.M. (electropulverización): 958,5 (M+H)+ y 956,5 (M-H)-
HPLC de fase inversa: Rt = 7,2 minutos (homogeneidad = 95%).

✓25-

EJEMPLO 9**Ensayo de proteasa NS3-NS4**

El ensayo enzimático usado para evaluar el compuesto de la presente invención está descrito en los documentos
5 de patente WO 00/09543 y WO 00/59929.

EJEMPLO 10**Ensayo de replicación de ARN de VHC en células****Cultivo celular**

10 Se utilizaron células Huh7 que mantienen de manera estable un replicón de VHC subgenómico, como se ha descrito previamente (Lohman et al., 1999, Science 285: 110-113), y se designaron como la estirpe celular S22.3. Las células S22.3 se mantuvieron en un medio de Earle
15 modificado por Dulbecco (DMEM), complementado con SBF al 10% y 1 mg/ml de neomicina (medio normal). Durante el ensayo, se usó un medio DMEM complementado con SBF al 10%, que contenía DMSO al 0,5% y no contenía neomicina (medio de ensayo). 16 horas antes de la adición del
20 compuesto, las células S22.3 se trataron con tripsina y se diluyeron hasta 50.000 células/ml en medio normal. Se distribuyeron 200 µl (10.000 células) en cada pocillo de una placa de 96 pocillos. Luego, la placa se incubó a 37° con CO₂ al 5% hasta el día siguiente.

25

30

Reactivos y materiales:

Producto	Compañía	N° catálogo	Almacenamiento
DMEM	Wisent Inc	10013CV	4°C
DMSO	Sigma	D-2650	t.a.
PBS (disolución salina tamponada con fosfato) de Dulbecco	Gibco-BRL	14190-136	t.a.
Suero de bovino fetal	Bio-Whittaker	14-901F	-20°C/4°C
Neomicina (G418)	Gibco-BRL	10131-027	-20°C/4°C
Tripsina-EDTA	Gibco-BRL	25300-054	-20°C/4°C
placas de 96 pocillos	Costar	3997	t.a.
unidad de filtración de PVDF de 0,22 µm	Millipore	SLGV025LS	t.a.
placa de valoración de pocillos profundos de polipropileno	Beckman	267007	t.a.

Preparación del compuesto de ensayo

Se añadieron 10 µl del compuesto de ensayo (en DMSO al 100%) a 2 ml del medio de ensayo, para alcanzar una concentración final de DMSO de 0,5%, y la disolución se sometió a ultrasonidos durante 15 min, y se filtró a través de una unidad de filtración Millipore de 0,22 µm. Se transfirieron 900 µl en la fila A de una placa de valoración de pocillos profundos de polipropileno. Las

filas de la B a la H contenían partes alícuotas de 400 µl de medio de ensayo (que contenía DMSO al 0,5%), y se usaron para preparar diluciones en serie (1/2) transfiriendo 400 µl de fila a fila (no se incluyó compuesto en la fila H).

Aplicación del compuesto de ensayo a las células

Se aspiró el medio de cultivo celular de la placa de 96 pocillos que contenía las células S22.3. Se transfirieron 175 µl de medio de ensayo con la dilución apropiada del compuesto de ensayo desde cada pocillo de la placa con el compuesto hasta el pocillo correspondiente de la placa de cultivo celular (la fila H se usó como el "testigo de no inhibición"). La placa de cultivo celular se incubó a 37° con CO₂ al 5% durante 72 h.

