

No. 05-070211- -00003-0000

Fecha: 2007-07-31 08:07:20 Dep. 2020 NUECREACIONE
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 400 OPOSIOBSERVTER Folios: 145

FORMULARIO UNICO DE OPOSICION

①

OPOSICION A:

- | | |
|--|--|
| ☒ Patente de Invención. | ☐ Marca Colectiva. |
| ☐ Patente de Modelo de Utilidad. | ☐ Marca de Certificación. |
| ☐ Diseño Industrial. | ☐ Lema Comercial. |
| ☐ Esquema de Trazado para Circuitos Integrados. | |
| ☐ Marcas de Productos o Servicios. | |

②

Numero del expediente: 05-70211

Solicitante: BOEHRINGER INGELHEIM INTERNATIONAL GMBH

SOLICITUD
PUBLICADA

Apoderado: EMILIO FERRERO

Título de la nueva creación o denominación del signo: "USO DE LA ASOCIACIÓN QUE CONTIENE UN INHIBIDOR NO NUCLEOSIDO DE LA TRANSCRIPTASA INVERSA (NNRTI) CON UN INHIBIDOR DE CITOCROMO P450, TALES COMO LOS INHIBIDORES DE LA PROTEASA".

Clase: 5/5

③ OPOSICION PRESENTADA POR:

SOLICITANTE	Nombre: PROCAPS S.A.	IDENTIFICACION	
	Dirección: CALLE 80 No. 78B -201	C.C. ☐	NIT ☒
	Nacionalidad o Domicilio: COLOMBIA	C.E. ☐	Otro ☐
	Lugar de Constitución: BARRANQUILLA	Cual	
	Teléfono: (571)3719900 Fax: (571)3730818	Número 896.106.527-5	
	E-mail:		
REPRESENTANTE O APODERADO	Nombre: LAURA CATALINA SILVA BARRERA	IDENTIFICACION	
	Dirección: CARRERA 13 No. 119-95	C.C. ☐	NIT ☒
	OF: 104	C.E. ☐	Otro ☐
	Teléfono: (571) 2131213 Fax: (571) 6121979	Cual	
	E-mail: negocios@optimum-co.com	Número 52.455.803 TP 147399	

④ FUNDAMENTOS DE LA OPOSICION:

Causal: Art.14, 16, 18, 20 y 30 de la Decisión 486 de la CAN

No cumple con los requisitos de patentabilidad.

Signo opositor:

Justificación:

La presente solicitud no cumple con los requisitos de patentabilidad dado que lo revelado, ya se encontraba en el estado de la técnica o es posible derivarlo de la misma tal y como se demostrará en el capítulo "Fundamento de Hechos".

⑤ Comprobante de pago No. _____

Fecha _____

83

⑥ ANEXOS

- ☒ Comprobante de pago de la tasa.
- ☒ Poderes, si fuere el caso.
- ☒ Pruebas de los fundamentos de la oposición.
- ☐ Documentos que acrediten el legítimo interés, si fuere el caso.
- ☒ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.

⑦

NOMBRE: LAURA CATALINA SILVA BARRERA

FIRMA:



C.C. 52.455.803

TP. 147399

Señora Jefe,

DIVISIÓN DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

Referencia : Expediente N° 05070211

Asunto : Oposición a la solicitud de patente de Invención titulada
"USO DE UNA ASOCIACIÓN QUE CONTIENE UN
INHIBIDOR NO NUCLEOSIDO DE LA TRANSCRIPTASA
INVERSA (NNRTI) CON UN INHIBIDOR DE
CITOCROMO P450, TALES COMO LOS INHIBIDORES
DE LA PROTEASA".

Solicitante : BOEHRINGER INGELHEIM INTERNACIONALGMBH.

Opositora : PROCAPS S.A.

Publicada en la Gaceta de la Propiedad Industrial

N° 575 del 30 de Abril de 2007

Yo, **LAURA CATALINA SILVA BARRERA**, abogada con tarjeta profesional N° 147399 del Consejo Superior de la Judicatura, en mi condición de apoderada de la sociedad **PROCAPS S.A.** conformada bajo las leyes de la República de Colombia y domiciliada en la ciudad de Barranquilla, Colombia, atentamente manifiesto que estando dentro del término de ley y por orden expresa de mi representada, presento oposición a la concesión del registro de la Patente de Invención titulada **"USO DE UNA ASOCIACIÓN QUE CONTIENE UN INHIBIDOR NO NUCLEOSIDO DE LA TRANSCRIPTASA INVERSA (NNRTI) CON UN INHIBIDOR DE**

84
85

CITOCROMO P450, TALES COMO LOS INHIBIDORES DE LA PROTEASA",
solicitada por *BOEHRINGER INGELHEIM INTERNACIONALGMBH*, y pido a
Usted declarar fundada esta oposición y en consecuencia negar el registro de la patente
solicitada.

1. INTERÉS PARA ACTUAR

Conforme a lo dispuesto en el artículo 42 de la Decisión 486 de 2000 de la
CAN, estando dentro del término estipulado por la ley, manifiesto el legítimo interés
de nuestra representada para oponerse al registro de la patente de la referencia,
atendiendo a que la solicitud pretendida no cumple con los requisitos dispuestos en la
ley como se analizará más adelante y de ser concedida afectaría directamente la
comercialización de algunos productos de mi representada, que contienen, en
particular Ritonavir. Por lo tanto, solicito respetuosamente a ese Despacho se sirva
tener en cuenta la presente oposición dentro del trámite administrativo que se surte en
esta Superintendencia.

2. FUNDAMENTOS DE HECHOS

2.1. La solicitud objeto de la presente fue presentada el 15 de julio de 2005,
reivindicando prioridad bajo la solicitud US60/433690 del 2002-12-16 y fue
publicada en Colombia, en la Gaceta de la Propiedad Industrial N° 575 del 30 de
Abril de 2007, página 482, N° publicación 1391, cuya publicación internacional
corresponde a la WO2004054586 del 2004-07-01.

2.2. La solicitud colombiana 05070211 objeto de la presente oposición, se refiere a una asociación de una cantidad terapéuticamente eficaz del compuesto de la fórmula I o una sal farmacéuticamente aceptable del mismo y una cantidad de un inhibidor de los citocromos P450, que es eficaz para mejorar la farmacocinética del compuesto de la fórmula I, donde el inhibidor de los citocromos P450 es un inhibidor de la CYP3A, donde el inhibidor de los citocromos P450 se selecciona del grupo que consiste en: amprenavir, atazanavir, claritromicina, ciclosporina, diiltiazem, eritomicina, itraconazol, indinavir, cetoconazol, mibefradil, nefazodona, nelfinavir, ritonavir, vitamina E, bergamotina, dihidroxibergamotina, zumo de pomelo y las sales farmacéuticamente aceptables de los mismos, donde el inhibidor de los citocromos P450 es ritonavir o una sal farmacéuticamente aceptable de los mismos, donde se provee en una cantidad de 50 mg a 3.000 mg del compuesto de la fórmula I o una sal farmacéuticamente aceptable del mismo, donde provee una cantidad de 30 mg a 500 mg de ritonavir o una sal aceptable del mismo. La solicitud también se refiere a una asociación caracterizada por que el compuesto de la fórmula I o una sal farmacéuticamente aceptable del mismo y el inhibidor de los citocromos P450 se formulan como una composición única, o donde el compuesto de la fórmula I o una sal farmacéuticamente aceptable del mismo y el inhibidor de los citocromos P450 se formulan como composiciones independientes.

2.3. Ahora bien, con base en el artículo 14 de la Decisión 486 de la CAN consagra lo siguiente: *"Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial"*. Así las cosas, las invenciones objeto de patente deben ser nuevas y tener nivel inventivo. Bien, pues antes de la fecha de prioridad de esta solicitud se encuentran los siguientes documentos que afectan la novedad y el nivel inventivo de la presente solicitud:

- D1: WO9844913. 1998-10-15
- D2 WO0196338. 2001-12-20
- D3: US6391919. 2002-05-21.

2.4. Ahora bien, el documento D1, particularmente en las reivindicaciones 1 a 7 y 17 a 22, revela el uso de una cantidad efectiva de un análogo de nucleósido (MKC-442) que funciona como un inhibidor no nucleosido de la transcriptasa inversa (Ver descripción página 2, líneas 10 y 11) que en combinación con un inhibidor de proteasa (ver reivindicación 1) es útil en el tratamiento de pacientes infectados con VIH. Como tal, este documento D1 evidencia la preexistencia de una asociación que contiene un inhibidor no nucleosido de la transcriptasa inversa (NNRTI) con un inhibidor de la proteasa, así como su utilidad. Por lo tanto, a los ojos de este documento D1, la presente solicitud no es novedosa.

2.5. Por su parte, el documento D2 evidencia la preexistencia de inhibidores no nucleosido de la transcriptasa inversa (NNRTI). Adicionalmente, a partir de la página línea 6, dicho documento D2 se refiere a que este, combinado con otros agentes terapéuticos, son útiles en el tratamiento de la infección por VIH. Particularmente, este documento D2 se refiere a un inhibidor no nucleosido de la transcriptasa inversa (NNRTI), que combinado, es útil en el tratamiento de la infección por VIH. Ahora bien, el documento D3, revela en el capítulo reivindicatorio, particularmente en las cláusulas 21 a 24, una combinación útil en el tratamiento de pacientes infectados con VIH, que comprende al menos un inhibidor de la transcriptasa inversa en combinación con un inhibidor de proteasa tal como el ritonavir (ver cláusula 23). A todas luces, cualquier persona versada en la materia, a partir de lo aquí referenciado en estos documentos D2 y D3, en combinación con la materia expuesta en el documento D1, de manera obvia puede

llegar a la solución propuesta en la presente solicitud objeto de oposición, de manera que afecta evidentemente su altura o nivel inventivo. En efecto, cualquier persona, a partir de lo indicado en estos documentos D2 y D3 combinados con D1, puede llegar a una asociación que contiene un inhibidor no nucleosido de la transcriptasa inversa (NNRTI) ampliamente definido en D2, con un inhibidor de la proteasa, combinación conocida como lo evidencia D1, donde el inhibidor de la proteasa se puede seleccionar a partir de ritonavir, tal como lo revelan las reivindicaciones 21 a 24 de D3. Por lo tanto, la presente solicitud también carece de altura o nivel inventivo. Por lo anterior la materia revelada en la presente solicitud no comprende altura o nivel inventivo toda vez que a los ojos de los documentos D2 y D3 en combinación con D1, se puede llegar a la solución propuesta, además que dicha solución se basa en la mera combinación conocida de diferentes entidades conocidas del arte anterior para solucionar o tratar un problema ya tratado de igual forma con anterioridad.

2.6. Por lo tanto, este documento objeto de oposición a la luz de los documentos D1 a D3, carece evidentemente de novedad y de altura o nivel inventivo.

2.7. En consecuencia y de acuerdo a lo descrito anteriormente, se deriva que la solicitud colombiana 05070211 no puede pretender un monopolio por patente toda vez que lo que pretende no cumple con dos de los tres requisitos necesarios para su protección.

2.8. Que por consiguiente, respecto a la solicitud pretendida se tiene que:

2.8.1. **CARECE DE NOVEDAD:** Según la decisión 486 de la Comunidad Andina de Naciones, Artículo 16.- *"Una invención se considerará nueva cuando no está*

comprendida en el estado de la técnica. El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida.” A la fecha de prioridad, 16 de diciembre de 2002, ya era conocido tal y como lo revela el arte anterior con base en el documento D1, una asociación que contiene un inhibidor no nucleosido de la transcriptasa inversa (NNRTI) con un inhibidor de la proteasa, así como su utilidad, sobre el cual busca protección en el capítulo reivindicatorio. Por lo tanto la presente solicitud no es novedosa.

2.8.2. CARENCIA DE ALTURA INVENTIVA. Según la Decisión 486 de la Comunidad Andina de Naciones, Artículo 18.- “Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica.” A la fecha de prioridad, el 16 de diciembre de 2002, a partir de los documentos D2 y D3 en combinación con D1, es posible deducir una asociación que contiene un inhibidor no nucleosido de la transcriptasa inversa (NNRTI) ampliamente definido en D2, con un inhibidor de la proteasa, combinación conocida como lo evidencia D1, donde el inhibidor de la proteasa se puede seleccionar a partir de ritonavir, tal como lo revelan las reivindicaciones 21 a 24 de D3, según lo definido en las reivindicaciones de la presente solicitud. De esta forma, la presente solicitud tampoco comprende altura o nivel inventivo

2.9. Dado lo anterior, es posible deducir que la solicitud y lo reivindicado no es objeto de patentabilidad pues es contrario a lo que dicta la Decisión 486 de la Comunidad Andina de Naciones, Artículo 14.- “Los Países Miembros otorgarán

89
90

patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial.”. La solicitud pretendida no cumple con dos de los tres requisitos de patentabilidad.

2.10. Por todo lo anterior, atentamente solicito a usted que se sirva declarar fundada la presente oposición y negar el registro Patente de invención denominada ***“USO DE UNA ASOCIACIÓN QUE CONTIENE UN INHIBIDOR NO NUCLEOSIDO DE LA TRANSCRIPTASA INVERSA (NNRTI) CON UN INHIBIDOR DE CITOCROMO P450, TALES COMO LOS INHIBIDORES DE LA PROTEASA”***, solicitada por ***BOEHRINGER INGELHEIM INTERNACIONALGMBH***, solicitud Colombiana que apareció publicada en la Gaceta de la Propiedad Industrial N° 575.

3. PRUEBAS

De conformidad con lo dispuesto por las normas vigentes, solicito a usted tener como prueba en este asunto los siguientes:

- D1: WO9844913. 1998-10-15
- D2 WO0196338. 2001-12-20
- D3: US6391919. 2002-05-21.

4. FUNDAMENTO DE DERECHO

Fundamento esta observación en las normas legales siguientes:

- 4.1. Numeral 1 del artículo 586 del Código de Comercio.
- 4.2. Artículo 42 de la Decisión 486 de la Comisión del Acuerdo de Cartagena.
- 4.3. En las demás normas concordantes.

5. ANEXOS

Para los efectos correspondientes anexo al presente escrito los siguientes documentos:

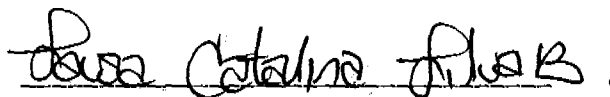
1. Poder para actuar en el presente.
2. Documento que acredita al representante legal de PROCAPS S.A.
3. Recibo de caja del respectivo pago de tasa.
4. Copias de los documentos:

- D1: WO9844913. 1998-10-15
- D2: WO0196338. 2001-12-20
- D3: US6391919. 2002-05-21.

6. NOTIFICACIONES

Para efectos de notificaciones que por disposición del artículo 50 del Decreto 01 de 1984 debe hacer su Despacho, informo que mi representada y la suscrita recibiremos notificaciones en la Secretaría de su Despacho o en mis oficinas situadas en la Carrera 13 N° 119-95, Of. 104 de esta ciudad de Bogotá.

Señor Superintendente,



LAURA CATALINA SILVA BARRERA

C.C. 52'455.803 de Bogotá

T.P.A. 147399 del C.S. de la J.

92
05

ANEXO 1

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: A61K 31/00	A2	(11) International Publication Number: WO 98/44913 (43) International Publication Date: 15 October 1998 (15.10.98)
(21) International Application Number: PCT/US98/07248 (22) International Filing Date: 7 April 1998 (07.04.98) (30) Priority Data: 60/041,909 7 April 1997 (07.04.97) US (71) Applicant (for all designated States except US): TRIANGLE PHARMACEUTICALS, INC. [US/US]; 4 University Place, 4611 University Drive, Durham, NC 27707 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): FURMAN, Phillip, A. [US/US]; 901 Bluestone Road, Durham, NC 27713 (US). MOXHAM, Carey, P. [US/US]; 7 Hickorywood Square, Durham, NC 27713 (US). BARRY, David [US/US]; 1810 Southlakeshore Drive, Chapel Hill, NC 27515 (US). DORROTO-ESODO, Katyna [US/US]; 12408 Tappersfield Court, Raleigh, NC 27613 (US). (74) Agent: KNOWLES, Sherry, M.; King & Spalding, 191 Peachtree Street, Atlanta, GA 30303-1763 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, ~~BB~~ , BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, ~~GB~~ , GE, GH, GM, GW, HU, IL, IS, JP, KE, KG, KP, KR, ~~KZ~~ , LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, ~~MX~~ , NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, ~~IE~~ , IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: USE OF MKC-442 IN COMBINATION WITH OTHER ANTIVIRAL AGENTS		
(57) Abstract Use of MKC-442 in combination or alternation with other antiviral agents for the treatment of patients infected with HIV is disclosed.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

USE OF MKC-442 IN COMBINATION WITH OTHER ANTIVIRAL AGENTS

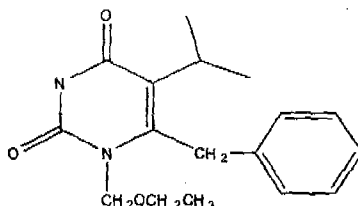
In 1983, the etiological cause of AIDS was determined to be the human immunodeficiency virus (HIV). In 1985, it was reported that the synthetic nucleoside 3-azido-3'-deoxythymidine (AZT) inhibits the replication of human immunodeficiency virus. Since then, a number of other synthetic nucleosides, including 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), and 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), have been proven to be effective against HIV. After cellular phosphorylation to the 5'-triphosphate by cellular kinases, these synthetic nucleosides are incorporated into a growing strand of viral DNA, causing chain termination due to the absence of the 3'-hydroxyl group. They can also inhibit the viral enzyme reverse transcriptase.

The success of various synthetic nucleosides in inhibiting the replication of HIV *in vivo* or *in vitro* has led a number of researchers to design and test nucleosides that substitute a heteroatom for the carbon atom at the 3'-position of the nucleoside. European Patent Application Publication No. 0 337 713 and U.S. Patent No. 5,041,449, assigned to BioChem Pharma, Inc., disclose racemic 2-substituted-4-substituted-1,3-dioxolanes that exhibit antiviral activity. U.S. Patent No. 5,047,407 and European Patent Application No. 0 382 526, also assigned to BioChem Pharma, Inc., disclose that a number of racemic 2-substituted-5-substituted-1,3-oxathiolane nucleosides have antiviral activity, and specifically report that the racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (referred to below as BCH-189) has approximately the same activity against HIV as AZT, with little toxicity. The (-)-enantiomer of the racemate BCH-189, known as 3TC, which is covered by U.S. Patent No. 5,539,116 to Liotta *et al.*, is currently sold for the treatment of HIV in combination with AZT in humans in the U.S.

It has also been disclosed that cis-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC") has potent HIV activity. Schinazi, *et al.*, "Selective Inhibition of Human Immunodeficiency Viruses by Racemates and Enantiomers of cis-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolane-5-yl]Cytosine" *Antimicrobial Agents and Chemotherapy*, November 1992, pp. 2423-2431. See also U.S. Patent No. 5,210,085; WO 91/11186, and WO 92/14743.

Another compound that exhibits efficacy against HIV both *in vitro* and *in vivo* is 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil, which is also known as MKC-442.

MKC-442



MKC-442 is described, for example, in U.S. Patent No. 5,461,060.

MKC-442, although a nucleoside analogue, functions as a non-nucleoside reverse transcriptase inhibitor. It is considered an allosteric inhibitor because it appears to exert its activity by binding to an "allosteric position", i.e., one other than the binding site, of the enzyme. Preclinical tests suggest that MKC-442 may possess characteristics that address several of the therapeutic challenges of HIV. When tested in cell culture assay systems against wild-type (drug-sensitive) and several mutant strains of HIV known to be resistant to established non-nucleoside reverse transcriptase inhibitors, MKC-442 retained much of its ability to inhibit HIV replication. In these studies, MKC-442 displayed greater potency than nevirapine against wild-type and mutant strains of HIV. Preclinical studies of MKC-442 in two drug combinations with AZT or with DDI and in three drug combinations with AZT and saquinavir have demonstrated synergistic inhibition of HIV replication.

Studies in animals suggest a favorable safety and pharmacokinetic profile for MKC-442. Animal pharmacokinetic analyses showed good oral bioavailability and excellent penetration into the central nervous system, a significant site of HIV replication that is poorly penetrated by many currently marketed anti-HIV drugs. In rats, for example, the concentration of MKC-442 in the brain was 100% of that seen in the plasma.