Extracción del ARN celular total

Después del periodo de incubación de 72 h, el ARN celular total se extrajo de las células S22.3 de la placa de 96 pocillos usando el equipo RNeasy 96 (Qiagen®, RNeasy Handbook, 1999). En resumen, el medio de ensayo se retiró completamente de las células, y se añadieron 100 µl de tampón RLT (Qiagen®) que contenían β-mercaptoetanol 143 mM a cada pocillo de la placa de cultivo celular de 96 pocillos. La microplaca se agitó suavemente durante 20 s. Luego se añadieron 100 µl de etanol al 70% a cada pocillo de la microplaca, y se mezclaron con una pipeta. El lisado se retiró y se aplicó a los pocillos de una placa RNeasy 96 (Qiagen®), que se colocó en la parte superior de un bloque de pocillos cuadrados de Qiagen®. La placa RNeasy 96 se selló con una lámina adhesiva, y el bloque de pocillos cuadrados con la placa RNeasy 96 se

cargó en el soporte, y se situó en un contenedor del rotor de una centrifugadora 4K15C. La muestra se centrifugó a 6000 rpm (~5600 x g) durante 4 min a temperatura ambiente. Se retiró la lámina adhesiva de la

5 placa y se añadieron 0,8 ml de tampón RW1 (equipo RNeasy 96 de Qiagen®) a cada pocillo de la placa RNeasy 96. La placa RNeasy 96 se selló con un nuevo trozo de lámina adhesiva y se centrifugó a 6000 rpm durante 4 min a temperatura ambiente. La placa RNeasy 96 se colocó en la

10 parte superior de otro bloque de pocillos cuadrados de Qiagen® limpio, se retiró la lámina adhesiva y se añadieron 0,8 ml de tampón RPE (equipo RNeasy 96 de Qiagen®) a cada pocillo de la placa RNeasy 96. La placa RNeasy 96 se selló con un nuevo trozo de lámina adhesiva

15 y se centrifugó a 6000 rpm durante 4 min a temperatura ambiente. Se retiró la lámina adhesiva y se añadieron otros 0,8 ml de tampón RPE (equipo RNeasy 96 de Qiagen®) a cada pocillo de la placa RNeasy 96. La placa RNeasy 96 se selló con un nuevo trozo de lámina adhesiva y se

20 centrifugó a 6000 rpm durante 10 min a temperatura ambiente. Se retiró la lámina adhesiva, se colocó la placa RNeasy 96 en la parte superior de una gradilla que contenía microtubos de recolección de 1,2 ml. Se eluyó el ARN añadiendo 50 µl de agua sin ribonucleasa a cada

25 pocillo, se selló la placa con un nuevo trozo de lámina adhesiva y se incubó durante 1 min a temperatura ambiente. La placa se centrifugó luego a 6000 rpm durante 4 min a temperatura ambiente. La etapa de elución se repitió con un segundo volumen de 50 µl de agua sin

30 ribonucleasa. Los microtubos con el ARN celular total se almacenaron a -70°.

Cuantificación del ARN celular total

El ARN se cuantificó con el sistema STORM® (Molecular Dynamics®), usando el equipo de cuantificación de ARN RiboGreen® (Molecular Probes®). En resumen, el reactivo RiboGreen se diluyó 200 veces en TE (tris-HCl 10 mM pH=7,5, EDTA 1 mM). Generalmente, se diluyeron 50 µl de reactivo en 10 ml de TE. Se diluyó una curva patrón de ARN ribosómico en TE hasta 2 µg/ml, y luego se transfirieron cantidades predeterminadas (100, 50, 40, 20, 10, 5, 2 y 0 µl) de la disolución de ARN ribosómico a una nueva placa de 96 pocillos. (COSTAR # 3997), y el volumen se completó hasta 100 µl con TE. Generalmente, se usó la columna 1 de la placa de 96 pocillos para la curva patrón, y los otros pocillos se usaron para cuantificar las muestras de ARN. Se transfirieron 10 µl de cada muestra de ARN que iba a ser cuantificado al correspondiente pocillo de la placa de 96 pocillos, y se añadieron 90 µl de TE. Se añadió un volumen (100 µl) de reactivo RiboGreen diluido a cada pocillo de la placa de 96 pocillos, se incubó durante 2 a 5 minutos a temperatura ambiente, y se protegió de la luz (una muestra de ARN de 10 µl en un volumen final de 200 µl genera una dilución de 20 X). Se midió la intensidad de fluorescencia de cada pocillo con el sistema STORM® (Molecular Dynamics®). Se creó una curva patrón sobre la base de las cantidades conocidas de ARN ribosómico y las intensidades de fluorescencia resultantes. Se determinó la concentración de ARN en las muestras experimentales a partir de la curva patrón y se corrigió para la dilución de 20 X.

Reactivos y materiales:

Producto	Compañía	N° catálogo	Almacenamiento
DEPC	Sigma	D5758	4°C
EDTA	Sigma	E5134	t.a.
Trizma-Base	Sigma	T8524	t.a.
Trizma-HCl	Sigma	T7149	t.a.
tiras de tubos de recolección	Qiagen	19562	t.a.
equipo de cuantificación de ARN RiboGreen	Molecular Probe	R11490	-20°C
equipo RNeasy 96	Qiagen	74183	t.a.
Bloques de pocillos cuadrados	Qiagen	19573	t.a.

R.T.-PCR (Transcripción Inversa-Reacción en Cadena de la Polimerasa) en tiempo real

- 5 La R.T.-PCR en tiempo real se llevó a cabo en el sistema de detección de secuencias ABI Prism 7700, usando el equipo TaqMan EZ R.T.-PCR (Perkin-Elmer Applied Biosystems®). La R.T.-PCR se optimizó para la cuantificación de la región 5' IRES del ARN del VHC,
- 10 usando la tecnología TaqMan (Roche Molecular Diagnostics Systems), similar a la técnica descrita previamente (Martel et al., 1999, J. Clin. Microbiol. 37: 327-332). El sistema se basa en la actividad 5'-3' nucleolítica de la ADN polimerasa AmpliTaq. En resumen, el método utiliza
- 15 una sonda de hibridación fluorogénica doblemente marcada (sonda PUTR), que se une específicamente al molde entre los cebadores de la PCR (cebadores 8125 y 7028). El extremo 5' de la sonda contiene un indicador fluorescente (6-carboxifluoresceína [FAM]) y el extremo 3' contiene un
- 20 extintor de fluorescencia (6-carboxitetrametilrodamina

[TAMRA]). El espectro de emisión del indicador FAM fue suprimido por el extintor en la sonda de hibridación intacta. La degradación de la sonda de hibridación mediante la actividad nucleasa libera al indicador, dando
5 como resultado un aumento de la emisión de fluorescencia. El detector de secuencias ABI Prism 7700 mide continuamente el aumento de la emisión de fluorescencia durante la amplificación por PCR, de tal manera que el producto amplificado fue directamente proporcional a la
10 señal. Se analizó en una etapa temprana de la reacción el perfil de amplificación, en un punto que representara la fase logarítmica de acumulación de producto. Se definió como ciclo umbral (C_T), para el detector de secuencias, un punto que representara un umbral de detección definido
15 del aumento en la señal fluorescente asociado con el crecimiento exponencial del producto de PCR. Los valores de C_T son inversamente proporcionales a la cantidad de ARN de VHC inicial; de tal manera que bajo idénticas condiciones de PCR, cuanto mayor sea la concentración
20 inicial de ARN de VHC, menor es el C_T . Se creó automáticamente una curva patrón por el sistema de detección ABI Prism 7700, representando el C_T frente a cada dilución patrón de concentración conocida de ARN de VHC.

25 Se incluyeron muestras de referencia para la curva patrón en cada placa R.T.-PCR. El ARN replicón de VHC se sintetizó (mediante transcripción T7) *in vitro*, se purificó y se cuantificó mediante una lectura de DO_{260} . Considerando que 1 μ g de este ARN= $2,15 \times 10^{11}$ copias de
30 ARN, se hicieron diluciones para tener 10^8 , 10^7 , 10^6 , 10^5 , 10^4 , 10^3 o 10^2 copias del ARN genómico/5 μ l. Se incorporó también el ARN celular total de Huh-7 en cada dilución (50 ng/5 μ l). Se combinaron 5 μ l de cada patrón de referencia (replicón de VHC + ARN de Huh-7) con 45 μ l de

mezcla de reactivo, y se usaron en la reacción R.T.-PCR en tiempo real.