A Phase I study evaluated the pharmacokinetics and tolerance of single escalating doses of MKC-442 in HIV-infected volunteers. The compound was generally well tolerated, with only a few participants experiencing minor adverse effects at the higher dose levels. In the groups receiving higher doses, concentrations of the drug in the plasma reached levels much higher than the levels required to suppress 90% of the virus in culture.

97
3

Preliminary data from a Phase I/II double-blind, placebo controlled trial designed to evaluate the safety and efficacy of repeated multiple oral doses of MKC-442 in HIV-infected patients has now also been evaluated. A total of 49 patients were treated with MKC-442 for up to two months. Doses ranging from 100 mg to 1000 mg twice a day were given to groups of six to eight patients at each dosage level. At the highest doses tested (705 mg and 1000 mg twice a day), the viral load was reduced by an average of 96% in all patients after one week. This reduction was mostly sustained at two weeks whereafter it was followed by a gradual increase in viral load from the nadir toward baseline levels. A single point mutation at position 13 of the reverse transcriptase that may be associated with resistance was found in the virus obtained from some patients. In over 308 patient-weeks of drug exposure, MKC-442 was well tolerated.

It is known that over a period of time, agents such as MKC-442 that are active against HIV induce mutations in the virus which reduce the efficacy of the drug. There is a need to improve the durability of antiviral efficacy produced by antiretroviral drugs, including MKC-442, by decreasing the rate at which such mutations arise. Further, although MKC-442 exhibits a favorable pharmacokinetic and biodistribution profile, there is always a desire to improve these parameters. There is also a need to decrease the metabolism of the drug, which can lead to an increase in the plasma concentration of or exposure to MKC-442.

U.S. Patent No. 5,604,209, issued on February 18, 1997 to Ubasawa et al., and assigned to Mitsubishi Chemical Corporation, discloses that certain 6-benzyl-1-ethoxymethyl-5-substituted uracil derivatives, including MKC-442, and certain 2',3'-dideoxyribonucleosides, including 2',3'-dideoxyinosine (DDI), 3'-azido-3'-deoxythymidine (AZT), AZT triphosphate, and 2',3'-dideoxycytidine (DDC), exhibit a synergistic effect against HIV.

Japanese Patent Application No. 9-18384 filed on January 31, 1997, by Mitsubishi Chemical Corporation, discloses a method for the treatment of HIV that includes the administration of a 6-benzyl-1-ethoxymethyl-5-substituted uracil derivative, including MKC-442, in combination with two or more nucleoside-type reverse transcriptase inhibitors or their esters, and in particular, those selected from the group consisting of AZT, 2',3'-dideoxy-3'-thiacytidine (3TC), PMEA (9-(2-phosphonylmethoxyethyl) adenine (Gilead); PMPA: (R)-9-(2-phosphonyl-methoxypropyl)adenine); 1592U89 succinate ((1S,4R)-4-[2-amino-6-

98
25

cyclopropyl-amino)-9H-purin-9-yl]-2-cyclopentene-1-methanol succinate); 2',3'-dideoxyinosine (DDI); and 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), and esters thereof.

In light of the strong activity of MKC-442 against HIV, it is an object of the present invention to provide a method and composition that includes MKC-442 for the treatment of patients infected with HIV that exhibits advantageous or improved pharmacokinetic, biodistribution, metabolic, resistance or other parameters over administration of MKC-442 alone.

It is also an object of the invention to improve the efficacy of MKC-442 during short periods of administration and over extended time periods.

It is yet another object of the present invention to provide a method and composition for the treatment of patients infected with HIV in which MKC-442 is administered in combination or alternation with a second compound that acts synergistically with MKC-442 against the virus.

It is still another object of the present invention to provide a method and composition for the treatment of patients infected with HIV in which MKC-442 is administered in combination or alternation with a second compound (or at least one other compound) that acts synergistically with MKC-442 against the virus.

SUMMARY OF THE INVENTION

It has been discovered that MKC-442 can be administered in combination with one or more antiviral agents to achieve an advantageous therapeutic effect against HIV. In some cases, the enhanced therapeutic effect is not attainable by administration of either agent alone. In a preferred but not necessary embodiment, the effect of administration of the two agents in combination or alternation is synergistic.

In one preferred embodiment, MKC-442 is administered in combination with a protease inhibitor. In particular embodiments, MKC-442 is administered in combination or alternation with indinavir (Crixivan), nelfinavir ([3S-[2(2S*,3S*),3-alpha,4-a-beta,8a-beta-]]-N-(1,1-dimethylethyl)decahydro-2-)-2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylthio)butyl]-3-isoquinolincarboxamide mono-methanesulfonate) (Viracept), saquinavir (Invirase), or 141W94 (amprenavir; (S)-tetrahydrofuran-3-yl-N-[(1S,2R)-3-

97
100

[N-[(4-aminophenyl)sulfonyl]-N-isobutylamino]-1-benzyl-2-hydroxypropyl]carbamate; or efavirenz (S)-6-chloro-4-(cyclopropylethynyl)-1, 4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one.).

In another preferred embodiment, MKC-442 is administered in combination or alternation with a nucleoside analog, including FTC, 3TC, or abacavir (1592U89) which is
5 (1S,4R)-4-[2-amino-6-cyclopropyl-amino)-9H-purin-9-yl]-2-cyclopentene-1-methanol succinate.

In another embodiment, MKC-442 is administered in combination with a nonnucleoside reverse transcriptase inhibitor such as DMP-266 (efavirenz; 1,4-dihydro-2H-3,1-benzoxazin-2-one); delavirdine, (1-[3-(1-methyl-ethyl)amino]-2-pyridinyl-4-
10 [[5-[(methylsulfonyl)amino]-1H-indol-2-yl]carbonyl]-, monoethanesulfonate); or nelfinavir ([3S-(3R*, 4aR*, 8aR*, 2'S*, 3'S*)]-2-[2'-hydroxy-3'-phenylthiomethyl-4'-aza-5'-oxo-5'-(2"-methyl-3"-hydroxyphenyl)pentyl]-decahydroiso-quinoline-3-N-t-butylcarboxamide methanesulfonic acid salt.

In general, during alternation therapy, an effective dosage of each agent is
15 administered serially, whereas in combination therapy, an effective dosage of two or more agents are administered together. The dosages will depend on such factors as absorption, biodistribution, metabolism and excretion rates for each drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject,
20 specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. Examples of suitable dosage ranges for protease inhibitors, for example, nelfinavir and indinavir, can be found in the scientific literature and in the Physicians Desk Reference. Many examples of suitable dosage ranges for other
25 compounds described herein are also found in public literature or can be identified using known procedures. These dosage ranges can be modified as desired to achieve a desired result.

The disclosed combination and alternation regimens are useful in the prevention and treatment of HIV infections and other related conditions such as AIDS-related complex
30 (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological

conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma, thrombocytopenia purpura and opportunistic infections. In addition, these compounds or formulations can be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is an isobologram analysis of the combination of MKC-442 and indinavir in cell culture.

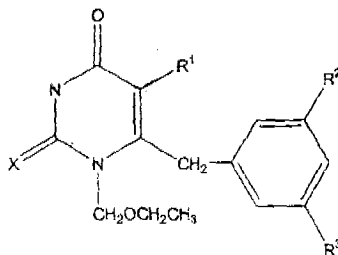
Figure 2 is an isobologram analysis of the combination of MKC-442 and nelfinavir in cell culture.

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that MKC-442 can be administered in combination with one or more antiviral agents to achieve an advantageous therapeutic effect to inhibit HIV replication. In most cases, the enhanced therapeutic effect is not attainable by administration of either agent alone. In a preferred but not necessary embodiment, the effect of administration of the two agents in combination or alternation is synergistic.

I. MKC-442 and Related Compounds

In one embodiment, an effective HIV-treatment amount of a compound of the formula:



wherein X is oxygen or sulfur, R¹ is ethyl or isopropyl, R² and R³ are independently hydrogen, C₁-C₄ alkyl or halogen (chlorine, bromine, iodine, or fluorine) is administered to a patient in need thereof in combination with a protease inhibitor or with another anti-HIV compound as described herein. In a preferred embodiment, the compound is MKC-442.

5 Any of the compounds described herein for combination or alternation therapy can be administered as any derivative that upon administration to the recipient, is capable of providing directly or indirectly, the parent compound, or that exhibits activity itself. Nonlimiting examples are the pharmaceutically acceptable salts (alternatively referred to as "physiologically acceptable salts"), and a compound which has been alkylated or acylated at
10 an appropriate position. The modifications can affect the biological activity of the compound, in some cases increasing the activity over the parent compound. This can easily be assessed by preparing the derivative and testing its antiviral activity according to known methods.

As used herein, the term pharmaceutically acceptable salts refers to salts that
15 retain the desired biological activity of the herein-identified compounds and exhibit minimal undesired toxicological effects. Non-limiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as amino acid, acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid,
20 benzoic acid, tannic acid, palmoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, naphthalenedisulfonic acid, and polygalacturonic acid; (b) base addition salts formed with metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with a cation formed from ammonia, N,N-dibenzylethylene-diamine, D-glucosamine, tetraethylammonium, or
25 ethylenediamine; or (c) combinations of (a) and (b); e.g., a zinc tannate salt or the like.

10222
103

II. Combination or Alternation Therapy

It has been recognized that drug-resistant variants of HIV can emerge after prolonged treatment with an antiviral agent. Drug resistance most typically occurs by mutation of a gene that encodes for an enzyme used in the viral life cycle, and most typically in the case of HIV, in either the reverse transcriptase or protease genes. It has been demonstrated that the efficacy of a drug against HIV infection can be prolonged, augmented, or restored by administering the compound in combination or alternation with a second, and perhaps third, antiviral compound that induces a different mutation(s) from that selected for by the principle drug. Alternatively, the pharmacokinetics, biodistribution, or other parameter of the drug can be altered by such combination or alternation therapy. In general, combination therapy is typically preferred over alternation therapy because it induces multiple simultaneous stresses on the virus.

The second antiviral agent for the treatment of HIV, in one embodiment, can be a protease inhibitor, or a reverse transcriptase inhibitor (a "RTI"), which can be either a synthetic nucleoside (a "NRTI") or a non-nucleoside compound (a "NNRTI"). In other embodiments, the second (or third) compound can be a pyrophosphate analog, or a fusion binding inhibitor. A list compiling resistance data collected *in vitro* and *in vivo* for a number of antiviral compounds is found in Schinazi et al., mutations in retroviral genes associated with drug resistance, *International Antiviral News*, Volume 1 (4), International Medical Press 1996.

In preferred embodiments, MKC-442 is administered in combination or alternation with FTC (2',3'-dideoxy-3'-thia-5-fluorocytidine); 141W94 (amprenavir, GlaxoWellcome, Inc.); Viramune (nevirapine), Rescriptor (delavirdine); or DMP-266 (efavirenz). In another preferred embodiment, MKC-442 is administered in combination or alternation with abacavir (1592U89), which is (1S,4R)-4-[2-amino-6-cyclopropyl-amino)-9H-purin-9-yl]-2-cyclopentene-1-methanol succinate.

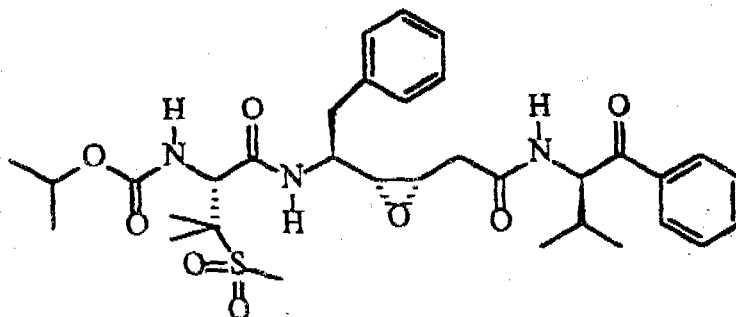
Other examples of antiviral agents that can be used in combination or alternation with the compounds disclosed herein for HIV therapy include 3TC; foscarnet; carbovir, acyclovir, interferon, stavudine, CS-92 (3'-azido-2',3'-dideoxy-5-methyl-cytidine), and b-D-

dioxolane nucleosides such as b-D-dioxolanylguanine (DXG), b-D-dioxolanyl-2,6-diaminopurine (DAPD), and b-D-dioxolanyl-6-chloropurine (ACP).

Preferred protease inhibitors include indinavir ({1(1,S,2R),5(S)}-2,3,5-trideoxy-N-(2,3-dihydro-2-hydroxy-1H-inden-1-yl)-5-[2-[[{(1,1-dimethylethyl)amino]carbonyl}-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-D-erythro-pentoamide sulfate; Merck),
 5 nelfinavir (Agouron), ritonavir (Abbot), saquinavir (Roche) and DMP-450 {[4R-4(r-a,5-a,6-b,7-6)]-hexahydro-5,6-bis(hydroxy)-1,3-bis(3-amino)phenyl)methyl)-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one}-bismesylate (Triangle Pharmaceuticals, Inc.).

Nonlimiting examples of other compounds that can be administered in combination or alternation with MKC-442 to augment the properties of the drug on
 10 administration include abacavir: (1S,4R)-4-[2-amino-6-cyclopropyl-amino)-9H-purin-9-yl]-2-cyclopentene-1-methanol succinate (1592U89, a carbovir analog; Glaxo Wellcome); AzddU: 3'-azido-2',3'-dideoxyuridine; zidovudine: AZT, 3'-azido-3'-deoxythymidine (Glaxo Wellcome); BILA 1906: N-{1S-[[[3-[2S-{(1,1-dimethylethyl)amino]carbonyl}-4R]-3-pyridinylmethyl]thio]-1-piperidinyl]-2R-hydroxy-1S-(phenylmethyl)propyl]amino]carbonyl]-
 15 2-methylpropyl}-2-quinolinecarboxamide (Bio Mega/Boehringer-Ingelheim); BILA 2185: N-(1,1-dimethylethyl)-1-[2S-[[[2-2,6-dimethylphenoxy)-1-oxoethyl]amino]-2R-hydroxy-4-phenylbutyl]4R-pyridinylthio)-2-piperidinecarboxamide (Bio Mega/Boehringer-Ingelheim); BM+51.0836: triazoloisoindolinone derivative; BMS 186,318: aminodiol derivative HIV-1 protease inhibitor (Bristol-Myers-Squibb); d4API: 9-[2,5-dihydro-5-(phosphonomethoxy)-2-furanyl]adenine (Gilead); stavudine: d4T, 2',3'-didehydro-3'-deoxythymidine (Bristol-Myers-Squibb); HBY097: S-4-isopropoxycarbonyl-6-methoxy-3-(methylthio-methyl)-3,4-dihydroquinoxalin-2(1H)-thione; HEPT: 1-[(2-hydroxyethoxy)methyl]6-(phenylthio)thymine; KNI-272: (2S,3S)-3-amino-2-hydroxy-4-phenylbutyric acid-containing tripeptide; L-697,593; 5-ethyl-6-methyl-3-(2-phthalimido-ethyl)pyridin-2(1H)-one; L-
 20 735,524: hydroxy-aminopentane amide HIV-1 protease inhibitor (Merck); L-697,661: 3-{{(-4,7-dichloro-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methylpyridin-2(1H)-one; L-FDDC: (-)-b-L-5-fluoro-2',3'-dideoxycytidine; L-FDOC: (-)-b-L-5-fluoro-dioxolane cytosine; 6-benzyl-1-ethoxymethyl-5-isopropyluracil (I-EBU; Triangle/Mitsubishi); nevirapine: 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyridol[3,2-b:2',3'-c]diazepin-6-one (Boehringer-
 25 30 Ingelheim); PFA: phosphonoformate (foscarnet; Astra); PMEA: 9-(2-

phosphonylmethoxyethyl) adenine (Gilead); PMPA: (R)-9-(2-phosphonyl-methoxypropyl)adenine (Gilead); Ro 31-8959: hydroxyethylamine derivative HIV-1 protease inhibitor (Roche); RPI-3121: peptidyl protease inhibitor, 1-[(3s)-3-(n-alpha-benzyloxycarbonyl)-1-asparginyl)-amino-2-hydroxy-4-phenylbutyryl]-n-tert-butyl-1-proline amide; 2720: 6-chloro-3,3-dimethyl-4-(isopropenyloxycarbonyl)-3,4-dihydro-quinoxalin-2(1H)thione; SC-52151: hydroxyethylurea isostere protease inhibitor (Searle); SC-55389A: hydroxyethyl-urea isostere protease inhibitor (Searle); TIBO R82150: (+)-(5S)-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1-jk][1,4]-benzodiazepin-2(1H)-thione (Janssen); TIBO 82913: (+)-(5S)-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1jk]-[1,4]benzodiazepin-2(1H)-thione (Janssen); TSAO-m3T: [2',5'-bis-O-(tert-butyl)dimethylsilyl]-3'-spiro-5'-(4'-amino-1',2'-oxathiole-2',2'-dioxide)]-b-D-pentofuranosyl-N3-methylthymine; U90152: 1-[3-[(1-methylethyl)-amino]2-pyridinyl]-4-[[5-[(methylsulphonyl)-amino]-1H-indol-2yl]carbonyl]piperazine; UC: thiocarboxanilide derivatives (Uniroyal); UC-781 = N-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2-methyl-3-furancarbothioamide; UC-82 = N-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2-methyl-3-thiophenecarbothioamide; VB 11,328: hydroxyethylsulphonamide protease inhibitor (Vertex); VX-478: amprenavir, 141W94, hydroxyethylsulphonamide protease inhibitor (Vertex/Glaxo Wellcome); XM 323: cyclic urea protease inhibitor (Dupont Merck), famciclovir, ganciclovir, and penciclovir. In another embodiment, MKC-442 is administered in combination with a protease inhibitor (LG 1350) of the structure:



105-25
106

Prodrug Formulations

Any of the nucleosides or other compounds which are described herein for use in combination or alternation therapy with MKC-442 or its related compounds can be administered as an acylated prodrug or a nucleotide prodrug, as described in detail below.

5 The term acyl refers to a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic alkyl or lower alkyl, alkoxyalkyl including methoxymethyl, aralkyl including benzyl, aryloxyalkyl such as phenoxymethyl, aryl including phenyl optionally substituted with halogen, C₁ to C₄ alkyl or C₁ to C₄ alkoxy, sulfonate esters such as alkyl or aralkyl sulphonyl including
10 methanesulfonyl, the mono, di or triphosphate ester, trityl or monomethoxytrityl, substituted benzyl, trialkylsilyl (e.g. dimethyl-t-butylsilyl).

Any of the nucleosides described herein or other compounds that contain a hydroxyl or amine function can be administered as a nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A
15 number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the hydroxyl group of the compound or of the mono, di or triphosphate of the nucleoside will increase the stability of the nucleotide. Examples of substituent groups that can replace one or more hydrogens on the phosphate moiety or hydroxyl are alkyl, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and
20 alcohols. Many are described in R. Jones and N. Bischofberger, *Antiviral Research*, 27 (1995) 1-17. Any of these can be used in combination with the disclosed nucleosides or other compounds to achieve a desired effect.

The active nucleoside or other hydroxyl containing compound can also be provided as an ether lipid (and particularly a 5'-ether lipid for a nucleoside), as disclosed in
25 the following references, which are incorporated by reference herein: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E.K., D.L.W., and C. Piantadosi. 1990. "Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation." *AIDS Res. Hum. Retro Viruses*. 6:491-501; Piantadosi, C., J. Marasco C.J., S.L. Morris-Natschke, K.L. Meyer, F. Gumus, J.R. Surles, K.S. Ishaq, L.S.
30 Kucera, N. Iyer, C.A. Wallen, S. Piantadosi, and E.J. Modest. 1991. "Synthesis and

10066
107

evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity." *J. Med. Chem.* 34:1408-1414; Hosteller, K.Y., D.D. Richman, D.A. Carson, L.M. Stuhmiller, G.M. T. van Wijk, and H. van den Bosch. 1992. "Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3'-deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 3'-deoxythymidine." *Antimicrob. Agents Chemother.* 36:2025-2029; Hostetler, K.Y., L.M. Stuhmiller, H.B. Lenting, H. van den Bosch, and D.D. Richman, 1990. "Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides." *J. Biol. Chem.* 265:61127.