Se llevó a cabo la reacción R.T.-PCR en tiempo real para las muestras experimentales que fueron purificadas en placas de pocillos RNeasy 96, combinando 5 µl de cada muestra de ARN celular total con 45 µl de mezcla de reactivo.

Reactivos y materiales:

Producto	Compañía	N° Catálogo	Almacenamiento
Equipo TaqMan EZ R.T.-PCR	PE Applied Biosystems	N808-0236	-20°C
Tapas ópticas MicroAmp	PE Applied Biosystems	N801-0935	t.a.
Placa óptica de reacción de 96 pocillos MicroAmp	PE Applied Biosystems	N801-0560	t.a.

Preparación de la mezcla de reactivo:

Componente	Volumen para una muestra (μL)	Volumen para una placa (μL) (91 muestras + volumen muerto)	Conc. Final
Agua sin ribonucleasa	16,5	1617	
tampón Taqman EZ 5X	10	980	1X
Mn(OAc) ₂ (25 mM)	6	588	3 mM
dATP (10 mM)	1,5	147	300 μM
dCTP (10 mM)	1,5	147	300 μM
dGTP (10 mM)	1,5	147	300 μM
dUTP (20 mM)	1,5	147	600 μM
Cebador directo (10 μM)	1	98	200 nM
Cebador inverso (10 μM)	1	98	200 nM
Sonda PUTR (5 μM)	2	196	200 nM
ADN polimerasa rTth (2,5 U/μl)	2	196	0,1 U/μl
AmpErase UNG (1 U/μl)	0,5	49	0,01 U/μl
Volumen Total	45	4410	

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

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

Nota: Estos cebadores amplifican una región de 256-nt que se localiza en la región 5' no codificante de VHC.

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

Testigos sin molde (NTC): En cada placa, se usaron 4
5 pocillos como "NTC". Para estos testigos, se añadieron 5
µl de agua al pocillo en lugar de ARN.

Condiciones de ciclos térmicos:

	50°C 2 min	
10	60°C 30 min	
	95°C 5 min	
	95°C 15 s	durante 2 ciclos
	60°C 1 min	
	90°C 15 s	durante 40 ciclos
15	60°C 1 min	

Después de la finalización de la reacción R.T.-PCR,
el análisis de los datos requirió el establecimiento de
una señal de fluorescencia umbral para la placa de PCR, y
20 se construyó una curva patrón representando el valor de C_T
frente al número de copias de ARN usado en cada reacción
de referencia. Los valores de C_T obtenidos para las
muestras de ensayo se usaron para interpolar un número de
copias de ARN basado en la curva patrón.

25 Finalmente, se normalizó el número de copias de ARN
(basado en la cuantificación de ARN RiboGreen del ARN
total extraído del pocillo del cultivo celular) y se
expresó como equivalentes de genoma/µg de ARN total
[e.g./µg].

30 El número de copias de ARN [e.g./µg] de cada pocillo
de la placa del cultivo celular fue una medida de la
cantidad de ARN de VHC replicante en presencia de
diversas concentraciones de inhibidor. El % de inhibición
se calculó con la siguiente ecuación:

135

100 - [(e.g./ μ g inh) / (e.g./ μ g test) x 100].

Se aplicó una curva no lineal ajustada con el modelo de Hill a los datos de inhibición-concentración, y se calculó la concentración eficaz al 50% (EC₅₀) usando el software SAS (Statistical Software System; SAS Institute, Inc. Cary, N.C.).