Nonlimiting examples of U.S. patents that disclose suitable lipophilic substituents that can be covalently incorporated into the nucleoside or other hydroxyl or amine containing compound, preferably at the 5'-OH position of the nucleoside or lipophilic preparations, include U.S. Patent Nos. 5,149,794 (Sep. 22, 1992, Yatvin et al.); 5,194,654 (Mar. 16, 1993, Hostetler et al.); 5,223,263 (June 29, 1993, Hostetler et al.); 5,256,641 (Oct. 26, 1993, Yatvin et al.); 5,411,947 (May 2, 1995, Hostetler et al.); 5,463,092 (Oct. 31, 1995, Hostetler et al.); 5,543,389 (Aug. 6, 1996, Yatvin et al.); 5,543,390 (Aug. 6, 1996, Yatvin et al.); 5,543,391 (Aug. 6, 1996, Yatvin et al.); and 5,554,728 (Sep. 10, 1996; Basava et al.), all of which are incorporated herein by reference. Foreign patent applications that disclose lipophilic substituents that can be attached to the nucleosides of the present invention, or lipophilic preparations, include WO 89/02733, WO 90/00555, WO 91/16920, WO 91/18914, WO 93/00910; WO 94/26273, WO 96/15132, EP 0 350 287, EP 93917054.4, and WO 91/19721.

Nonlimiting examples of nucleotide prodrugs are described in the following references: Ho, D.H.W. (1973) "Distribution of Kinase and deaminase of 1 β -D-arabinofuranosylcytosine in tissues of man and mouse." *Cancer Res.* 33, 2816-2820; Holy, A. (1993) Isopolar phosphorous-modified nucleotide analogues," In: De Clercq (Ed.), Advances in Antiviral Drug Design, Vol. I, JAI Press, pp. 179-231; Hong, C.I., Nechaev, A., and West, C.R. (1979a) "Synthesis and antitumor activity of 1 β -D-arabino-furanosylcytosine conjugates of cortisol and cortisone." *Biochem. Biophys. Res. Commun.* 88, 1223-1229; Hong, C.I., Nechaev, A., Kirisits, A.J. Buchheit, D.J. and West, C.R. (1980) "Nucleoside conjugates as potential antitumor agents. 3. Synthesis and antitumor activity of 1-(β -D-arabinofuranosyl) cytosine conjugates of corticosteroids and selected lipophilic alcohols." *J. Med. Chem.* 28, 171-177; Hosteller, K.Y., Stuhmiller, L.M., Lenting, H.B.M. van den Bosch, H. and Richman

19727
108

- J. Biol. Chem.* **265**, 6112-6117; Hosteller, K.Y., Carson, D.A. and Richman, D.D. (1991); "Phosphatidylazidothymidine: mechanism of antiretroviral action in CEM cells." *J. Biol. Chem.* **266**, 11714-11717; Hosteller, K.Y., Korba, B. Sridhar, C., Gardener, M. (1994a) "Antiviral activity of phosphatidyl-dideoxycytidine in hepatitis B-infected cells and enhanced hepatic uptake in mice." *Antiviral Res.* **24**, 59-67; Hosteller, K.Y., Richman, D.D., Sridhar, C.N. Felgner, P.L. Felgner, J., Ricci, J., Gardener, M.F. Selleseth, D.W. and Ellis, M.N. (1994b) "Phosphatidylazidothymidine and phosphatidyl-ddC: Assessment of uptake in mouse lymphoid tissues and antiviral activities in human immunodeficiency virus-infected cells and in raucher leukemia virus-infected mice." *Antimicrobial Agents Chemother.* **38**, 2792-2797; Hunston, R.N., Jones, A.A. McGuigan, C., Walker, R.T., Balzarini, J., and DeClercq, E. (1984) "Synthesis and biological properties of some cyclic phosphotriesters derived from 2'-deoxy-5-fluorouridine." *J. Med. Chem.* **27**, 440-444; Ji, Y.H., Moog, C., Schmitt, G., Bischoff, P. and Luu, B. (1990); "Monophosphoric acid esters of 7- β -hydroxycholesterol and of pyrimidine nucleoside as potential antitumor agents: synthesis and preliminary evaluation of antitumor activity." *J. Med. Chem.* **33** 2264-2270; Jones, A.S., McGuigan, C., Walker, R.T., Balzarini, J. and DeClercq, E. (1984) "Synthesis, properties, and biological activity of some nucleoside cyclic phosphoramidates." *J. Chem. Soc. Perkin Trans. I*, 1471-1474; Juodka, B.A. and Smrt, J. (1974) "Synthesis of diribonucleoside phosph (P-N) amino acid derivatives." *Coll. Czech. Chem. Comm.* **39**, 363-968; Kataoka, S., Imai, J., Yamaji, N., Kato, M., Saito, M., Kawada, T. and Imai, S. (1989) "Alkylated cAMP derivatives; selective synthesis and biological activities." *Nucleic Acids Res. Sym. Ser.* **21**, 1-2; Kataoka, S., Uchida, " (cAMP) benzyl and methyl triesters." *Heterocycles* **32**, 1351-1356; Kinchington, D., Harvey, J.J., O'Connor, T.J., Jones, B.C.N.M., Devine, K.G., Taylor-Robinson D., Jeffries, D.J. and McGuigan, C. (1992) "Comparison of antiviral effects of zidovudine phosphoramidate and phosphorodiamidate derivatives against HIV and ULV *in vitro*." *Antiviral Chem. Chemother.* **3**, 107-112; Kodama, K., Morozumi, M., Saithoh, K.I., Kuninaka, H., Yosino, H. and Saneyoshi, M. (1989) "Antitumor activity and pharmacology of 1- β -D-arabinofuranosylcytosine -5'-stearylphosphate; an orally active derivative of 1- β -D-arabinofuranosylcytosine." *Jpn. J. Cancer Res.* **80**, 679-685; Korty, M. and Engels, J. (1979) "The effects of adenosine- and guanosine 3',5' phosphoric and acid benzyl esters on guinea-pig ventricular myocardium." *Naunyn-Schmiedeberg's Arch. Pharmacol.* **310**, 103-111;

- 108-28
109
- Kumar, A., Goe, P.L., Jones, A.S. Walker, R.T. Balzarini, J. and DeClercq, E. (1990) "Synthesis and biological evaluation of some cyclic phosphoramidate nucleoside derivatives." *J. Med. Chem.* **33**, 2368-2375; LeBec, C., and Huynh-Dinh, T. (1991) "Synthesis of lipophilic phosphate triester derivatives of 5-fluorouridine and arabinocytidine as anticancer prodrugs." *Tetrahedron Lett.* **32**, 6553-6556; Lichtenstein, J., Barner, H.D. and Cohen, S.S. (1960) "The metabolism of exogenously supplied nucleotides by *Escherichia coli*," *J. Biol. Chem.* **235**, 457-465; Luthy, J., Von Daeniken, A., Friederich, J. Manthey, B., Zweifel, J., Schlatter, C. and Benn, M.H. (1981) "Synthesis and toxicological properties of three naturally occurring cyanoepithioalkanes". *Mitt. Geg. Lebensmittelunters. Hyg.* **72**, 131-133 (*Chem. Abstr.* 95, 127093); McGuigan, C. Tollerfield, S.M. and Riley, P.a. (1989) "Synthesis and biological evaluation of some phosphate triester derivatives of the anti-viral drug Ara." *Nucleic Acids Res.* **17**, 6065-6075; McGuigan, C., Devine, K.G., O'Connor, T.J., Galpin, S.A., Jeffries, D.J. and Kinchington, D. (1990a) "Synthesis and evaluation of some novel phosphoramidate derivatives of 3'-azido-3'-deoxythymidine (AZT) as anti-HIV compounds." *Antiviral Chem. Chemother.* **1** 107-113; McGuigan, C., O'Connor, T.J., Nicholls, S.R. Nickson, C. and Kinchington, D. (1990b) "Synthesis and anti-HIV activity of some novel substituted dialkyl phosphate derivatives of AZT and ddCyd." *Antiviral Chem. Chemother.* **1**, 355-360; McGuigan, C., Nicholls, S.R., O'Connor, T.J., and Kinchington, D. (1990c) "Synthesis of some novel dialkyl phosphate derivative of 3'-modified nucleosides as potential anti-AIDS drugs." *Antiviral Chem. Chemother.* **1**, 25-33; McGuigan, C., Devin, K.G., O'Connor, T.J., and Kinchington, D. (1991) "Synthesis and anti-HIV activity of some haloalkyl phosphoramidate derivatives of 3'-azido-3' deoxythylmidine (AZT); potent activity of the trichloroethyl methoxyalaninyl compound." *Antiviral Res.* **15**, 255-263; McGuigan, C., Pathirana, R.N., Balzarini, J. and DeClercq, E. (1993b) "Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT." *J. Med. Chem.* **36**, 1048-1052.
- Alkyl hydrogen phosphate derivatives of the anti-HIV agent AZT may be less toxic than the parent nucleoside analogue. *Antiviral Chem. Chemother.* **5**, 271-277; Meyer, R. B., Jr., Shuman, D.A. and Robins, R.K. (1973) "Synthesis of purine nucleoside 3', 5'-cyclic phosphoramidates." *Tetrahedron Lett.* 269-272; Nagyvary, J. Gohil, R.N., Kirchner, C.R. and Stevens, J.D. (1973) "Studies on neutral esters of cyclic AMP," *Biochem. Biophys. Res. Commun.* **55**, 1072-1077; Namane, A. Gouyette, C., Fillion, M.P., Fillion, G. and Huynh-

109 39
110

- Dinh, T. (1992) "Improved brain delivery of AZT using a glycosyl phosphotriester prodrug." *J. Med. Chem.* 35, 3039-3044; Nargeot, J. Nerbonne, J.M. Engels, J. and Leser, H.A. (1983) *Natl. Acad. Sci. U.S.A.* 80, 2395-2399; Nelson, K.A., Bentrude, W.G. Stser, W.N. and Hutchinson, J.P. (1987) "The question of chair-twist equilibria for the phosphate rings of nucleoside cyclic 3', 5' monophosphates. ¹HNMR and x-ray crystallographic study of the diastereomers of thymidine phenyl cyclic 3', 5'-monophosphate." *J. Am. Chem. Soc.* 109, 4058-4064; Nerbonne, J.M., Richard, S., Nargeot, J. and Lester, H.A. (1984) "New photoactivatable cyclic nucleotides produce intracellular jumps in cyclic AMP and cyclic GMP concentrations." *Nature* 301, 74-76; Neumann, J.M., Hervé, M., Debouzy, J.C., Guerra, F.I., Gouyette, C., Dupraz, B. and Huyny-Dinh, T. (1989) "Synthesis and transmembrane transport studies by NMR of a glucosyl phospholipid of thymidine." *J. Am. Chem. Soc.* 111, 4270-4277; Ohno, R., Tatsumi, N., Hirano, M., Imai, K. Mizoguchi, H., Nakamura, T., Kosaka, M., Takatuski, K., Yamaya, T., Toyama K., Yoshida, T., Masaoka, T., Hashimoto, S., Ohshima, T., Kimura, I., Yamada, K. and Kimura, J. (1991) "Treatment of myelodysplastic syndromes with orally administered 1-β-D-arabinouranosylcytosine -5' stearylphosphate." *Oncology* 48, 451-455. Palomino, E., Kessle, D. and Horwitz, J.P. (1989) "A dihydropyridine carrier system for sustained delivery of 2', 3' dideoxynucleosides to the brain." *J. Med. Chem.* 32, 22-625; Perkins, R.M., Barney, S. Wittrock, R., Clark, P.H., Levin, R. Lambert, D.M., Petteway, S.R., Serafinowska, H.T., Bailey, S.M., Jackson, S., Harnden, M.R. Ashton, R., Sutton, D., Harvey, J.J. and Brown, A.G. (1993) "Activity of BRL47923 and its oral prodrug, SB203657A against a rauscher murine leukemia virus infection in mice." *Antiviral Res.* 20 (Suppl. I). 84; Piantadosi, C., Marasco, C.J., Jr., Norris-Natschke, S.L., Meyer, K.L., Gumus, F., Surles, J.R., Ishaq, K.S., Kucera, L.S. Iyer, N., Wallen, C.A., Piantadosi, S. and Modest, E.J. (1991) "Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV-1 activity." *J. Med. Chem.* 34, 1408-1414; Pompon, A., Lefebvre, I., Imbach, J.L., Kahn, S. and Farquhar, D. (1994). "Decomposition pathways of the mono- and bis(pivaloyloxymethyl) esters of azidothymidine-5'-monophosphate in cell extract and in tissue culture medium; an application of the 'on-line ISRP-cleaning HPLC technique." *Antiviral Chem Chemother.* 5, 91-98; Postemark, T. (1974) "Cyclic AMP and cyclic GMP." *Annu. Rev. Pharmacol.* 14, 23-33; Prisbe, E.J., Martin, J.C.M., McGhee, D.P.C., Barker, M.F., Smee, D.F. Duke, A.E., Matthews, T.R. and Verheyden, J.P.J. (1986)

4030
111

- "Synthesis and antiherpes virus activity of phosphate and phosphonate derivatives of 9-[(1, 3-dihydroxy-2-propoxy)methyl] guanine." *J. Med. Chem.* 29, 671-675; Pucchi, F., Gosselin, G., Lefebvre, I., Pompon, A., Aubertin, A.M., Dirn, and Imbach, J.L. (1993) "Intracellular delivery of nucleoside monophosphate through a reductase-mediated activation process." *Antiviral Res.* 22, 155-174; Pugaeva, V.P., Klochkeva, S.I., Mashbits, F.D. and Eizengart, R.S. (1969).
- 5 "Toxicological assessment and health standard ratings for ethylene sulfide in the industrial atmosphere." *Gig. Trf. Prof. Zabol.* 14, 47-48 (*Chem. Abstr.* 72, 212); Robins, R.K. (1984) "The potential of nucleotide analogs as inhibitors of Retro viruses and tumors." *Pharm. Res.* 11-18; Rosowsky, A., Kim, S.H., Ross and J. Wick, M.M. (1982) "Lipophilic 5'-(alkylphosphate) esters of 1- β -D-arabinofuranosylcytosine and its N⁴-acyl and 2,2'-anhydro-
- 10 3'-O-acyl derivatives as potential prodrugs." *J. Med. Chem.* 25, 171-178; Ross, W. (1961) "Increased sensitivity of the walker turnout towards aromatic nitrogen mustards carrying basic side chains following glucose pretreatment." *Biochem. Pharm.* 8, 235-240; Ryu, E.K., Ross, R.J., Matsushita, T., MacCoss, M., Hong, C.I. and West, C.R. (1982). "Phospholipid-nucleoside conjugates. 3. Synthesis and preliminary biological evaluation of 1- β -D-
- 15 arabinofuranosylcytosine 5' diphosphate [-], 2-diacylglycerols." *J. Med. Chem.* 25, 1322-1329; Saffhill, R. and Hume, W.J. (1986) "The degradation of 5-iododeoxyuridine and 5-bromoethoxyuridine by serum from different sources and its consequences for the use of these compounds for incorporation into DNA." *Chem. Biol. Interact.* 57, 347-355; Saneyoshi, M., Morozumi, M., Kodama, K., Machida, J., Kuninaka, A. and Yoshino, H.
- 20 (1980) "Synthetic nucleosides and nucleotides. XVI. Synthesis and biological evaluations of a series of 1- β -D-arabinofuranosylcytosine 5'-alkyl or arylphosphates." *Chem. Pharm. Bull.* 28, 2915-2923; Sastry, J.K., Nehete, P.N., Khan, S., Nowak, B.J., Plunkett, W., Arlinghaus, R.B. and Farquhar, D. (1992) "Membrane-permeable dideoxyuridine 5'-monophosphate analogue inhibits human immunodeficiency virus infection." *Mol. Pharmacol.* 41, 441-445;
- 25 Shaw, J.P., Jones, R.J., Arimilli, M.N., Louie, M.S., Lee, W.A. and Cundy, K.C. (1994) "Oral bioavailability of PMEA from PMEA prodrugs in male Sprague-Dawley rats." *9th Annual AAPS Meeting*. San Diego, CA (Abstract). Shuto, S., Ueda, S., Imamura, S., Fukukawa, K., Matsuda, A. and Ueda, T. (1987) "A facile one-step synthesis of 5' phosphatidyl nucleosides by an enzymatic two-phase reaction." *Tetrahedron Lett.* 28, 199-202; Shuto, S., Itoh, H.,
- 30 Ueda, S., Imamura, S., Fukukawa, K., Tsujino, M., Matsuda, A. and Ueda, T. (1988) *Pharm.*

711 ST
112

Bull. 36, 209-217. An example of a useful phosphate prodrug group is the S-acyl-2-thioethyl group, also referred to as "SATE".

These compounds can be tested for synergistic activity with MKC-442 against HIV according to a number of assays, including that described below.

5 **Example 1 Evaluation of Inhibition of HIV Infection Using Combined Antiviral Agents**

10 The inhibitory effects of combined antiviral agents were evaluated using the human T cell line, MT2. Infection of this cell line with HIV results in cell death. Inhibition of the HIV induced cytopathic effect by the combined agents is measured in a MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) based assay. The mitochondrial dehydrogenases of viable cells will cleave the tetrazolium ring of MTT, yielding purple formazan crystals which are soluble in acidified isopropanol. The resulting purple solution is measured spectrophotometrically. The amount of purple dye formed is directly related to the number of viable cells. Therefore, a decrease in color in this assay indicates cell death due to viral cytopathic effects (CPE). This assay has been employed in the assessment of a variety of antiretroviral compounds (Pauwels, R. et al, 1988, *J. Virol. Methods*, 20: 309-321.) and is readily adaptable to any system with a lytic virus.

20

Preparation of drug plates.

25 The assays are performed in a 96 well microtiter plate. Each compound to be tested is made up at 4 times the final concentration to allow for dilution by cells and other test compounds. Compounds are prepared in complete RPMI media (RPMI 1640 plus 10% fetal bovine serum plus 20 mg/mL gentamicin). Two drugs are tested simultaneously on each plate using a checkerboard design. Serial dilutions of the first drug are added to the vertical wells (50 mL/well) of the plate (columns 2 – 11). Serial dilutions of the second drug (50 mL/well) are then added to the plate across horizontal rows (rows A – G). Column #2 and row H contain dilutions of only one compound. EC₅₀ values obtained from these wells are a measure of the antiviral activity of each compound alone. Column #1 contains virus control,

30

32
42
113

infected cells grown in the absence of compound, and Column 12 contains cell control, mock infected cells.

Preparation of cells.

MT2 cells are infected with the LAI strain of HIV at a multiplicity of infection of 0.01 in complete RPMI containing 2 mg/mL polybrene for 2 hours at 37°C. Following infection, cells diluted to 2.5×10^5 cells/mL in complete RPMI media. 100 mL of the cell suspension are seeded onto 96 well plates, 2.5×10^4 cells/well, containing test compounds as described above. The final volume in each well is 200 mL. Plates are sealed and incubated at 37°C in a humidified 5% CO₂ incubator for 5 days.

Developing the assay.

Following the 5 day incubation, 80 mL of cell free supernatant is removed from each well and 10 mL of a 5 mg/mL solution of MTT is added. The plates are returned to the incubator for 2 hours followed by the addition of 100 mL of acidified isopropanol (10% Triton X-100 plus 0.1N HCl in anhydrous isopropanol) to each well. Formazan crystals are dissolved by repeated pipetting in the wells. Plates are read at 570 nm in a Biotek plate reader.

Calculation of Data.

A dose response curve for each individual compound is generated using the absorption values of the cell controls as 100% protection and no drug virus infected cells as 0% protection. From this dose response curve a EC₅₀ value is calculated and is defined as the concentration of drug that inhibits 50% of viral induced CPE. The effects of the combined compounds were obtained using the isobologram technique (Elion, G.B. et al, 1954, J. Biol. Chem., 208: 477-488). The data plotted were obtained by dividing the EC₅₀ of compound #1 in the presence of a fixed concentration of compound #2 by the EC₅₀ of compound #1 alone. In a similar fashion data can be obtained by dividing the EC₅₀ of compound #2 in the presence of a fixed concentration of compound #1 by the EC₅₀ of compound #2 alone. The EC₅₀ ratios are plotted against the ratio of compound at each combination. If the curve

33
113
114

generated by these points falls on the theoretic diagonal line, the combination is additive. If the curve is above the theoretical line, the combination is antagonistic. And, if the curve lies below the theoretical line, then the combination is synergistic. Figures 1 and 2 illustrate isobologram analysis of the combination of MKC-442 with indinavir and with neftinavir.