Cuando se evaluaron los compuestos de esta invención con los ensayos enzimáticos y en células precedentes, se encontró que los compuestos eran altamente activos. Más específicamente, los compuestos tuvieron valores de IC₅₀ inferiores a 0,1 μ M en el ensayo de proteasa NS3-NS4, y valores de EC₅₀ inferiores a 0,5 μ M en el ensayo de replicación de ARN de VHC en células.

15 EJEMPLO 11

Ensayos de especificidad

Los ensayos de especificidad usados para evaluar la selectividad de este compuesto están descritos en el documento de patente WO 00/09543.

Cuando se evaluaron los compuestos en los ensayos de especificidad, se encontró que los compuestos de fórmula 1 eran selectivos por cuanto no mostraban una inhibición significativa en los ensayos de elastasa de leucocitos humanos y Catepsina B.

25

EJEMPLO 12

Propiedades farmacocinéticas

Los compuestos de la presente invención muestran también buenas propiedades farmacocinéticas tales como niveles detectables en el plasma en ratas a 1 hora y a las 2 horas después de una dosis oral de 4 ó 5 mg/kg.

Más explícitamente, se usó el siguiente ensayo, un chequeo de absorción oral *in vivo*, para determinar los

niveles en el plasma de compuestos de ensayo en ratas después de una administración oral.

Materiales y Métodos:

5 1. **Método usado para agrupar compuestos ("selección de cassettes"):**

La selección de compuestos para ser agrupados en una "cassette" se basó en su semejanza estructural y propiedades fisicoquímicas. Se estableció un método de
10 extracción en fase sólida aplicable a todos los compuestos seleccionados. Basándose en los ensayos iniciales en los que cada compuesto se inoculó en plasma de rata y se hizo pasar a través de HPLC o HPLC/EM a una concentración de 0,5 μ M, el tiempo de retención, la masa
15 iónica, y la separación posible entre los compuestos por HPLC y/o HPLC/EM se usaron como base para agrupar 3-4 compuestos en una "cassette".

2. **Preparación del vehículo oral y del compuesto:**

Cada "cassette" contenía 3-4 compuestos, a 5 ó 4
20 mg/kg para cada compuesto. Las cassettes se prepararon como una suspensión oral en metilcelulosa acuosa al 0,5% y monooleato de polioxietileno (20) sorbitán (Tween-80) al 0,3%. El volumen de dosificación fue 10 ml/kg por alimentación forzada por vía oral.

25 3. **Dosificación y muestreo de plasma:**

Se privó de alimento durante la noche a ratas Sprague Dawley machos, que estaban en jaulas individuales, con acceso a dextrosa acuosa al 10%. Se administraron dosis a dos ratas con cada "cassette". Se
30 recogieron muestras de plasma (~1 ml) de las dos ratas a 1 y 2 h después de la dosificación, y se agruparon para extracción y análisis.

4. **Extracción y análisis de los compuestos:**

Se extrajeron de cada cassette, mediante el método de extracción en fase sólida, muestras de plasma a 1 y 2 h, plasma testigo, plasma testigo en el que se inocularon todos los compuestos a una concentración 0,5 μM cada uno.

- 5 Las muestras se analizaron por HPLC y HPLC/EM con un propósito de comparación. Las concentraciones de plasma se estimaron basándose en la concentración simple del patrón 0,5 μM .

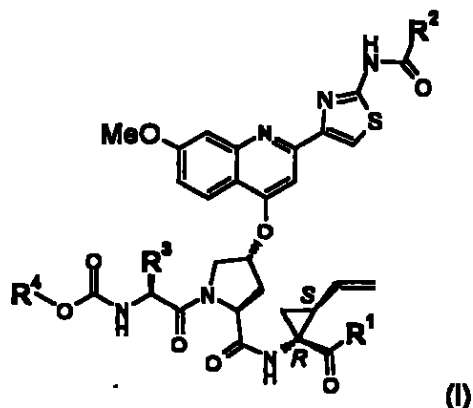
10 Resultados

- Cuando se ensayaron en el muestreo anterior los compuestos de los ejemplos 1 a 8 de esta invención, se encontró que estaban presentes en el plasma a niveles significativos en los intervalos de 1 hora y 2 horas
15 después de la administración oral, promediando niveles en el plasma sanguíneo de 0,83 μM y 0,75 μM respectivamente.