Even though a combination exhibits additive and not synergistic effects according to this assay, the combination can still provide an effect that is different from the separate administration of the two agents. For example, the biodistribution, pharmacokinetics, or metabolism of one can be affected by the other. Further, MKC-442 is advantageously delivered in combination or alternation with another antiviral agent that exhibits a different mutation profile than MKC-442.

PREPARATION OF PHARMACEUTICAL COMPOSITIONS

Humans suffering from effects caused by any of the diseases described herein, and in particular, HIV infection, can be treated by administering to the patient an effective amount of MKC-442 in combination with one or more agents described above or a pharmaceutically acceptable derivative or salt thereof in the presence of a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, enterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The active compounds are included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount of compound to inhibit viral replication *in vivo*, especially HIV replication, without causing serious toxic effects in the treated patient. By "inhibitory amount" is meant an amount of active ingredient sufficient to exert an inhibitory effect as measured by, for example, an assay such as the ones described herein.

A preferred dose of the compound for all the above-mentioned conditions will be in the range from about 1 to 50 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent nucleoside to be delivered. If the derivative exhibits activity in

34
115

itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art.

The compounds are conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. An oral dosage of 50 to 1000 mg is usually convenient.

5 Ideally, the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 mM, preferably about 1.0 to 10 mM. This may be achieved, for example, by the intravenous injection of a 0.1 to % solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

10 The concentration of active compound in the drug composition will depend on absorption, distribution, metabolism and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual
15 need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

20 A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible bind agents, and/or adjuvant
25 materials can be included as part of the composition.

30 The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a

25
#5
116

flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

5 The compounds can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

 The compounds or their pharmaceutically acceptable derivative or salts thereof can also be mixed with other active materials that do not impair the desired action, or with
10 materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, protease inhibitors, or other nucleoside or non-nucleoside antiviral agents, as discussed in more detail above. Solutions or suspensions used for parental, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,
15 glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose
20 vials made of glass or plastic.

 If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

 In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release
25 formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation.

36
H6
112

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. these may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearyl phosphatidyl ethanolamine, stearyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of this invention.

37
AT
118

We Claim.

1. Use of an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a protease inhibitor, or its pharmaceutically acceptable salt in the treatment of a patient infected with HIV.

2. Use of an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a compound selected from the group consisting of: indinavir, nelfinavir, saquinavir, amprenavir, and DMP-450 or its pharmaceutically acceptable salt, in the treatment of a patient infected with HIV.

3. The use of claim 2, wherein the compound is indinavir.

4. The use of claim 2, wherein the compound is nelfinavir.

5. The use of claim 2, wherein the compound is saquinavir.

6. The use of claim 2, wherein the compound is amprenavir.

7. The use of claim 2, wherein the compound is DMP-450.

8. Use of an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a compound selected from the group consisting of abacavir, FTC, or 3TC or its pharmaceutically acceptable salt, in the treatment of a patient infected with HIV.

9. The use of claim 8, wherein the compound is abacavir.

10. The use of claim 8, wherein the compound is FTC.

11. The use of claim 8, wherein the compound is 3TC.

12. Use of an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a compound selected from the group consisting of DMP-266, delavirdine, and nevirapine or its pharmaceutically acceptable salt, in the treatment of a patient infected with HIV.

13. The use of claim 12, wherein the compound is DMP-266.

14. The use of claim 12, wherein the compound is delavirdine.

15. The use of claim 12, wherein the compound is nevirapine.

16. A pharmaceutical composition for the treatment of a patient infected with HIV, comprising an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination with a protease inhibitor, or its pharmaceutically acceptable salt.

38
18
119

17. A pharmaceutical composition for the treatment of a patient infected with HIV, comprising an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a compound selected from the group consisting of: indinavir, nelfinavir, saquinavir, amprenavir, and DMP-450 or its pharmaceutically acceptable salt.

5 18. The composition of claim 17, wherein the compound is indinavir.

19. The composition of claim 17, wherein the compound is nelfinavir.

20. The composition of claim 17, wherein the compound is saquinavir.

21. The composition of claim 17, wherein the compound is amprenavir.

22. The composition of claim 17, wherein the compound is DMP-450.

10 23. A pharmaceutical composition for the treatment of a patient infected with HIV, comprising an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a compound selected from the group consisting of abacavir, FTC, or 3TC or its pharmaceutically acceptable salt.

24. The composition of claim 8, wherein the compound is abacavir.

15 25. The composition of claim 8, wherein the compound is FTC.

26. The composition of claim 8, wherein the compound is 3TC.

27. A pharmaceutical composition for the treatment of a patient infected with HIV, comprising an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination with a compound selected from the group consisting of DMP-266, delavirdine, and nevirapine or its pharmaceutically acceptable salt.

20 28. The composition of claim 27, wherein the compound is DMP-266.

29. The composition of claim 27, wherein the compound is delavirdine.

30. The composition of claim 27, wherein the compound is nevirapine.

31. Use of an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a protease inhibitor, or its pharmaceutically acceptable salt in the preparation of a medicament for medical therapy.

32. Use of an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a compound selected from the group consisting of: indinavir, nelfinavir, saquinavir, amprenavir, and DMP-450 or its pharmaceutically acceptable salt, in the preparation of a medicament for medical therapy.

39
119 120

33. The use of claim 2, wherein the compound is indinavir.

34. The use of claim 2, wherein the compound is nelfinavir.

35. The use of claim 2, wherein the compound is saquinavir.

36. The use of claim 2, wherein the compound is amprenavir.

37. The use of claim 2, wherein the compound is DMP-450.

5 38. Use of an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a compound selected from the group consisting of abacavir, FTC, or 3TC or its pharmaceutically acceptable salt, in the preparation of a medicament for medical therapy.

39. The use of claim 8, wherein the compound is abacavir.

10 40. The method of claim 8, wherein the compound is FTC.

41. The use of claim 8, wherein the compound is 3TC.

42. Use of an effective treatment amount of MKC-442 or its pharmaceutically acceptable salt in combination or alternation with a compound selected from the group consisting of DMP-266, delavirdine, and nevirapine or its pharmaceutically acceptable salt, in
15 the preparation of a medicament for medical therapy.

43. The use of claim 12, wherein the compound is DMP-266.

44. The use of claim 12, wherein the compound is delavirdine.

45. The use of claim 12, wherein the compound is nevirapine.

40
120
121

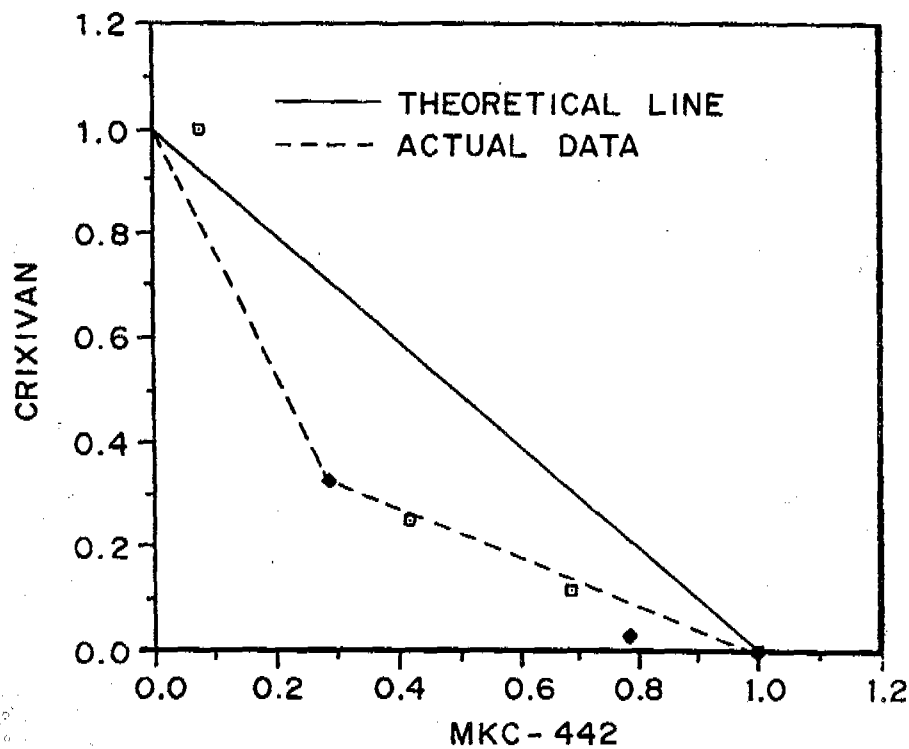


FIG. 1

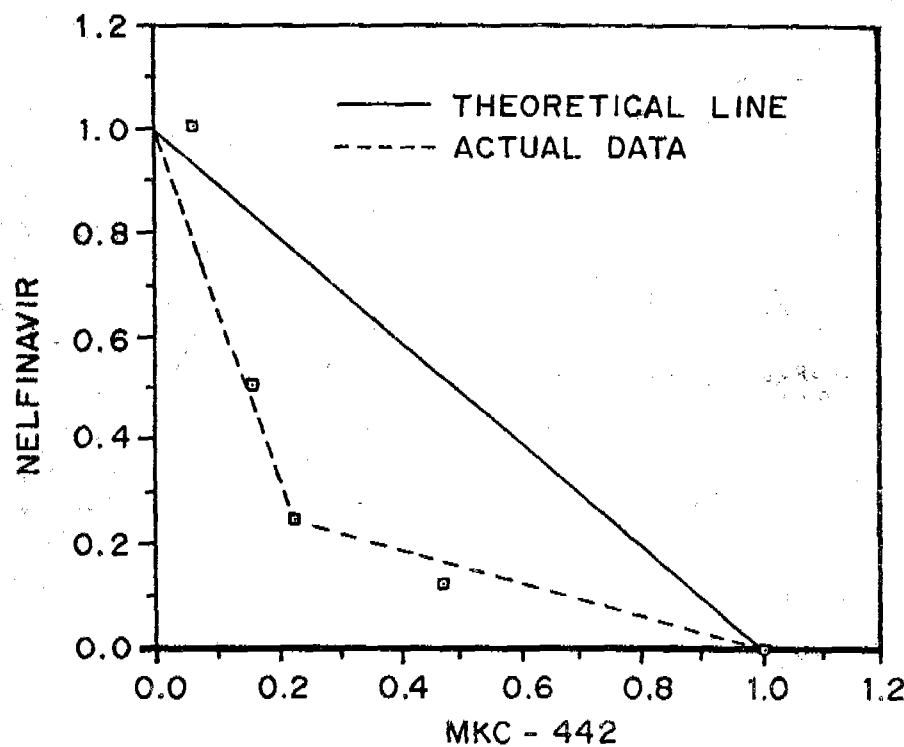


FIG. 2

41
124
122

ANEXO 2

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 December 2001 (20.12.2001)

PCT

(10) International Publication Number
WO 01/96338 A1

(51) International Patent Classification⁷: **C07D 471/14**,
A61K 31/55 // (C07D 471/14, 243:00, 221:00, 221:00)

[CA/CA]; 2615, de la Volière, Laval, Québec H7L 3V6
(CA).

(21) International Application Number: PCT/CA01/00890

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

(22) International Filing Date: 14 June 2001 (14.06.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/212,329 16 June 2000 (16.06.2000) US
60/256,638 18 December 2000 (18.12.2000) US

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

(71) Applicant (*for all designated States except US*):
BOEHRINGER INGELHEIM (CANADA) LTD.
[CA/CA]; 2100 Cunard Street, Laval, Québec H7S 2G5
(CA).

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

(72) Inventor; and

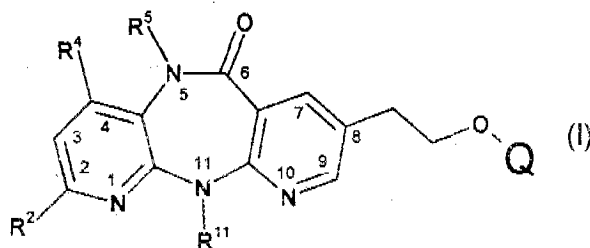
(75) Inventor/Applicant (*for US only*): **SIMONEAU, Bruno**

Published:

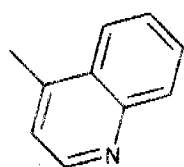
with international search report

[Continued on next page]

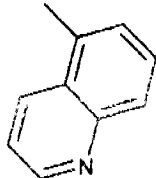
(54) Title: NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS



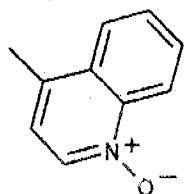
(57) Abstract: Provided are compounds of general formula (I), wherein R² is selected from the group consisting of H, F, Cl, (C₁₋₄) alkyl, (C₃₋₄) cycloalkyl and CF₃; R⁴ is H or Me; R⁵ is H, Me or Et, with the proviso that R⁴ and R⁵ are not both Me, and if R⁴ is Me then R⁵ cannot be Et; R¹¹ is Et, cyclopropyl, propyl, isopropyl, or isobutyl; and Q is selected from the group consisting of (II), (III), (IV) and (V); and pharmaceutically acceptable salts thereof, as inhibitors of HIV reverse transcriptase, wild-type and several mutant strains.



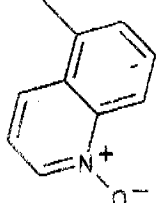
(II)



(III)



(IV)



(V)

WO 01/96338 A1

93
123
124

WO 01/96338 A1



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

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

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS**TECHNICAL FIELD OF THE INVENTION**

5

The invention relates to novel compounds and pharmaceutically acceptable salts thereof, their use, either alone or in combination with other therapeutic agents, in the treatment or prophylaxis of HIV infection, and to pharmaceutical compositions comprising the compounds.

10

BACKGROUND OF THE INVENTION

The disease known as acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV), particularly the strain known as HIV-1. In order for HIV to be replicated by a host cell, the information of the viral genome must be integrated into the host cell's DNA. However, HIV is a retrovirus, meaning that its genetic information is in the form of RNA. The HIV replication cycle therefore requires a step of transcription of the viral genome (RNA) into DNA, which is the reverse of the normal chain of events. The transcription of the viral RNA into DNA is accomplished by an enzyme that has been aptly dubbed reverse transcriptase (RT). The HIV virion includes a copy of RT along with the viral RNA.

Reverse transcriptase has three known enzymatic functions; it acts as an RNA-dependent DNA polymerase, as a ribonuclease, and as a DNA-dependent DNA polymerase. Acting as an RNA-dependent DNA polymerase, RT transcribes a single-stranded DNA copy of the viral RNA. Acting as a ribonuclease, RT destroys the original viral RNA, and frees the DNA just produced from the original RNA. Finally, acting as a DNA-dependent DNA polymerase, RT makes a second, complementary DNA strand, using the first DNA strand as a template. The two strands form

double-stranded DNA, which is integrated into the host cell's genome by another enzyme called integrase.

Compounds that inhibit the enzymatic functions of HIV-1 reverse
5 transcriptase will inhibit replication of HIV-1 in infected cells. Such
compounds are useful in the prevention or treatment of HIV-1 infection in
human subjects, as demonstrated by known RT inhibitors such as 3'-azido-3'-
deoxythymidine (AZT), 2',3'-dideoxyinosine (ddI), 2',3'-dideoxycytidine
(ddC), d4T, 3TC, Nevirapine, Delavirdine, Efavirenz and Abacavir, the main
10 drugs thus far approved for use in the treatment of AIDS.

As with any antiviral therapy, use of RT inhibitors in the treatment of AIDS
eventually leads to a virus that is less sensitive to the given drug. Resistance
(reduced sensitivity) to these drugs is the result of mutations that occur in the
15 reverse transcriptase segment of the *pol* gene. Several mutant strains of HIV
have been characterised, and resistance to known therapeutic agents is due
to mutations in the RT gene. Some of the most commonly observed mutants
clinically are: the Y181C mutant, in which a tyrosine (Y), at codon 181, has
been mutated to a cysteine (C) residue, and K103N where the lysine (K) at
20 position 103 has been replaced by asparagine (N). Other mutants which
emerge with increasing frequency during treatment with known antivirals
include the single mutants V106A, G190A, Y188C, and P236L; and the double
mutants K103N/Y181C, K103N/P225H, K103N/V108I, and K103N/L100I.

25 As therapy and prevention of HIV infection using antivirals continues, the
emergence of new resistant strains is expected to increase. There is therefore
an ongoing need for new inhibitors of RT, with different patterns of
effectiveness against the various mutants.

30 Compounds having tricyclic structures which are inhibitors of HIV-1 are

described in U.S. Pat. No. 5,366,972. Other inhibitors of HIV-1 reverse transcriptase are described in Hargrave et al., J. Med Chem., 34, 2231 (1991).

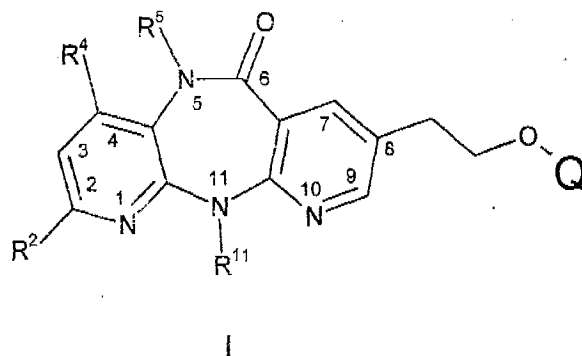
U.S. Pat. No. 5,705,499 proposes 8-arylalkyl- and 8-arylheteroalkyl-5,11-dihydro-6H-dipyrido[3,2-B:2',3'-E][1,4]diazepines as inhibitors of RT. The exemplified compounds are shown to have some activity against wild type and mutant HIV-1 RT, particularly Y181C and other single mutants such as K103N albeit less effectively.

Specifically, the compounds of the present invention are effective at inhibiting the Y181C and K103N mutants as well as a broad range of other single mutants and certain commonly found double mutants such as K103N/Y181C and K103N/P225H.

SUMMARY OF THE INVENTION

The invention reduces the difficulties and disadvantages of the prior art by providing novel compounds that are potent inhibitors of single and double mutant strains of HIV-1 RT.

In a first aspect the invention provides a compound of the general formula I:



wherein

R² is selected from the group consisting of H, F, Cl, C₁₋₄ alkyl, C₃₋₄ cycloalkyl and CF₃;

47
128 128

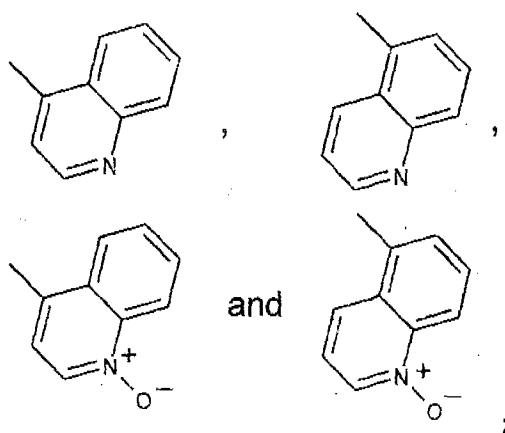
4

R⁴ is H or Me;

R⁵ is H, Me or Et, with the proviso that R⁴ and R⁵ are not both Me, and if R⁴ is Me then R⁵ cannot be Et;

R¹¹ is Me, Et, cyclopropyl, propyl, isopropyl, or cyclobutyl;

5 Q is selected from the group consisting of:



or a pharmaceutically acceptable salt thereof.

10 In a second aspect, the invention provides an inhibitor of HIV replication, of the general formula I, or a pharmaceutically acceptable salt thereof.

In a third aspect, the invention provides an inhibitor of a reverse transcriptase enzyme of HIV, of the general formula I, or a pharmaceutically acceptable salt thereof.

15

In a fourth aspect, the invention provides a method for the treatment or prevention of HIV infection, comprising administering to a patient an HIV inhibiting amount of a compound of formula I, or a pharmaceutically acceptable salt thereof.

20

In a fifth aspect, the invention provides a pharmaceutical composition for the

48
118
129

5

treatment or prevention of HIV infection, comprising a compound of formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

- 5 In a sixth aspect, the invention provides a method for preparation of a compound of formula I, or a pharmaceutically acceptable salt thereof.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

- 10 As used herein, the term "C₁₋₄ alkyl" is intended to mean linear or branched alkyl radicals containing from one to four carbon atoms and includes methyl, ethyl, propyl, isopropyl, butyl, *sec*-butyl and *tert*-butyl.

- As used herein, the term "C₃₋₄ cycloalkyl" is intended to mean saturated
15 cyclic hydrocarbon radicals containing three to four carbon atoms and includes cyclopropyl and cyclobutyl.

Detailed description of preferred embodiments

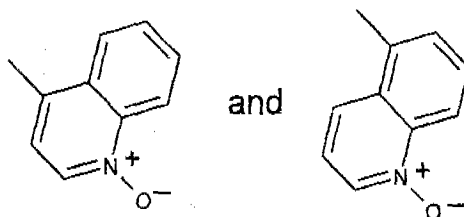
- According to a preferred embodiment, compounds of the invention are defined according to formula I wherein R² is preferably Cl, F, or H. More
20 preferably, R² is Cl or H. Most preferably, R² is H.

According to a preferred embodiment, compounds of the invention are defined according to formula I wherein R⁴ is preferably H.

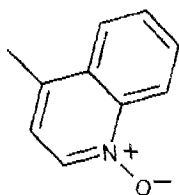
- 25 According to an alternative embodiment, compounds of the invention are defined according to formula I wherein preferably R⁵ is Me.

Preferably, compounds of the invention are defined according to formula I wherein R¹¹ is Et or cyclopropyl. More preferably, R¹¹ is Et.

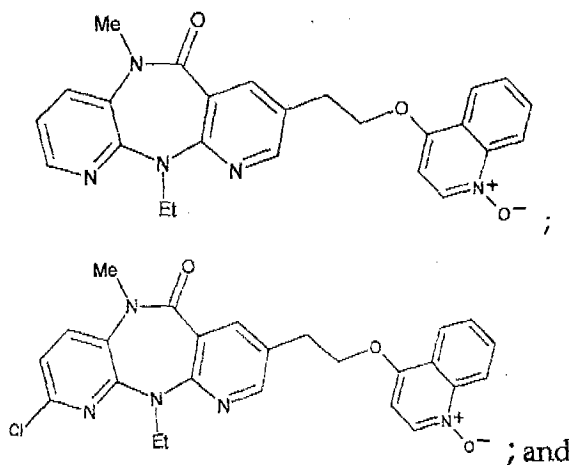
According to a preferred embodiment, compounds of the invention are defined according to formula I wherein Q is preferably selected from the group consisting of:

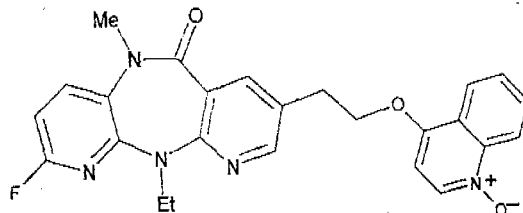


More preferably, Q is:



Alternatively, preferred embodiments of the invention include compounds selected from the group consisting of:





The compounds of the invention are effective inhibitors of wild type reverse transcriptase as well as inhibiting, for example, the single mutation enzymes Y181C, K103N, V106A, G190A, Y188C, and P236L. The compounds also
5 inhibit the double mutation enzymes K103N/Y181C, K103N/P225H, K103N/V108I and K103N/L100I.

The compounds of formula I possess inhibitory activity against HIV-1 reverse transcriptase. When administered in suitable dosage forms, they are
10 useful in the treatment of AIDS, ARC and related disorders associated with HIV-1 infection. Another aspect of the invention, therefore, is a method for treating HIV-1 infection which comprises administering to a human being, infected by HIV-1, a therapeutically effective amount of a novel compound of formula I, as described above. Whether it be termed treatment or
15 prophylaxis, the compounds may also be used to prevent perinatal transmission of HIV-1 from mother to baby, by administration to the mother prior to birth.

The compounds of formula I may be administered in single or divided doses
20 by the oral or parenteral routes. A suitable oral dosage for a compound of formula I would be in the range of about 0.5 mg to 1 g per day. A preferred oral dosage for a compound of formula I would be in the range of about 100 mg to 800 mg per day for a patient weighing 70 kg. In parenteral formulations, a suitable dosage unit may contain from 0.1 to 250 mg of said
25 compounds, preferably 1 mg to 200 mg. It should be understood, however,

51
131
132

that the dosage administration from patient to patient will vary and the dosage for any particular patient will depend upon the clinician's judgement, who will use as criteria for fixing a proper dosage the size and condition of the patient as well as the patient's response to the drug.

5

When the compounds of the present invention are to be administered by the oral route, they may be administered as medicaments in the form of pharmaceutical preparations, which contain them in association with a compatible pharmaceutical carrier material. Such carrier material can be an inert organic or inorganic carrier material suitable for oral administration. Examples of such carrier materials are water, gelatin, talc, starch, magnesium stearate, gum arabic, vegetable oils, polyalkylene-glycols, petroleum jelly and the like.

15 The pharmaceutical preparations can be prepared in a conventional manner and finished dosage forms can be solid dosage forms, for example, tablets, dragees, capsules, and the like, or liquid dosage forms, for example solutions, suspensions, emulsions and the like. The pharmaceutical preparations may be subjected to conventional pharmaceutical operations such as sterilisation.

20 Further, the pharmaceutical preparations may contain conventional adjuvants such as preservatives, stabilisers, emulsifiers, flavour-improvers, wetting agents, buffers, salts for varying the osmotic pressure and the like. Solid carrier material which can be used include, for example, starch, lactose, mannitol, methyl cellulose, microcrystalline cellulose, talc, silica, dibasic

25 calcium phosphate, and high molecular weight polymers (such as polyethylene glycol).

For parenteral use, a compound of formula I can be administered in an aqueous or non-aqueous solution, suspension or emulsion in a

52
132
133

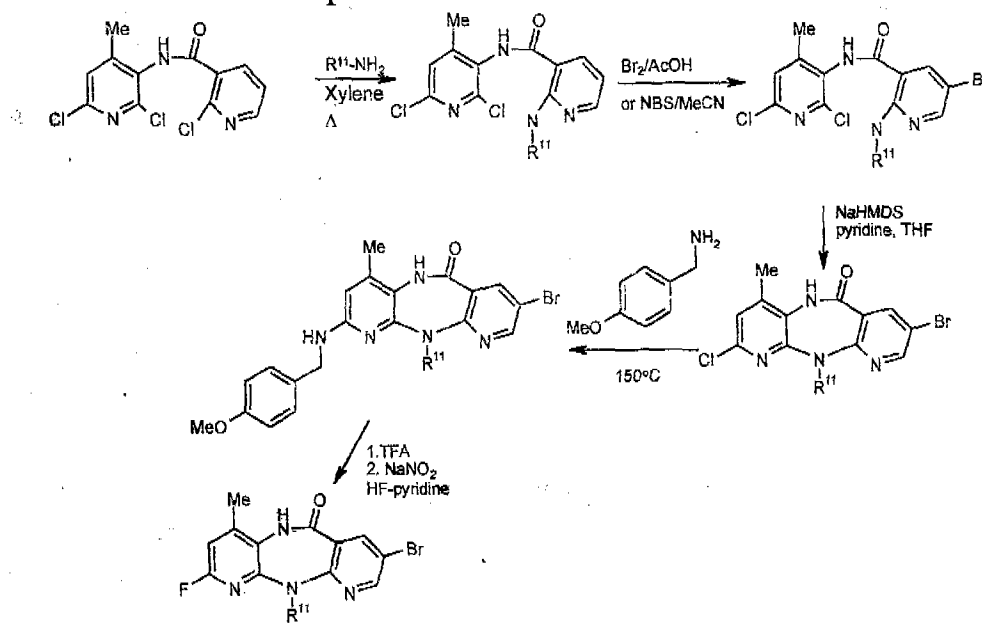
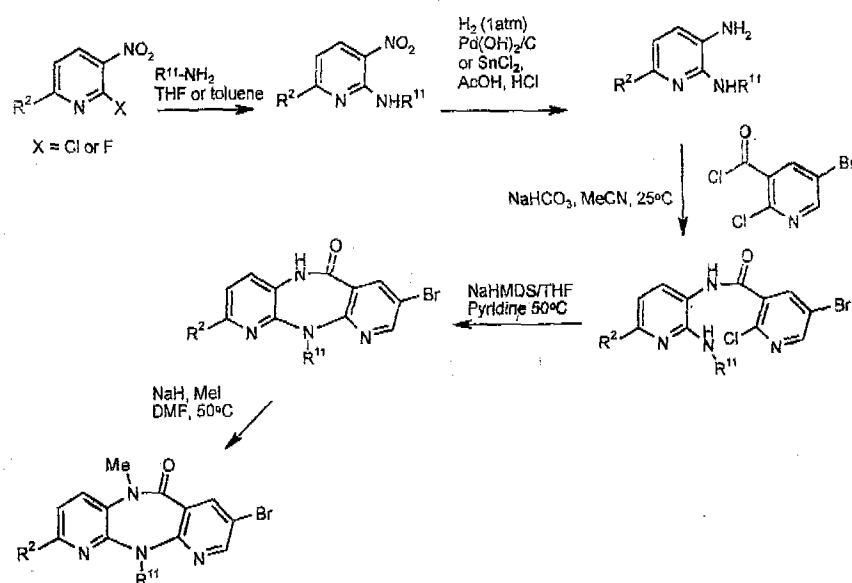
pharmaceutically acceptable oil or a mixture of liquids, which may contain bacteriostatic agents, antioxidants, preservatives, buffers or other solutes to render the solution isotonic with the blood, thickening agents, suspending agents or other pharmaceutically acceptable additives. Additives of this type
5 include, for example, tartrate, citrate and acetate buffers, ethanol, propylene glycol, polyethylene glycol, complex formers (such as EDTA), antioxidants (such as sodium bisulfite, sodium metabisulfite, and ascorbic acid), high molecular weight polymers (such as liquid polyethylene oxides) for viscosity regulation and polyethylene derivatives of sorbitol anhydrides. Preservatives
10 may also be added if necessary, such as benzoic acid, methyl or propyl paraben, benzalkonium chloride and other quaternary ammonium compounds.

The compounds of this invention may also be administered as solutions for
15 nasal application and may contain in addition to the compounds of this invention suitable buffers, tonicity adjusters, microbial preservatives, antioxidants and viscosity-increasing agents in an aqueous vehicle. Examples of agents used to increase viscosity are polyvinyl alcohol, cellulose derivatives, polyvinylpyrrolidone, polysorbates or glycerin. Microbial
20 preservatives added may include benzalkonium chloride, thimerosal, chlorobutanol or phenylethyl alcohol.

Additionally, the compounds provided by the invention can be administered by suppository.

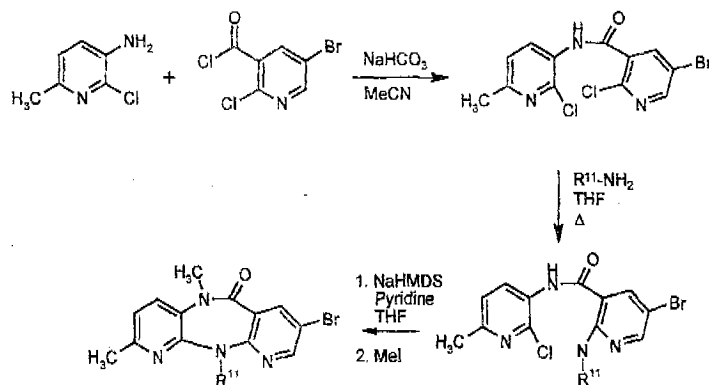
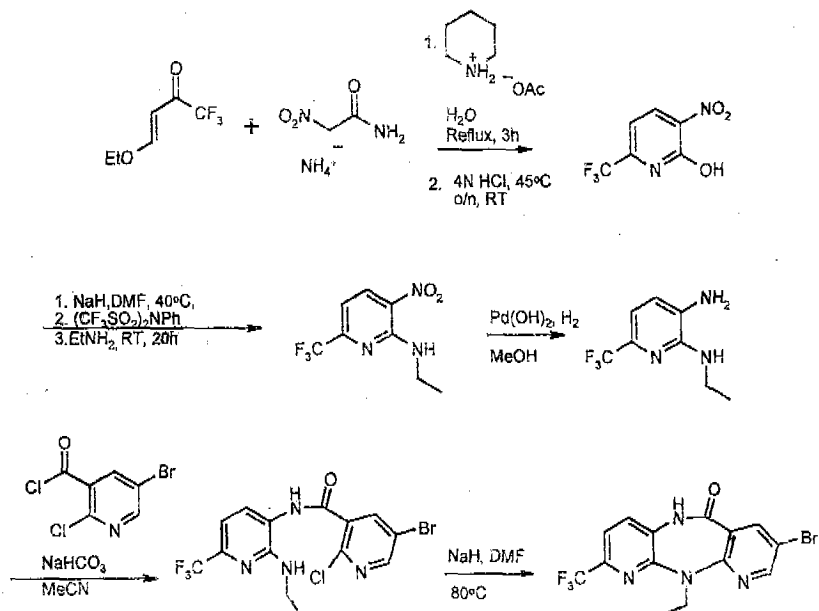
25

The compounds of the invention may be made using the skills of a synthetic organic chemist. An exemplary reaction scheme is shown in schemes 1 to 6.

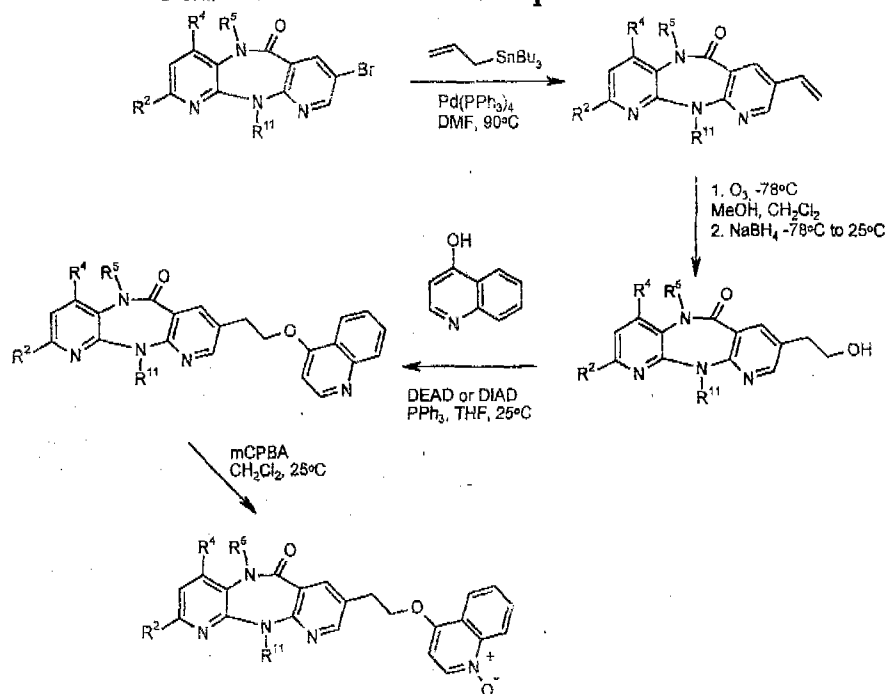
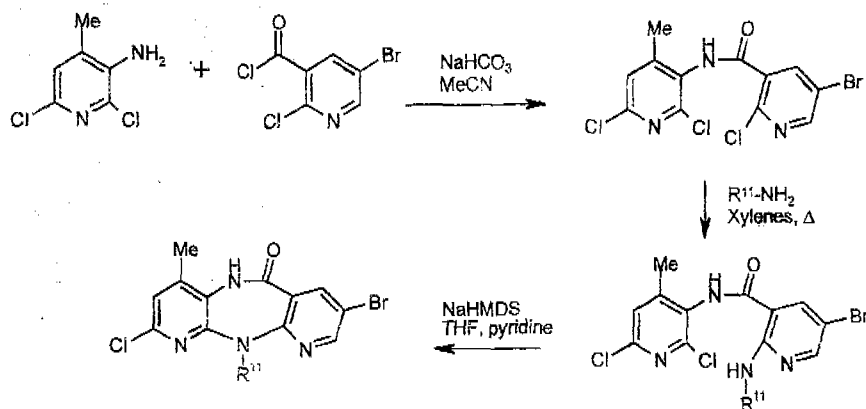
Scheme 1: Preparation of intermediates in which R⁴ is MeScheme 2: Preparation of intermediates in which R⁵ is Me

- 5 The sequence of scheme 2 is analogous to one described by J.M. Klunder *et al.*; *J. Med. Chem.* 1998, 41, 2960-71, and C.L. Cywin *et al.*; *J. Med. Chem.* 1998, 41, 2972-84.

11

Scheme 3: Preparation of intermediates in which R² is C₁₋₃ alkylScheme 4: Preparation of intermediates in which R² is CF₃

12

Scheme 5: Introduction of the quinoline nucleus**Scheme 6: Alternate route to compounds in which R^4 is Me**

5

As stated before, the compounds provided by the invention inhibit the enzymatic activity of HIV-1 RT. Based upon testing of these compounds, as described below, it is known that they inhibit the RNA-dependent DNA polymerase activity of HIV-1 RT. It is known (data not shown) that they also inhibit the DNA-dependent DNA polymerase activity of HIV-1 RT. Utilising the Reverse Transcriptase (RT) Assay described below, compounds can be

58
136
137

tested for their ability to inhibit the RNA-dependent DNA polymerase activity of HIV-1 RT. Certain specific compounds described in the Examples which appear below, were so tested. The results of this testing appear in Table 2 as IC₅₀ (nM) and Table 3 as EC₅₀ (nM).

5

EXAMPLES

The present invention is illustrated in further detail by the following non-limiting examples. All reactions were performed in a nitrogen or argon
10 atmosphere. Temperatures are given in degrees Celsius. Solution percentages or ratios express a volume to volume relationship, unless stated otherwise.

Abbreviations or symbols used herein include:

- DEAD: diethyl azodicarboxylate;
- 15 DIAD: diisopropyl azodicarboxylate;
- DIEA: diisopropylethylamine;
- DMAP: 4-(dimethylamino)pyridine;
- DMSO: dimethylsulfoxide;
- DMF: dimethylformamide;
- 20 ES MS: electron spray mass spectrometry;
- Et: ethyl;
- EtOAc: ethyl acetate;
- Et₂O: diethyl ether;
- HPLC: high performance liquid chromatography;
- 25 iPr: isopropyl;
- Me: methyl;
- MeOH: methanol;
- MeCN: acetonitrile;
- NBS: N-bromosuccinimide;

- Ph: phenyl;
TBE: tris-borate-EDTA;
TBTU: 2-(1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium
tetrafluoroborate;
5 TFA: trifluoroacetic acid;
THF: tetrahydrofuran;
MS (ES): electrospray mass spectrometry;
MS (FAB) or FAB/MS: fast atom bombardment mass spectrometry;
HRMS: high resolution mass spectrometry;
10 PFU: plaque forming units;
DEPC: diethyl pyrocarbonate;
DTT: dithiothreitol;
EDTA: ethylenediaminetetraacetate;
UMP: uridine 5'-monophosphate;
15 UTP: uridine 5'-triphosphate;
MES: 2-(n-morpholino)ethanesulfonic acid;
SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis;
MWCO: molecular weight cut-off;
Bis-Tris Propane: 1,3-Bis[tris(hydroxymethyl)-methylamino]propane;
20 GSH: reduced glutathione;
OBG: n-Octyl- β -D-glucoside.

SYNTHESES

- The following examples illustrate methods for preparing compounds of the
25 invention.

Example I (entry 24, Table 1)

5,11-Dihydro-11-ethyl-5-methyl-8-[2-(4-quinolinylloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

a) 2-(Ethylamino)-3-nitropyridine

To a solution of 2-chloro-3-nitropyridine (51 g, 325 mmol) in THF (650 mL) was added a 2 M solution of ethylamine in THF (365 mL, 731 mmol). The reaction was stirred at room temperature overnight. The reaction mixture
5 was poured into water (~1.5 L) and the resulting solid was filtered and dried under reduced pressure to give the title compound (52 g).

b) 3-Amino-2-(ethylamino)pyridine

A solution of 2-(ethylamino)-3-nitropyridine (52 g) in MeOH (600 mL) was
10 stirred overnight at room temperature under hydrogen (1 atm.) in the presence of 20% Pd(OH)₂/C (10.4 g). The catalyst was removed by filtration through diatomaceous earth. The filtrate was concentrated under reduced pressure to give the title compound as a black solid (39 g, 88% yield over steps a) and b).