- Es notable esta demostración de absorción oral *in vivo* para los compuestos tripeptídicos de esta invención, en vista de la pobre absorción oral atribuida
20 generalmente a esta clase de péptidos. La fácil absorción oral hace estos compuestos útiles para tratar una infección por VHC.

REIVINDICACIONES

1. Un compuesto de fórmula (1):



5 en la que R¹ es hidroxil o N¹HSO₂R^{1A} en el que R^{1A} es alquilo(C₁₋₈), cicloalquilo(C₃₋₇) o {alquilo(C₁₋₈)-cicloalquilo(C₃₋₇)}, estando todos ellos sustituidos opcionalmente de 1 a 3 veces con halo, ciano, nitro, O-alquilo(C₁₋₈), amido, amino o fenilo, o R^{1A} es arilo C₆ o
 10 C₁₀ que está sustituido opcionalmente de 1 a 3 veces con halo, ciano, nitro, alquilo(C₁₋₈), O-alquilo(C₁₋₈), amido, amino o fenilo; R² es t-butilo, -CH₂-C(CH₃)₃, o -CH₂-ciclopentilo; R³ es t-butilo o ciclohexilo y R⁴ es ciclobutilo, ciclopentilo o ciclohexilo; o una sal
 15 farmacéuticamente aceptable del mismo.

2. El compuesto de fórmula 1 conforme a la reivindicación 1, en el que R¹ es hidroxil, N¹HSO₂Me, N¹HSO₂-ciclopropilo, o N¹HSO₂-Ph.

3. El compuesto de fórmula I conforme a la
 20 reivindicación 2, en el que R¹ es N¹HSO₂Me o hidroxil.

4. El compuesto de fórmula I conforme a la reivindicación 3, en el que R¹ es hidroxil.

5. El compuesto de fórmula 1 conforme a una cualquiera de las reivindicaciones 1 a 4, en el que R² es
 25 t-butilo o -CH₂-C(CH₃)₃.

6. El compuesto de fórmula I conforme a la reivindicación 5, en el que R² es CH₂-C(CH₃)₃.

7. El compuesto de fórmula I conforme a una cualquiera de las reivindicaciones 1 a 6, en el que R^3 es t-butilo.

8. El compuesto de fórmula I conforme a una cualquiera de las reivindicaciones 1 a 7, en el que R^4 es ciclopentilo o ciclohexilo.

9. El compuesto de fórmula I conforme a la reivindicación 8, en el que R^4 es ciclopentilo.

10. El compuesto de fórmula I conforme a la reivindicación 1, en el que R^1 es hidroxilo, R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es t-butilo y R^4 es ciclopentilo.

11. El compuesto de fórmula I conforme a la reivindicación 1, en el que R^1 es hidroxilo, R^2 y R^3 es cada uno t-butilo y R^4 es ciclopentilo.

12. El compuesto de fórmula I conforme a la reivindicación 1, en el que R^1 es hidroxilo, R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es ciclohexilo y R^4 es ciclopentilo.

13. El compuesto de fórmula I conforme a la reivindicación 1, en el que R^1 es hidroxilo, R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, y R^3 y R^4 es cada uno ciclohexilo.

14. El compuesto de fórmula I conforme a la reivindicación 1, en el que R^1 es hidroxilo, R^2 es ciclopentilmetilo, R^3 es t-butilo y R^4 es ciclobutilo.