15

c) 2-Chloro-N-[2-(ethylamino)-3-pyridinyl]-5-bromo-3-pyridinecarboxamide

To a cooled solution of 3-amino-2-(ethylamino)pyridine (30.6 g, 223 mmol) in MeCN (740 mL) was added solid NaHCO₃ (56.3 g, 669 mmol). After 5 min,
20 crude 5-bromo-2-chloro-3-pyridinecarbonyl chloride (prepared from 5-bromo-2-hydroxy-3-pyridinecarboxylic acid and SOCl₂ [as described by T. W. Gero *et al.* in *Synth. Commun.* 1989, 19, 553-559 (incorporated herein by reference) but with omission of the aqueous work-up] was added (1 equiv., 223 mmol). After 2 h, the reaction mixture was poured over ice/H₂O (1.5 L)
25 and the resulting solid was filtered, rinsed with H₂O and then hexane. After drying under reduced pressure overnight, the title compound was obtained as a black solid (54.9 g, 69% yield).

30

d) 8-Bromo-5,11-dihydro-11-ethyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

To a solution of 2-chloro-N-[2-(ethylamino)-3-pyridinyl]-5-bromo-3-

pyridinecarboxamide (54.9 g, 154.4 mmol) in pyridine (308 mL) at 50 °C was added drop-wise a 1 M solution of NaHMDS (sodium hexamethyldisilazide) in THF (355 mL, 355 mmol). After 10 min, the reaction was allowed to cool to room temperature, and then was poured over ice water (2 L). The resulting solid was filtered, rinsed with water and then hexane. The solid was dried under reduced pressure to give the title compound (36 g, 75% yield) as a dark green solid.

e) 8-Bromo-5,11-dihydro-11-ethyl-5-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

To a solution of the 8-bromo-5,11-dihydro-11-ethyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (36.7 g, 115 mmol) in DMF (380 mL) was added NaH (3.5 g, 138 mmol), and the mixture was heated to 50 °C for 30 min. The reaction mixture was cooled to room temperature and treated with MeI (14.3 mL, 230 mmol). After 1.5 h, the reaction mixture was poured over ice water. The solid was filtered, washed with water and then hexane to give after drying the title compound (37.9 g 99% yield) as a dark grey solid.

f) 5,11-Dihydro-11-ethyl-5-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

Allyltributyltin (30.7 mL, 99.0 mmol) and Pd(PPh₃)₄ (5.20 g, 4.50 mmol) were added to a degassed (N₂ through solution for 30 min) solution of 8-bromo-5,11-dihydro-11-ethyl-5-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (30.0 g, 90.0 mmol) in DMF (450 mL) at room temperature. The mixture was stirred at 90 °C for 1.5 h then was cooled to room temperature and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane: EtOAc, 8:2 to 7:3) to give the title compound (22.19 g, 84% yield).

g) 5,11-Dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-

b:2',3'-e][1,4]diazepin-6-one

A stream of ozonised oxygen was bubbled through a cold (-78 °C) solution of 5,11-dihydro-11-ethyl-5-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (22.19 g, 75.4 mmol) in CH₂Cl₂ (150 mL) and MeOH (150 mL) for 2.5 h. A stream of N₂ was next bubbled through the solution for 15 min and then solid NaBH₄ (4.99 g, 132 mmol) was added to the solution. The reaction mixture was allowed to warm to room temperature. After 1 h, aqueous saturated NH₄Cl (200 mL) was added and the mixture was stirred at room temperature for 2 h. The organic solvents were removed under reduced pressure. Water (300 mL) and CHCl₃ (300 mL) were added to the residue. The phases were separated and the aqueous layer was extracted with CHCl₃ (3 × 300 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc:CHCl₃, 4:1) to give the title compound (16.1 g, 72% yield) as a white solid.

h) 5,11-Dihydro-11-ethyl-5-methyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2b:2',3'-e] [1,4]diazepin-6-one

Diethyl azodicarboxylate (DEAD) (12.8 mL, 81.0 mmol) was added dropwise to a solution of 5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (16.1 g, 54.0 mmol), 4-hydroxyquinoline (11.6 g, 81.0 mmol) and Ph₃P (21.3 g, 81.0 mmol) in THF (270 mL) at room temperature. The mixture was stirred at room temperature for 1h then was concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc:MeOH; 95:5) to give the title compound (17.7 g, 77% yield) as a white solid: MS (ESI) m/z 426 (MH)⁺.

Example II (entry 2, Table 1)

2-Chloro-5,11-dihydro-11-ethyl-5-methyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

a) 6-Chloro-2-(ethylamino)-3-nitropyridine

An ice-cold solution of EtNH₂ (49.8 g, 1.10 mol) in toluene (200 mL) was added over 15 min to an ice-cold solution of 2,6-dichloro-3-nitropyridine (100.0 g, 0.52 mol) in toluene (225 mL). The mixture was stirred at 0 °C for 45 min. Water (500 mL) and EtOAc (500 mL) were added and the phases were separated. The organic layer was successively washed with water (200 mL) and brine (200 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The residual solid was recrystallised from MeOH to give the title compound (83.7 g, 80% yield) as yellow needles.

10

b) 3-Amino-6-chloro-2-(ethylamino)pyridine

A solution of SnCl₂·H₂O (616.3 g, 2.73 mol) in aqueous 12 N HCl (500 mL) was rapidly added to a solution of 6-chloro-2-(ethylamino)-3-nitropyridine (169.5 g, 0.84 mol) in AcOH (1.7 L) at room temperature. After 20 min, the mixture was cooled to 0 °C and water (250 mL) was added. Solid NaOH (240 g) was then added in small portions. The resulting suspension was filtered to remove tin salts. The filtrate was diluted with water (3.5 L), the solution rendered basic by addition of aqueous 10 N NaOH and then extracted with EtOAc (3 × 1.7 L). The combined organic layers were washed with brine (1 L), dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 3:2) to give the title compound (89.9 g, 62% yield) as a brown solid: MS (ESI) m/z 172/174 (MH)⁺.

15

20

c) 5-Bromo-2-chloro-N-[2-(ethylamino)-6-chloro-3-pyridinyl]-3-pyridinecarboxamide

A solution of 5-bromo-2-chloro-3-pyridinecarbonyl chloride (30.0 g, 97.0 mmol) in MeCN (100 mL) was added via cannula to a solution of 3-amino-6-chloro-2-(ethylamino)pyridine (16.6 g, 97.0 mmol) in MeCN (180 mL) containing solid NaHCO₃ (14.2 g, 169 mmol) at room temperature. The

25

30

5 mixture was stirred at room temperature for 1 h. Water (200 mL) was added and the mixture was stirred for 10 min. The resulting suspension was filtered. The solid was washed with Et₂O (50 mL) and concentrated from pyridine solution (3 × 50 mL) to give the title compound (28.4 g, 75% yield).

d) 8-Bromo-2-chloro-5,11-dihydro-11-ethyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

A 1 M solution of NaHMDS in THF (167.5 mL, 167.5 mmol) was slowly added to a solution of 5-bromo-2-chloro-N-{2-(ethylamino)-6-chloro-3-pyridinyl}-3-pyridinecarboxamide (28.4 g, 72.8 mmol) in pyridine (146 mL) heated to 50 °C. The reaction mixture was stirred at 50 °C for 1.5 h. The mixture was then poured into a mixture of water and ice (1 L) and, after 1 h, the resulting suspension was filtered. The solid was washed with water and dried under reduced pressure to give the title compound (23.4 g, 91% yield).

e) 8-Bromo-2-chloro-5,11-dihydro-11-ethyl-5-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

Solid NaH (60% oil dispersion, 3.46 g, 86.1 mmol) was added over 30 min to a solution of 8-bromo-2-chloro-5,11-dihydro-11-ethyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (23.4 g, 66.3 mmol) in DMF (220 mL) at 50 °C. The mixture was stirred at 50 °C for 1 h then was allow to cool to room temperature. The mixture was poured into water (1 L) and the resulting suspension was filtered. The solid was successively washed with water and hexane then dried under reduced pressure to give the title compound (23.0 g, 94% yield).

f) 2-Chloro-5,11-dihydro-11-ethyl-5-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

Allyltributyltin (21.3 mL, 68.7 mmol) and Pd(Ph₃P)₄ (3.61 g, 3.12 mmol) were added to a degassed (N₂ through solution for 30 min) solution of 8-bromo-2-chloro-5,11-dihydro-11-ethyl-5-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (23.0 g, 62.5 mmol) in DMF (312 mL). The mixture was

heated to 90 °C for 2 h. The mixture was concentrated under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 7:3) to give the title compound (13.4 g, 65% yield).

5 g) **2-Chloro-5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

Ozonised oxygen was introduced into a cold (-78 °C) solution of 2-chloro-5,11-dihydro-11-ethyl-5-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (13.4 g, 40.7 mmol) in MeOH (102 mL) and CH₂Cl₂ (102 mL) until complete disappearance of the alkene. Nitrogen was bubbled through the solution to remove excess O₃. Solid NaBH₄ (2.69 g, 71.1 mmol) was next added in small portions and the mixture was allowed to warm slowly to room temperature. After 1 h, aqueous saturated NH₄Cl (150 mL) was added and the mixture stirred for 20 min. The organic solvents were removed under reduced pressure. Water (100 mL) was added to the aqueous solution. The solution was extracted with CHCl₃ (3 × 200 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc:CHCl₃, 4:1) to give the title compound (10.4 g, 77% yield).

20

h) **2-Chloro-5,11-dihydro-11-ethyl-5-methyl-8-[2-(4-quinolinylloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

Diethyl azodicarboxylate (DEAD) (4.26 mL, 27.0 mmol) was added dropwise to a solution of 2-chloro-5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (6.00 g, 18.0 mmol), 4-hydroxyquinoline (3.92 g, 27.0 mmol) and Ph₃P (7.09 g, 27.0 mmol) in THF (90 mL) at room temperature. The mixture was stirred at room temperature for 1h then was concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc:MeOH, 95:5) to give the title compound (6.22 g, 75% yield) as a white solid: MS (ESI) m/z 460/462.

30

Example III (entry 6, Table 1)

2-Chloro-11-cyclopropyl-5,11-dihydro-5-methyl-8-[2-(4-quinolinyl-ethoxy)-ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

5

a) 6-Chloro-2-(cyclopropylamino)-3-nitropyridine

A solution of cyclopropylamine (1.25 g, 22.0 mmol) in toluene (11 mL) was added over 10 min to an ice-cold solution of 2,6-dichloro-3-nitropyridine (2.00 g, 10.4 mmol) in toluene (10 mL). The mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. Water (50 mL) was added to the mixture and the phases were separated. The organic layer was washed with brine (25 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 3:2) to give the title compound (1.97 g, 89% yield) as a yellow solid.

15

b) 2-Chloro-11-cyclopropyl-5,11-dihydro-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

The title compound was prepared from 6-chloro-2-(cyclopropylamino)-3-nitropyridine using a method similar to that described for the 11-ethyl analog in example II.

20

c) 2-Chloro-11-cyclopropyl-5,11-dihydro-5-methyl-8-[2-(4-quinolinyl-ethoxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

Diethyl azodicarboxylate (DEAD) (85.6 µL, 0.54 mmol) was added dropwise to a solution of 2-chloro-11-cyclopropyl-5,11-dihydro-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (125 mg, 0.36 mmol), 4-hydroxyquinoline (78.9 mg, 0.54 mmol) and Ph₃P (143 mg, 0.54 mmol) in THF (1.8 mL) at room temperature. The mixture was stirred at room temperature for 3 h then was concentrated under reduced pressure. The residue was partially purified by flash chromatography (EtOAc:MeOH; 95:5). The solid was further purified by reverse phase HPLC (CombiPrep ADS-AQ 50 × 20 mm, 5 µ, 120 Å, 5-100% MeCN + 0.10% TFA / water + 0.10% TFA in

30

25 min) to give the trifluoroacetic acid salt of the title compound (17.7 g, 77% yield) as a white solid: MS (ESI) m/z 472/474 (MH)⁺.

Example IV (entry 4, Table 1)

5 **5,11-Dihydro-11-ethyl-2-fluoro-5-methyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one**

a) 2,6-Difluoro-3-nitropyridine

To a mixture of concentrated sulphuric acid (600 mL) and fuming nitric acid (90%, 400mL) in a ice-bath (internal temperature maintained at 5-10 °C) was added drop-wise 2,6-difluoropyridine (200 g, 1.74 mol). The resulting mixture was stirred overnight at room temperature. The mixture was poured slowly into 3 kg of ice and extracted with Et₂O (2 × 2 L). The combined organic layers were washed with aqueous 1.5 N NaOH (2 × 1 L), then with aqueous saturated NaHCO₃ (400 mL) or until pH is around 8-9. The organic layers were dried over MgSO₄, filtrated and concentrated under reduced pressure until constant weight (to remove unreacted 2,6-difluoropyridine: 10-12%). The title compound was obtained as a yellow liquid (207.3 g, 74% yield).

20

b) 2-(Ethylamino)-6-fluoro-3-nitropyridine

To a solution of 2,6-difluoro-3-nitropyridine (45.7 g, 285 mmol) in THF (500 mL) at -40 °C was added drop-wise a solution of ethylamine (25.7 g, 570 mmol) in THF (250 mL). After 30 min, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in EtOAc. The organic phase was washed with brine, dried (MgSO₄), filtered and concentrated. The resulting yellow solid was purified by flash chromatography (15% EtOAc in hexane) to give the title compound (43.2 g, 82% yield) as a yellow solid.

30

5
1245
146

c) 3-Amino-2-(ethylamino)-6-fluoropyridine

A solution of 2-(ethylamino)-6-fluoro-3-nitropyridine (43.2 g, 230 mmol) in THF (1 L) was stirred overnight at room temperature under hydrogen (1 atm.) in the presence of 20% Pd(OH)₂/C (4.35 g). The catalyst was removed
5 by filtration through diatomaceous earth. The filtrate was concentrated under reduced pressure to give the title compound (36.3 g, 95% yield) as a black solid.

d) 2-Chloro-N-[2-(ethylamino)-6-fluoro-3-pyridinyl]-5-bromo-3-pyridinecarboxamide

10 To a cooled solution (4 °C) of 3-amino-2-(ethylamino)-6-fluoropyridine (31.0 g, 200 mmol) in MeCN (160 mL) was added solid NaHCO₃ (50.4 g, 600 mmol). After 15 min, a solution of 5-bromo-2-chloro-3-pyridinecarbonyl chloride (1 equiv., 200 mmol) in MeCN (155 mL) was added. After 60 min at
15 room temperature, the reaction mixture was poured into water (1.2 L) and stirred for 30 min. The resulting solid was filtered, dried under reduced pressure at 50 °C overnight. The title compound (73.7 g, 99% yield) was obtained as a black solid.

e) 8-Bromo-5,11-dihydro-11-ethyl-2-fluoro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

20 To a solution of the 2-chloro-N-[2-(ethylamino)-6-fluoro-3-pyridinyl]-5-bromo-3-pyridinecarboxamide (73.5 g, 216 mmol) in pyridine (435 mL) at 50 °C was added drop-wise a 1 M solution of NaHMDS in THF (520 mL, 520
25 mmol). After 10 min, the reaction was allowed to cool to room temperature, then poured over ice water (2 L). The resulting solid was filtered, rinse with water and then hexane. The solid was dried under reduced pressure to give the title compound (50.6 g, 69% yield) as a dark green solid.

f) 8-Bromo-5,11-dihydro-11-ethyl-2-fluoro-5-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one56
146
147

To a solution of the 8-bromo-5,11-dihydro-11-ethyl-2-fluoro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (44 g, 130.5 mmol) in DMF (520 mL) was added NaH (4.28 g, 178 mmol), and the mixture was heated to 50 °C for 30 min. The reaction mixture was cooled to room temperature and treated with MeI (24.4 mL, 522 mmol). After 1.5 h, the reaction mixture was poured over ice water. The solid was filtered, washed with water and then hexane, dried under reduced pressure to give the title compound (43.2 g, 94% yield) as dark grey solid.

g) **5,11-Dihydro-11-ethyl-2-fluoro-5-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one**
Allyltributyltin (32.0 mL, 103.4 mmol) and Pd(Ph₃P)₄ (5.43 g, 4.70 mmol) were added to a degassed (N₂ through solution for 45 min) solution of 8-bromo-5,11-dihydro-11-ethyl-2-fluoro-5-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (33.0 g, 94.0 mmol) in DMF (470 mL). Additional amounts of Pd(Ph₃)₄ (1.09 g, 0.94 mmol added after 1, 2, 3, 4, 5 h of reaction) were added to complete the reaction. The mixture was heated to 90 °C for 6 h. The mixture was concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc, 8:2 to 7:3) to give the title compound (22.4 g, 76% yield).

h) **5,11-Dihydro-11-ethyl-2-fluoro-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one**
A stream of ozonised oxygen was bubbled through a cold (-78 °C) solution of 5,11-dihydro-11-ethyl-2-fluoro-5-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (22.38 g, 71.6 mmol) in CH₂Cl₂ (100 mL) and MeOH (100 mL) for 3 h. A stream of N₂ was next bubbled through the solution for 15 min and then solid NaBH₄ (5.05 g, 133 mmol) was added to the solution. The reaction mixture was allowed to warm to room temperature. After 1 h, an additional portion of NaBH₄ (1.62 g, 43.0 mmol) was added to the reaction

68
148
149

mixture. After an additional hour, aqueous saturated NH_4Cl (150 mL) was added and the mixture was stirred at room temperature for 30 min. The organic solvents were removed under reduced pressure. Water (200 mL) was added and the mixture was extracted with CHCl_3 (3×300 mL). The combined organic layers were dried (MgSO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography ($\text{EtOAc}:\text{CHCl}_3$, 4:1) to give the title compound (19.7 g, 72% yield) as a white solid.

10 **i) 5,11-Dihydro-11-ethyl-2-fluoro-5-methyl-8-[2-(4-quinolinylloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

Diethyl azodicarboxylate (DEAD) (14.3 mL, 91.0 mmol) was added dropwise to a solution of 5,11-dihydro-11-ethyl-2-fluoro-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (19.2 g, 60.7 mmol), 4-hydroxyquinoline (13.2 g, 91.0 mmol) and Ph_3P (23.9 g, 91.0 mmol) in THF (300 mL) at room temperature. The mixture was stirred at room temperature for 1h then was concentrated under reduced pressure. The residue was purified by flash chromatography ($\text{EtOAc}:\text{MeOH}$; 95:5) to give the title compound (17.9 g, 67% yield) as a white solid: MS (ESI) m/z 444 (MH)⁺.