15. El compuesto de fórmula I conforme a la reivindicación 1, en el que R^1 es hidroxilo, R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es t-butilo y R^4 es ciclobutilo.

16. El compuesto de fórmula I conforme a la reivindicación 1, en el que R^1 es NHSO_2Me , R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es t-butilo y R^4 es ciclopentilo.

17. El compuesto de fórmula I conforme a la reivindicación 1, en el que R^1 es NHSO_2Ph , R^2 es $\text{CH}_2\text{-C}(\text{CH}_3)_3$, R^3 es t-butilo y R^4 es ciclopentilo.

18. Una composición farmacéutica que comprende una cantidad eficaz contra el virus de la hepatitis C de un compuesto de fórmula I conforme a una cualquiera de las

reivindicaciones 1 a 17, o una sal farmacéuticamente aceptable del mismo, mezclado con un vehículo o agente auxiliar farmacéuticamente aceptable.

19. La composición farmacéutica conforme a la
5 reivindicación 18, que comprende además una cantidad terapéuticamente eficaz de uno o más de otros agentes anti-VHC.

20. La composición farmacéutica conforme a la
10 reivindicación 19, en la que dicho otro agente anti-VHC se selecciona de: α -interferón, o α -interferón pegilado.

21. La composición farmacéutica conforme a la reivindicación 19 ó 20, en la que dicho otro agente anti-VHC es ribavirina.

22. La composición farmacéutica conforme a la
15 reivindicación 19, 20 ó 21, en la que dicho otro agente anti-VHC se selecciona de inhibidores de: helicasa, proteasa NS2/3 y sitio de entrada interno del ribosoma (IRES).

23. Un método para el tratamiento o prevención de
20 una infección viral de hepatitis C en un mamífero, administrando al mamífero una cantidad eficaz contra el virus de la hepatitis C de un compuesto de fórmula 1 conforme a una cualquiera de las reivindicaciones 1 a 17, o una sal farmacéuticamente aceptable del mismo.

25 24. Un método para el tratamiento o prevención de una infección viral de hepatitis C en un mamífero, administrando al mamífero una cantidad eficaz contra el virus de la hepatitis C de la composición conforme a una cualquiera de las reivindicaciones 18 a 22.

30 25. Un método para el tratamiento o prevención de una infección viral de hepatitis C en un mamífero, administrando al mamífero una cantidad eficaz contra el virus de la hepatitis C de un compuesto de fórmula I según una cualquiera de las reivindicaciones 1 a 17 o una

sal terapéuticamente aceptable del mismo en combinación con uno o más de otros agentes anti-HCV.

26.- El método conforme a la reivindicación 25, en el que dicho otro agente anti-HCV se selecciona entre: α -
5 interferón o α -interferón pegilado.

27.- El método conforme a la reivindicación 25 ó 26, en el que dicho otro agente anti-HCV es ribavirina.

28. El método conforme a la reivindicación 25, 26 ó 27, en el que dicho otro agente anti-HCV se selecciona
10 entre inhibidores de: helicasa, NS2/3-proteasa y sitio de entrada de ribosomas internos (IRES).

29. Uso de un compuesto de fórmula I conforme a una cualquiera de las reivindicaciones 1 a 17, para la elaboración de un medicamento para el tratamiento o
15 prevención de una infección viral de hepatitis C.

30. La composición farmacéutica conforme a la reivindicación 19, 20 ó 21, en la que dicho otro agente anti-HCV es un inhibidor de polimerasa de HCV.

31. El método conforme a la reivindicación 25, 26 ó
20 27, en el que dicho otro agente anti-HCV es un inhibidor de polimerasa de HCV.