20 **Example V (entry 1, Table 1)**

2-Chloro-5,11-dihydro-11-ethyl-4-methyl-8-[2-(4-quinolinylloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

25 **a) 2-Chloro-N-(2,6-dichloro-4-methyl-3-pyridinyl)-3-pyridinecarboxamide**

NaHCO_3 (21.72 g, 258.6 mmol) was added to a solution of 3-amino-2,6-dichloro-4-methylpyridine (41.61 g, 235.1 mmol; prepared as described by K. G. Grozinger *et al.* J. Heterocyclic Chem. 1995, 32, 259-263) in MeCN (350 mL) and the resulting suspension was stirred for 15 min. A solution of 2-chloronicotinyl chloride (43.44 g, 246.8 mmol) in MeCN (500 mL) was then introduced over 30 min. The resulting suspension was stirred at room temperature. After 24 h, the solution was found to be acidic and so an

additional amount of NaHCO_3 (3.00 g, 35.7 mmol) was added. The suspension was then stirred at room temperature for an additional 2 days. The mixture was then poured into a mixture of water (2 L) and ice (200 g) and stirred for 20 min. The solid was filtered and washed with water (500 mL) and the resulting solid was then dried over P_2O_5 under reduced pressure to give the title compound (62.55 g, 84% yield) as a white powder.

b) N-(2,6-dichloro-4-methyl-3-pyridinyl)-2-(ethylamino)-3-pyridinecarboxamide

10 A solution of 2-chloro-N-(2,6-dichloro-4-methyl-3-pyridinyl)-3-pyridinecarboxamide (63.17 g, 194.0 mmol) and ethylamine (28.0 g, 583 mmol) in xylenes (250 mL) was stirred at 120 to 125 °C in a steel autoclave for 7 h. The resulting suspension was poured into water (1 L), stirred for 15 min and filtered. The residue was dissolved in EtOAc and washed with water (3
15 ×), brine and was dried over MgSO_4 . The filtrate was extracted with EtOAc and the combined organic extracts were washed with water and brine and dried over MgSO_4 . The two fractions were then combined and excess xylenes removed by co-distillation with benzene to give the title compound (61.98 g, pink solid) as the major component of a mixture of compounds. The material,
20 not purified at this point, was used directly in the subsequent reaction.

c) 5-Bromo-N-(2,6-dichloro-4-methyl-3-pyridinyl)-2-(ethylamino)-3-pyridinecarboxamide

Solid KOAc (22.2 g, 229 mmol) was added to a solution of crude N-(2,6-dichloro-4-methyl-3-pyridinyl)-2-(ethylamino)-3-pyridinecarboxamide (61.98
25 g, < 190 mmol) in AcOH (600 mL). A solution of Br_2 (9.7 mL, 191 mmol) was then added over 30 min. After stirring 30 min, additional amounts of KOAc and Br_2 were introduced until TLC indicated that no starting material remained (a total of 11.2 g of KOAc and 5.91 mL of Br_2). The solution was
30 then poured into water (2 L) and stirred for 20 min. The solid was removed

by filtration and then dissolved in Et₂O (1 L). The ethereal solution was washed with water (3 ×), aqueous saturated NaHCO₃, brine then dried (MgSO₄) to give a mixture containing, as the major component, the title compound (77.7 g, yellow foam). The material was used directly in the subsequent reaction.

d) 8-Bromo-2-chloro-5,11-dihydro-11-ethyl-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

A solution of crude 5-bromo-*N*-(2,6-dichloro-4-methyl-3-pyridinyl)-2-(ethylamino)-3-pyridinecarboxamide (77.7 g, mmol) in anhydrous pyridine (1.0 L) was heated to 50 °C. A 1 M solution of NaHMDS in THF (418 mL, 418 mmol) was then added dropwise and stirring was continued for an additional 15 min. After cooling to room temperature, water (50 mL) was added and the mixture concentrated to two-thirds volume on a rotary evaporator. The residue was then poured into water (3 L). Filtration gave the title compound (23.44 g) as a tan solid. The filtrate was extracted with EtOAc (3 ×) and the combined extracts were washed with water and brine then dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 9:1 or CHCl₃) to give an additional amount of the title compound (14.06 g, for a total of 37.50 g, 50% yield for 3 steps).

e) 2-Chloro-5,11-dihydro-11-ethyl-4-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

A solution of 8-bromo-2-chloro-5,11-dihydro-11-ethyl-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (12.6 g, 34.3 mmol) in DMF (25.0 mL) was degassed under reduced pressure for 20 min. Pd(PPh₃)₄ (775 mg, 0.7 mmol) was then added followed by allyltributyltin (12.5 mL, 41.1 mmol). After degassing under reduced pressure for 10 min, the mixture was heated to 100 °C for 7 h. The mixture was then concentrated under reduced

pressure. The residue was purified by flash chromatography (hexane:EtOAc, 19:1) to give the title compound (8.04 g, 71% yield) as a yellow solid.

5 **f) 2-Chloro-5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

A stream of O₃ was introduced into a cold (-78 °C) solution of 2-chloro-5,11-dihydro-11-ethyl-4-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (8.04 g, 24.4 mmol) and Sudan III in CH₂Cl₂ (120 mL) and MeOH (120 mL). When the pink solution turned brown, O₂ was bubbled
10 through the solution for 10 min. NaBH₄ (1.12 g, 29.4 mmol) was then added and the solution was allowed to warm to room temperature. After 30 min, an additional amount of NaBH₄ (500 mg) was added. After 1 h, aqueous saturated NH₄Cl was then added and the mixture stirred for 20 min. The solution was concentrated under reduced pressure and extracted with
15 CH₂Cl₂ (3 ×). The combined organic extracts were washed with brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CHCl₃:EtOH, 97:3) to give the title compound (6.01 g, 74% yield) as a white solid.

20 **g) 2-Chloro-5,11-dihydro-11-ethyl-4-methyl-8-[2-(4-quinolinylloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

Diethyl azodicarboxylate (DEAD) (83 µL, 0.53 mmol) was added drop-wise to a solution of 2-chloro-5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (125 mg, 0.38 mmol), 4-
25 hydroxyquinoline (76.3 mg, 0.53 mmol) and Ph₃P (138 mg, 0.53 mmol) in THF (2.5 mL) at room temperature. The mixture was stirred at room temperature for 3 h then was concentrated under reduced pressure. The residue was dissolved in EtOAc (60 mL) and the solution was successively washed with 1 N aqueous NaOH (3 × 10 mL) and brine (15 mL) then was
30 dried (MgSO₄), filtered and concentrated under reduced pressure. The

residue was purified by flash chromatography (EtOAc:MeOH, 9:1) to give the title compound (52 mg, 30% yield) as a white solid: MS (ESI) m/z 460/462 (MH)⁺.

5 **Example VI (entry 7, Table 1)**

5,11-Dihydro-11-ethyl-2-fluoro-4-methyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

- 10 **a) 8-Bromo-5,11-dihydro-11-ethyl-2-(((4-methoxyphenyl)methyl)amino)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**
8-Bromo-2-chloro-5,11-dihydro-11-ethyl-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (2.50 g, 6.80 mmol) was dissolved in dioxane (10 mL) in a glass pressure bottle and 4-methoxybenzylamine (4.0 mL, 30.6 mmol) was added to the solution. The mixture was heated to 170 °C for 5 days. The
15 reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was taken up in EtOAc and washed with 10% aqueous NH₄Cl, dried over MgSO₄, filtered and concentrated under reduced pressure to give a brown oil which was chromatographed over silica gel (hexane:EtOAc, 4:1). The title compound was obtained as a yellow solid
20 (1.35 g, 42% yield).

b) 2-Amino-8-bromo-5,11-dihydro-11-ethyl-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

- 8-Bromo-5,11-dihydro-11-ethyl-2-(((4-methoxyphenyl)methyl)amino)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (6.52 g, 18.7 mmol) was
25 dissolved in trifluoroacetic acid (100 mL) and the solution was stirred magnetically at room temperature for 18 h. Trifluoroacetic acid was evaporated under reduced pressure and the residue was poured into a mixture containing water (450 mL), concentrated NH₄OH (50 mL) and
30 EtOAc (400 mL). The mixture was stirred magnetically for 2 h at room temperature. The organic phase was concentrated under reduced pressure.

73
153
154

The yellow precipitate was filtered off and washed with water, dried under reduced pressure to give 7.51 g of a yellow solid which was triturated in Et₂O to give the title compound (5.12 g, 105% yield) as a yellow solid.

5 **c) 8-Bromo-5,11-dihydro-11-ethyl-2-fluoro-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

A plastic bottle was charged with 2-amino-8-bromo-5,11-dihydro-11-ethyl-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (5.10 g, 14.6 mmol). HF-pyridine (100 g) was added and the resulting suspension was cooled to 0°
10 C. Sodium nitrite (1.12 g, 16.1 mmol) was added in several portions over 30 min to produce a purple solution. The mixture was then stirred for 16 h at room temperature. The reaction mixture was poured onto ice and 6 N NaOH. The beige suspension was extracted with EtOAc. The organic extracts were combined, dried over MgSO₄, filtered and concentrated. The residue was
15 triturated with Et₂O/hexane to give the title compound (4.30 g, 84% yield).

d) 5,11-Dihydro-11-ethyl-2-fluoro-4-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

Allyltributyltin (7.77 mL, 25.1 mmol) and Pd(Ph₃P)₄ (1.32 g, 1.14 mmol) were
20 added to a degassed (N₂ through solution for 30 min) solution of 8-bromo-5,11-dihydro-11-ethyl-2-fluoro-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (8.0 g, 22.8 mmol) in DMF (114 mL). The mixture was heated to 90 °C for 3 h. The mixture was poured into water (250 mL) and extracted with EtOAc (4 × 250 mL). The combined organic layers were
25 washed with 20% aqueous NH₄OH (250 mL) and brine (250 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc, 4:1 to 1:1) to give the title compound (3.16 g, 51% yield).

30 **e) 5,11-Dihydro-11-ethyl-2-fluoro-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

24
134
155

A stream of ozonised oxygen was bubbled through a cold (-78 °C) solution of 5,11-dihydro-11-ethyl-2-fluoro-4-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (3.16 g, 10.1 mmol) in CH₂Cl₂ (25 mL) and MeOH (25 mL) for 45 min. A stream of N₂ was next bubbled through the solution for 15 min and then solid NaBH₄ (669 mg, 17.1 mmol) was added to the solution. The reaction mixture was allowed to warm to room temperature. After 2 h, aqueous saturated NH₄Cl (50 mL) was added and the mixture was stirred at room temperature for 15 min. The organic solvents were removed under reduced pressure. The residual aqueous solution was extracted with CHCl₃ (3 × 100 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc:CHCl₃, 1:1) to give the title compound (2.40 g, 75% yield) as a white solid.

15 f) **5,11-Dihydro-11-ethyl-2-fluoro-4-methyl-8-[2-(4-quinolinylloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one**
Diisopropyl azodicarboxylate (DIAD) (117 µL, 0.59 mmol) was added dropwise to a solution of 5,11-dihydro-11-ethyl-2-fluoro-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (125 mg, 0.40 mmol), 4-hydroxyquinoline (86.0 mg, 0.59 mmol) and Ph₃P (156 mg, 0.59 mmol) in THF (1.9 mL) at room temperature. The mixture was stirred at room temperature for 3 h then was concentrated under reduced pressure. The residue was dissolved in EtOAc (40 mL) and the solution was successively washed with 3 N aqueous NaOH (3 × 20 mL), aqueous saturated NH₄Cl (15 mL), brine (15 mL) then was dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was partially purified by flash chromatography (EtOAc:MeOH; 95:5). The solid was further purified by reverse phase HPLC (CombiPrep ADS-AQ 50 × 20 mm, 5 µ, 120 Å, 5-100% MeCN + 0.10% TFA / water + 0.10% TFA in 25 min) to give the

20
25

trifluoroacetic acid salt of the title compound (106 mg, 48% yield) as a white solid: MS (ESI) m/z 444 (MH)⁺.

Example VII (entry 5, Table 1)

- 5 **11-(Cyclopropyl)-5,11-dihydro-2-fluoro-4-methyl-8-{2-(4-quinolinyl-ethoxy)-ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

a) 11-Cyclopropyl-5,11-dihydro-2-fluoro-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

- 10 The title compound was prepared from 8-bromo-2-chloro-11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one using a procedure similar to that of the corresponding 11-ethyl analog described in example VI.

- 15 **b) 11-Cyclopropyl-5,11-dihydro-2-fluoro-4-methyl-8-{2-(4-quinolinyl-ethoxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**
Diisopropyl azodicarboxylate (DIAD) (131 μ L, 0.66 mmol) was added dropwise to a solution of 11-(cyclopropyl)-5,11-dihydro-2-fluoro-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (140
20 mg, 0.44 mmol), 4-hydroxyquinoline (96.4 mg, 0.66 mmol) and Ph₃P (124 mg, 0.66 mmol) in THF (2.2 mL) at room temperature. The mixture was stirred at room temperature for 3 h then was concentrated under reduced pressure. The residue was dissolved in EtOAc (60 mL) and the solution was successively washed with 3 N aqueous NaOH (3 $\times$ 20 mL), aqueous
25 saturated NH₄Cl (20 mL) and brine (20 mL) then was dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was partially purified by flash chromatography (EtOAc:MeOH; 95:5). The solid was further purified by reverse phase HPLC (CombiPrep ADS-AQ 50 $\times$ 20 mm, 5 μ , 120 A, 5-100% MeCN + 0.10% TFA / water + 0.10% TFA in 25 min) to give the
30 trifluoroacetic acid salt of the title compound (104 mg, 43% yield) as a white solid: MS (ESI) m/z 456 (MH)⁺.

Example VIII (entry 8, Table 1)

5,11-Dihydro-11-ethyl-4-methyl-8-[2-(4-quinolinylloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

5

a) 5,11-Dihydro-11-ethyl-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

A mixture of 2-chloro-5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (168 mg, 0.51 mmol), ammonium formate (318 mg, 5.1 mmol) and 10% Pd/C (50 mg) in EtOH (6 mL) was stirred at room temperature. After 24 h, additional amounts of ammonium formate (200 mg, mmol) and 10% Pd/C (50 mg) were added. After stirring an additional 24 h, the reaction was concentrated under reduced pressure. The residue was dissolved in CHCl₃ and the mixture was washed with aqueous saturated NaHCO₃, dried (MgSO₄), filtered and concentrated under reduced pressure to give the title compound (122 mg, 81%) as a yellow oil.

15

b) 5,11-Dihydro-11-ethyl-4-methyl-8-[2-(4-quinolinylloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

20

Diisopropyl azodicarboxylate (DIAD) (42 µL, 0.21 mmol) was added dropwise to a solution of 5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (42.0 mg, 0.14 mmol), 4-hydroxyquinoline (31 mg, 0.21 mmol) and Ph₃P (55.4 mg, 0.21 mmol) in THF (0.7 mL) at room temperature. The mixture was stirred at room temperature for 2 h then was concentrated under reduced pressure. The residue was dissolved in EtOAc (60 mL) and the solution was successively washed with 3 N aqueous NaOH (3 × 20 mL), aqueous saturated NH₄Cl (20 mL) and brine (20 mL) then was dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was partially purified by flash chromatography (EtOAc:MeOH; 95:5). The solid was further purified by reverse phase HPLC

25

30

(CombiPrep ADS-AQ 50 × 20 mm, 5 μ , 120 Å, 5-100% MeCN + 0.10% TFA / water + 0.10% TFA in 25 min) to give the trifluoroacetic acid salt of the title compound (14.2 mg, 19% yield) as a white solid: MS (ESI) m/z 426 (MH)⁺.

5 **Example IX (entry 3, Table 1)**

11-(Cyclopropyl)-5,11-dihydro-4-methyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

10 **a) 5-Bromo-2-chloro-N-(2,6-dichloro-4-methyl-3-pyridinyl)-3-pyridinecarboxamide**

3-Amino-2,6-dichloro-4-methylpyridine (0.51 g, 2.85 mmol) was dissolved in toluene (35 mL) and pyridine (0.27 mL, 3.28 mmol) was added. 5-Bromo-2-chloro-3-pyridinecarbonyl chloride (0.80 g, 3.14 mmol) was then added dropwise over 30 min. The resulting mixture was stirred at room temperature for 1 h, diluted with water and extracted with toluene (2 ×). The combined organic extracts were dried (MgSO₄), filtered and concentrated under reduced pressure. The resulting thick oil was triturated with CH₂Cl₂, and the white solid collected via suction filtration to give the title compound (0.41 g, 36% yield).

20

b) 5-Bromo-2-(cyclopropylamino)-N-(2,6-dichloro-4-methyl-3-pyridinyl)-3-pyridinecarboxamide

A solution of 5-bromo-2-chloro-N-(2,6-dichloro-4-methyl-3-pyridinyl)-3-pyridinecarboxamide (3.00 g, 7.61 mmol) and cyclopropylamine (2.11 mL, 30.5 mmol) in xylenes was heated in a sealed tube at 120 °C for 24 h. The reaction was cooled to 0 °C, diluted with water and extracted with EtOAc (2 ×). The combined organic extracts were dried (MgSO₄), filtered and concentrated under reduced pressure. The resulting residue was purified by trituration (Et₂O:hexane, 3:1) to give the title compound (2.35 g, 75% yield) as a pale yellow solid.

30

c) 8-Bromo-2-chloro-11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

- A solution of 5-bromo-2-(cyclopropylamino)-*N*-(2,6-dichloro-4-methyl-3-pyridinyl)-3-pyridinecarboxamide (105 mg, 0.254 mmol) in pyridine (3.0 mL) was heated to 50 °C while a 1 M solution of NaHMDS in THF (0.53 mL, 0.53 mmol) was added. The resulting solution was stirred at 50 °C for 3 h. The reaction was quenched with aqueous saturated NH₄Cl, diluted with water and extracted with EtOAc (3 ×). The combined organic extracts were washed with brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The resulting residue was purified via flash chromatography (hexane:EtOAc, 7:3) to give the title compound (32 mg, 33% yield).

d) 2-Chloro-11-cyclopropyl-5,11-dihydro-4-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

- A solution of 8-bromo-2-chloro-11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (0.046 g, 0.122 mmol) in DMF (2.0 mL) was degassed by sparging with N₂ for 10 min. Pd(PPh₃)₄ (2.8 mg, 0.003 mmol) and allyltributyltin (0.45 mL, 0.146 mmol) were added and the resulting solution was heated to 90 °C for 2.5 h. The reaction mixture was diluted with water and extracted with CH₂Cl₂ (3 ×) and EtOAc (2 ×). The combined organic extracts were dried (MgSO₄), filtered and concentrated under reduced pressure. The resulting residue was purified via flash chromatography (hexane to hexane:EtOAc, 7:3) to give the title compound (0.033 g, 80% yield).

e) 2-Chloro-11-cyclopropyl-5,11-dihydro-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

- A cold (-78 °C) solution of 2-chloro-11-cyclopropyl-5,11-dihydro-4-methyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (0.82 g, 2.43 mmol) in CH₂Cl₂ (7.5 mL) and MeOH (7.5 mL) was saturated with O₃ until the solution turned pale blue. The reaction mixture was left at -78 °C for 10

min, then NaBH₄ (0.23 g, 9.71 mmol) was added and the mixture was allowed to warm slowly to room temperature over 2 h. The reaction was quenched with aqueous 10% citric acid solution and extracted with EtOAc (3 x). The organic extracts were combined, washed with brine, dried (MgSO₄),
5 filtered and concentrated under reduced pressure. The resulting residue was purified via flash chromatography (MeOH:CH₂Cl₂, 2 to 10%) to give the title compound (0.41 g, 60% yield) as a white solid.

10 **f) 11-Cyclopropyl-5,11-dihydro-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

The title compound was prepared using a method similar to that described for the 11-ethyl analog in example VIIIa.

15 **g) 11-Cyclopropyl-5,11-dihydro-4-methyl-8-[2-(4-quinolinyloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

Diisopropyl azodicarboxylate (DIAD) (167 µL, 0.85 mmol) was added dropwise to a solution of 11-(cyclopropyl)-5,11-dihydro-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (175 mg, 0.57 mmol), 4-hydroxyquinoline (123 mg, 0.85 mmol) and Ph₃P (223 mg, 0.85 mmol) in THF
20 (2.8 mL) at room temperature. The mixture was stirred at room temperature for 3 h then was concentrated under reduced pressure. The residue was dissolved in EtOAc (60 mL) and the solution was successively washed with 3 N aqueous NaOH (3 x 20 mL), aqueous saturated NH₄Cl (20 mL) and brine (20 mL) then was dried (MgSO₄), filtered and concentrated under reduced
25 pressure. The residue was purified by flash chromatography (EtOAc:MeOH; 95:5) to give the title compound (74 mg, 30% yield) as a white solid: MS (ESI) m/z 438 (MH)⁺.

Example X (entry 21, Table 1)

5,11-Dihydro-11-ethyl-5-methyl-8-{2-[(1-oxido-4-quinolinyl)oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

- 5 Solid mCPBA (80-85%, *m*-chloroperbenzoic acid) (2.21 g, 10.2 mmol) was added to a solution of 5,11-dihydro-11-ethyl-5-methyl-8-{2-(4-quinolinyl-oxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (2.56 g, 6.02 mmol) in CH₂Cl₂ (30 mL) and THF (30 mL) at room temperature (note that the reaction can be performed in CH₂Cl₂ alone). The mixture was stirred at room temperature for 1 h then was concentrated under reduced pressure. The residue was dissolved in CHCl₃ (400 mL), the solution was successively washed with aqueous 10% Na₂S₂O₃ (3 × 75 mL), aqueous saturated NaHCO₃ (3 × 75 mL) and brine (75 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CHCl₃:MeOH, 9:1) to give the title compound (2.01 g, 76% yield) as a white solid: MS (ESI) *m/z* 442 (MH)⁺.

Example XI (entry 17, Table 1)

20 **2-Chloro-5,11-dihydro-11-ethyl-5-methyl-8-{2-[(1-oxido-4-quinolinyl)oxy]-ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

- Using a procedure identical to that of example X, 2-chloro-5,11-dihydro-11-ethyl-5-methyl-8-{2-(4-quinolinyl-oxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (2.70 g, 5.87 mmol) gave the title compound (2.05 g, 73% yield) as a white solid: MS (ESI) *m/z* 476/478 (MH)⁺.