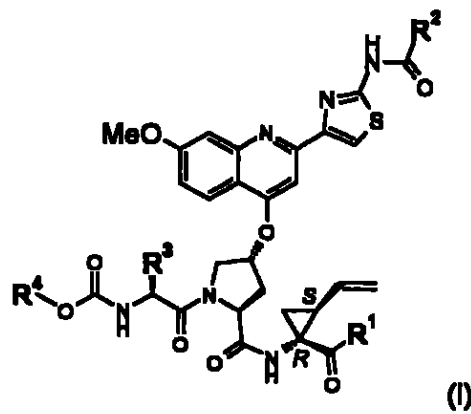
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30 COLO 09255-841-008-7
PRP/WPCL9255/ID-000242

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RESUMEN

Compuestos de fórmula (1):



(I)

en la que R¹ es hidroxilo o un derivado de sulfonamida; R²
 5 es t-butilo o -CH₂-C(CH₃)₃ o -CH₂-ciclopentilo; R³ es t-
 butilo o ciclohexilo y R⁴ es ciclobutilo, ciclopentilo o
 ciclohexilo; o una sal farmacéuticamente aceptable de los
 mismos, se describe que son útiles como inhibidores de la
 proteasa NS3 del VHC.

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25 COLO 09255-841-008-7
 PRP/WPCL9255/ID-000242

LISTADO DE SECUENCIAS

143

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FORMATO DE DIGITALIZACION
RELACION DE ANEXOS

Expediente No		34085343	No de Follo
Item		No de unidades	✓
1	CD		
2	DVD		
3	REVISTA		
4	LIBRO		
5	PERIODICO		
6	DISQUETES		
7	SOBRE DE MANILA		
8	SOBRE BLANCO TAMAÑO CARTA		
9	SOBRE BLANCO TAMAÑO OFICIO		
10	OTROS:		

Observaciones:

Sobra de arte final

145
145
SUPERINDUSTRIA Y COMERCIO
Radicacion : 04005343 00000000
Fecha (AMD): 2004-08-31 17:42:47
Tramite : 011 PCT-PATENT 379 FASE INCI 411 PRESENTAR
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

MIT : 800174089-2

RECIBO OFICIAL DE CAJA : 04 - 66,29R
FECHA : AGOSTO 23 DE 2004

SUPERINDUSTRIA Y COMERCIO
Radicacion : 04005343 00000000
Fecha (AMD): 2004-08-31 17:42:47
Tramite : 011 PCT-PATENT 1 REGISTRO/ 411 PRESENTAR
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

CONSIGNACION

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	23490083	23/08/2004	34,210,600.00

CONCEPTO

CANT. HISTORICO	CONCEPTO	TOTAL CONCEPTO
1 50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	422,000.00
	TOTAL :	\$ 422,000.00

SOM: CUATROCIENTOS VEINTIDOS MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

Cdo 09255-841-008-7

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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 ()	()
DISEÑO INDUSTRIAL ()		
- Indicación de que se solicita el registro de Diseño Industrial	()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud	()	()
- Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño	()	()
Comprobante de pago de las tasas establecidas	()	()
COMPLETA ()	INCOMPLETA ()	()
ESQUEMA DE TRAZADO ()		
- Indicación de que solicita el registro de un Esquema de trazado	()	()
- Datos de identificación del solicitante o de la persona que presenta La solicitud	()	()
Representación gráfica del esquema de trazado	()	()
Comprobante de pago de las tasas establecidas	()	()
COMPLETA ()	INCOMPLETA ()	()

Firma Responsable



Fecha

31 / 05 - 04



142
Industria y Comercio
SUPERINTENDENCIA

2020
Bogotá, D.C.,

Doctor:
EMILIO FERRERO
Apoderado
BOEHRINGER INGELHEIM INTERNATIONAL GMBH

Oficio N° 48852

ASUNTO:	Radicación N°	04-85343
	Solicitud internacional	PCT/CA2003/00091
	Fecha inicio fase nacional	01/09/2004
	Trámite	011
	Evento	379
	Actuación	430
	Folios	001

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

□ La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486).

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

El anterior requerimiento fue notificado por fijación en lista de fecha, 24 SET. 2004

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11/11