Example XII (entry 11, Table 1)

5,11-Dihydro-11-ethyl-2-fluoro-5-methyl-8-{2-[(1-oxido-4-quinolinyl)oxy]-ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

- 30 Solid mCPBA (457 mg, 2.12 mmol) was added to a solution of 5,11-dihydro-2-fluoro-11-ethyl-5-methyl-8-{2-(4-quinolinyl-oxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (470 mg, 1.06 mmol) in CH₂Cl₂ (10 mL). The

87
162
mixture was stirred at room temperature for 13 h then was concentrated under reduced pressure. The residue was dissolved in CHCl_3 (75 mL), the solution was successively washed with aqueous 10% $\text{Na}_2\text{S}_2\text{O}_3$ (3×25 mL), aqueous saturated NaHCO_3 (3×25 mL) and brine (25 mL), dried (MgSO_4),
5 filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CHCl_3 :MeOH, 19:1 to 9:1) to give the title compound (250 mg, 51% yield) as a white solid: MS (ESI) m/z 460 (MH)⁺.

Example XIII (entry 10, Table 1)

10 *2-Chloro-11-cyclopropyl-5,11-dihydro-5-methyl-8-[2-((1-oxido-4-quinolin-yl)oxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one*

Solid mCPBA (177 mg, 0.82 mmol) was added to a solution of 2-chloro-11-cyclopropyl-5,11-dihydro-5-methyl-8-[2-(4-quinolinyloxy)ethyl]-6H-
15 dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (143 mg, 0.30 mmol) in CH_2Cl_2 (3 mL) and THF (3 mL). The mixture was stirred at room temperature for 14 h then was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL), the solution was successively washed with aqueous 10% $\text{Na}_2\text{S}_2\text{O}_3$ (3×10 mL), aqueous saturated NaHCO_3 (3×10 mL) and brine (10
20 mL), dried (MgSO_4), filtered and concentrated under reduced pressure. The residue was partially purified by flash chromatography (EtOAc:MeOH, 95:5 to CH_2Cl_2 :MeOH, 9:1). The impure compound was further purified by reverse phase HPLC (CombiPrep ADS-AQ 50×20 mm, 5μ , 120 Å, 5-100% MeCN + 0.10% TFA / water + 0.10% TFA in 25 min). The pure
25 fractions were treated with aqueous saturated NaHCO_3 , extracted with EtOAc and the solution was dried (MgSO_4), filtered and concentrated under reduced pressure to give the title compound (28.3 mg, 19%) as a white solid: MS (ESI) m/z 488/490 (MH)⁺.

Example XIV (entry 14, Table 1)

2-Chloro-5,11-dihydro-11-ethyl-4-methyl-8-{2-[(1-oxido-4-quinolinyl)-oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

- 5 Solid mCPBA (46.9 mg, 0.22 mmol) was added to a solution of 2-chloro-5,11-dihydro-11-ethyl-5-methyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (50.0 mg, 0.11 mmol) in CH₂Cl₂ (2.2 mL). The mixture was stirred at room temperature for 1 h then was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL), the
- 10 solution was successively washed with aqueous 10% Na₂S₂O₃ (3 × 10 mL), aqueous saturated NaHCO₃ (3 × 10 mL) and brine (10 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by reverse phase HPLC (CombiPrep ADS-AQ 50 × 20 mm, 5 μ, 120 Å, 5-100% MeCN + 0.10% TFA / water + 0.10% TFA in 25 min). The pure
- 15 fractions were treated with aqueous saturated NaHCO₃, extracted with EtOAc and the solution was dried (MgSO₄), filtered and concentrated under reduced pressure to give the title compound (32.5 mg, 63%) as a white solid: MS (ESI) m/z 488/490 (MH)⁺.

Example XV (entry 9, Table 1)

5,11-Dihydro-11-ethyl-2-fluoro-4-methyl-8-{2-[(1-oxido-4-quinolinyl)-oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

- Using a procedure similar to that of example XIV, 5,11-dihydro-11-ethyl-2-fluoro-4-methyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (125 mg, 0.28 mmol) gave the title compound (40 mg, 31%) as a white solid: MS (ESI) m/z 460 (MH)⁺.
- 25

Example XVI (entry 12, Table 1)

- 5,11-Dihydro-11-ethyl-4-methyl-8-{2-[(1-oxido-4-quinolinyl)oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one**
- 30

Solid mCPBA (163 mg, 0.75 mmol) was added to a solution of 5,11-dihydro-

11-ethyl-4-methyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e]
[1,4]diazepin-6-one (144 mg, 0.34 mmol) in CH₂Cl₂ (7.5 mL). The solution
was stirred at room temperature for 2 h. The mixture was diluted with
CH₂Cl₂ (125 mL), washed successively with aqueous 10% Na₂S₂O₃ (2 × 25
5 mL), aqueous saturated NaHCO₃ (2 × 25 mL) and brine (25 mL), then was
dried (MgSO₄), filtered and concentrated under reduced pressure. The
residue was purified by flash chromatography (CH₂Cl₂:MeOH, 10:1) to give
the title compound (102 mg, 68% yield) as a white solid: MS (ESI) m/z 442
(MH).

10

Example XVII (entry 15, Table 1)

**11-Cyclopropyl-5,11-dihydro-11-ethyl-4-methyl-8-{2-[(1-oxido-4-quinolin-
yl)oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

15 Using a procedure similar to that of example XIV, 11-cyclopropyl-5,11-
dihydro-11-4-methyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e]
[1,4]diazepin-6-one (37.1 mg, 0.085 mmol) gave the title compound (35 mg,
91%) as a white solid: MS (ESI) m/z 454 (MH)⁺.

Example XVIII (entry 25, Table 1)

**2-Chloro-5,11-dihydro-11-ethyl-4-methyl-8-{2-(5-quinolinylloxy)ethyl}-6H-
dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one**

Diethyl azodicarboxylate (DEAD) (77 µL, 0.49 mmol) was added drop wise
25 to a solution of 2-chloro-5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-4-methyl-
6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (125 mg, 0.38 mmol), 5-
hydroxyquinoline (70.9 mg, 0.49 mmol) and Ph₃P (128 mg, 0.49 mmol) in
THF (2.5 mL) at room temperature. After 1.5 h, additional amounts of DEAD
(35 µL, 0.22 mmol) and PPh₃ (59 mg, 0.22 mmol) were added to the mixture.
30 The mixture was stirred at room temperature for 1 h then was diluted in
EtOAc (50 mL) and the solution was successively washed with aqueous 1 N

NaOH (4 × 10 mL) and brine (15 mL) then was dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CH₂Cl₂:acetone, 85:15). The resulting solid was triturated with Et₂O to give the title compound (38 mg, 22% yield) as a white solid: MS (ESI) m/z 460/462 (MH)⁺.

Example XIX (entry 20, Table 1)

2-Chloro-5,11-dihydro-11-ethyl-5-methyl-8-{2-[(1-oxido-5-quinolinyl)-oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

Diethyl azodicarboxylate (DEAD) (160 µL, 1.01 mmol) was added drop-wise to a solution of 2-chloro-5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (225 mg, 0.68 mmol), 5-hydroxyquinoline (147 mg, 1.01 mmol) and Ph₃P (266 mg, 1.01 mmol) in THF (3.4 mL) at room temperature. The mixture was stirred at room temperature for 3 h then was concentrated under reduced pressure. The residue was partially purified by flash chromatography (EtOAc:MeOH; 95:5). The fractions containing the 5-quinolinylloxy derivative were concentrated, dissolved in CH₂Cl₂ (2 mL) and THF (5 mL) and treated with mCPBA (80%, 248 mg, 1.15 mmol) at room temperature. The solution was stirred at room temperature for 2.5 h, then was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL), washed successively with aqueous 10% Na₂S₂O₃ (3 × 10 mL), aqueous saturated NaHCO₃ (3 × 10 mL) and brine (10 mL), then was dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc:MeOH, 95:5 to CH₂Cl₂:MeOH, 9:1) to give the title compound (113 mg, 35% yield for 2 steps) as a white solid: MS (ESI) m/z 476/478 (MH)⁺.

Example XX (entry 18, Table 1)

5,11-Dihydro-11-ethyl-2-fluoro-5-methyl-8-{2-[(1-oxido-5-quinolinyl)-oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

Using a procedure identical to that of example XIX, 5,11-dihydro-11-ethyl-2-fluoro-8-(2-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (267 mg, 0.84 mmol) gave the title compound (204 mg, 52% yield for 2 steps) as a white solid: MS (ESI) m/z 460 (MH)⁺.

Example XXI (entry 19, Table 1)

2-Chloro-5,11-dihydro-11-ethyl-4-methyl-8-{2-[(1-oxido-5-quinolinyl)-oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

Using a procedure similar to that of example XIX but using diisopropyl azodicarboxylate (DIAD) instead of diethyl azodicarboxylate, 2-chloro-5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (223 mg, 0.67 mmol) gave the title compound (43.4 mg, 14% yield for 2 steps) as a white solid: MS (ESI) m/z 476/478 (MH)⁺.

Example XXII (entry 23, Table 1)

5,11-Dihydro-11-ethyl-2-fluoro-4-methyl-8-{2-[(1-oxido-5-quinolinyl)-oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one Using a procedure similar to that of example XXI, 5,11-dihydro-11-ethyl-2-fluoro-8-(2-hydroxyethyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (250 mg, 0.79 mmol) gave the title compound (64.7 mg, 18% yield for 2 steps) as a white solid: MS (ESI) m/z 460 (MH)⁺.

Example XXIII, Entry 22, Table 1

2-Chloro-5,11-dihydro-11-ethyl-8-{2-[(1-oxido-4-quinolinyl)oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one
MS (ESI) m/z 462/464 (MH)⁺.

Example XXIV, Entry 26, Table 1

5,11-Dihydro-5,11-diethyl-8-{2-(4-quinolinylloxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

MS (ESI) m/z 440 (MH)⁺.

Example XXV, Entry 27, Table 1

5,11-Dihydro-5,11-diethyl-8-{2-[(1-oxido-4-quinolinyl)oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one MS (ESI) m/z 456 (MH)⁺.

5

Example XXVI (Entry 28, Table 1)

2,5-Dimethyl-5,11-dihydro-11-ethyl-8-{2-[(1-oxido-4-quinolinyl)oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

10 a) 5-Bromo-2-chloro-N-(6-chloro-2-methyl-3-pyridinyl)-3-pyridinecarboxamide

NaHCO₃ (3.9 g, 46.4 mmol) was added to a solution of 3-amino-2-chloro-6-methylpyridine (2.2 g, 15.4 mmol; prepared as described by K. G. Grozinger et al. J. Heterocyclic Chem. 1995, 32, 259-263) in MeCN (50 mL). The resulting suspension was stirred for 15 min. and a solution of crude 5-bromo-2-chloro-3-pyridinecarbonyl chloride (prepared from 5-bromo-2-hydroxy-3-pyridinecarboxylic acid and SOCl₂ [as described by T. W. Gero et al. in Synth. Commun. 1989, 19, 553-559 (incorporated herein by reference) but with omission of the aqueous work-up] (4 g,) in MeCN (10 mL) was introduced over 30 min. The resulting suspension was stirred at room temperature. After 24 h, the mixture was poured into a mixture of water (100 mL) and ice (10 g) and stirred for 20 min. The suspension was filtered and the resulting solid was washed with water (50 mL) and hexane (25 mL), then dried over P₂O₅ under reduced pressure to give the title compound (1.8 g, 31% yield) as a white powder.

25

b) 5-Bromo-N-(2-chloro-6-methyl-3-pyridinyl)-2-(ethylamino)-3-pyridinecarboxamide

A solution of 5-bromo-2-chloro-N-(2-chloro-6-methyl-3-pyridinyl)-3-

pyridinecarboxamide (1.7 g, 4.8 mmol) and ethylamine (2M in THF, 10.5 mmol, 5.2 mL) in THF (5 mL) was stirred at 90 to 95 °C in a steel autoclave

30

for 16 h. The resulting mixture was poured into water (100 mL), stirred for 15 min and filtered. The solid was washed with water followed by hexane to give the title compound as a yellow solid (1.58 g, 89% yield).

5 c) **8-Bromo-5,11-dihydro-2,5-dimethyl-11-ethyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one**

A solution of crude 5-bromo-*N*-(2-dichloro-6-methyl-3-pyridinyl)-2-(ethylamino)-3-pyridinecarboxamide (0.7 g, 1.8 mmol) in anhydrous pyridine (18 mL) was heated to 50 °C. A 1 M solution of NaHMDS in THF (7.2 mL, 7.2 mmol) was then added dropwise and stirring was continued for an additional 3h. After cooling to room temperature, methyl iodide (0.6 mL, 9 mmol) was added and the reaction was stirred overnight. Water (25 mL) was added and the mixture was extracted with EtOAc (3 ×) and the combined extracts were washed with water and brine then dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 8:2) to give the title compound (259 mg, 41% yield).

20 d) **5,11-Dihydro-2,5-dimethyl-11-ethyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one**

A solution of 8-bromo-5,11-dihydro-2,5-dimethyl-11-ethyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (258 mg, 0.7 mmol) in DMF (7.4 mL) was degassed under reduced pressure for 20 min. Pd(PPh₃)₄ (43 mg, 0.04 mmol) was then added followed by allyltributyltin (0.3 mL, 0.85 mmol). After degassing under reduced pressure for 10 min, the mixture was heated to 100 °C for 1.5 h. The mixture was then concentrated under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 8:2) to give the title compound (184 mg, 98% yield) as a yellow solid.

e) 5,11-Dihydro-2,5-dimethyl-11-ethyl-8-(2-hydroxyethyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

A stream of O₃ was introduced into a cold (-78 °C) solution of 5,11-dihydro-2,5-dimethyl-11-ethyl-8-(2-propenyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (187 mg, 0.6 mmol) and Sudan III in CH₂Cl₂ (3 mL) and MeOH (3 mL). When the pink solution turned brown, O₂ was bubbled through the solution for 10 min. NaBH₄ (57 mg, 1.52 mmol) was then added and the solution was allowed to warm to room temperature. After 30 min, an additional amount of NaBH₄ (20 mg) was added. After 2 h, aqueous saturated NH₄Cl was then added and the mixture stirred for 20 min. The solution was concentrated under reduced pressure and extracted with CH₂Cl₂ (3x). The combined organic extracts were washed with brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc:Hexane 6:4) to give the title compound (111 mg, 58% yield) as a white solid.

f) 2,5-Dimethyl-5,11-dihydro-11-ethyl-8-[2-(4-quinolinylloxy)ethyl]-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

Diethyl azodicarboxylate (DEAD) (139 µL, 0.88 mmol) was added dropwise to a solution of 5,11-dihydro-2,5-dimethyl-11-ethyl-8-(2-hydroxyethyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (110 mg, 0.35 mmol), 4-hydroxyquinoline (128 mg, 0.88 mmol) and Ph₃P (231 mg, 0.88 mmol) in THF (3.5 mL) at room temperature. The mixture was stirred at room temperature for 16h. The reaction mixture was diluted in EtOAc (60 mL) and the solution was successively washed with 1 N aqueous NaOH (3 × 10 mL) and brine (15 mL) then was dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc then EtOAc:MeOH, 9.5:0.5) to give the title compound (57 mg, 37% yield) as a white solid: MS (ESI) m/z 440 (MH)⁺.

g) 5,11-Dihydro-2,5-dimethyl-11-ethyl-8-{2-[(1-oxido-4-quinolinyl)oxy]ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one
Solid mCPBA (80-85%, m-chloroperbenzoic acid) (60 mg, 0.28 mmol) was

- 5 added to a solution of 5,11-dihydro-2,5-dimethyl-11-ethyl-8-{2-(4-quinolinyl-oxy)ethyl}-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one (55 mg, 0.13 mmol) in CH₂Cl₂ (1.3 mL) at room temperature. The mixture was stirred at room temperature for 2.5 h then diluted with CH₂Cl₂. The resulting solution was successively washed with aqueous 10% Na₂S₂O₃, aqueous
10 saturated NaHCO₃ and brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (MeOH/EtOAc, 2% to 5%) to give the title compound (59 mg, 99% yield) as a white solid: MS (ESI) m/z 456 (MH)⁺.

15 **Example XXVII (Entry 29, Table 1)**

5,11-Dihydro-11-ethyl-5-methyl-8-{2-[(1-oxido-4-quinolinyl)oxy]ethyl}-2-(trifluoromethyl)-6H-dipyrido[3,2-b:2',3'-e] [1,4]diazepin-6-one

a) 2-(Ethylamino)-3-nitro-6-(trifluoromethyl)pyridine

- 20 To a solution of 2-nitroacetamide ammonium salt (4 g, 33 mmol) in water (165 mL) was added piperidinium acetate (33 mmol) in water followed by slow addition of 4-ethoxy-1,1,1-trifluoro-3-buten-2-one (6.1 mL, 43 mmol) in MeOH (11 mL). The reaction was stirred for 2 h at room temperature and at reflux for 3 h. The reaction mixture was allowed to cool to 45 °C and aqueous
25 HCl (1N) was added until the pH was acidic. After 1 h at room temperature, the reaction mixture was extracted with CH₂Cl₂ (3x). The combined organic layer was successively washed with water and brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc, then EtOAc:MeOH, 9:1) to give 3-nitro-6-
30 (trifluoromethyl)-2(1H)-pyridinone (2.7 g, 40% yield) as a yellow solid.

To a solution of 3-nitro-6-(trifluoromethyl)-2(1H)-pyridinone (2.4 g, 11.5 mmol) in DMF (69 mL) was added sodium hydride (350 mg, 13.8 mmol). The reaction was stirred for 30 min at 45 °C, cooled to room temperature and N-phenyltrifluoromethanesulfonimide (4.9 g, 13.8 mmol) was added. After 1 h, a
5 2 M solution of ethylamine in THF (13 mL, 25 mmol) was added. The reaction was stirred at room temperature overnight. The reaction mixture was poured into water (100 mL) and extracted with EtOAc (3x). The combined organic layer was successively washed water and brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was
10 purified by flash chromatography (hexane:EtOAc, 9:1) to give the desired compound (1.1 g, 41% yield) as a yellow oil.

b) 3-Amino-2-(ethylamino)-6-(trifluoromethyl)pyridine

A solution of 2-(ethylamino)-3-nitro-6-(trifluoromethyl)pyridine (1.1 g, 4.9
15 mmol) in MeOH (40 mL) was stirred overnight at room temperature under hydrogen (1 atm.) in the presence of 20% Pd(OH)₂/C (100 mg). The catalyst was removed by filtration through diatomaceous earth. The filtrate was concentrated under reduced pressure to give the title compound as an orange oil (1 g).

20

c) 2-Chloro-N-{2-(ethylamino)-6-(trifluoromethyl)-3-pyridinyl}-5-bromo-3-pyridinecarboxamide

To a cooled solution of 3-amino-2-(ethylamino)-6-trifluoromethyl pyridine (1 g, 4.9 mmol) in MeCN (24 mL) was added solid NaHCO₃ (906 mg, 11 mmol).
25 After 5 min, crude 5-bromo-2-chloro-3-pyridinecarbonyl chloride (prepared from 5-bromo-2-hydroxy-3-pyridinecarboxylic acid and SOCl₂ [as described by T. W. Gero et al. in Synth. Commun. 1989, 19, 553-559 (incorporated herein by reference) but with omission of the aqueous work-up] was added (1 equiv., 4.9 mmol). After 2 h, the reaction mixture was poured over

ice/H₂O (1.5 L) and the resulting solid was filtered, rinsed with H₂O and then hexane. After drying under reduced pressure overnight, the title compound was obtained as a light brown solid (1.6 g, 77% yield).

5 **d) 8-Bromo-5,11-dihydro-11-ethyl-2-(trifluoromethyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one**

To a solution of 2-chloro-N-{2-(ethylamino)-6-(trifluoromethyl)-3-pyridinyl}-5-bromo-3-pyridinecarboxamide (766 mg, 1.8 mmol) in DMF (18 mL) was added NaH (137 mg, 5.4 mmol). The reaction was stirred at 80 °C. After 1 h
10 the reaction was allowed to cool to room temperature, and then was poured in water (20 mL). The resulting mixture was extracted with EtOAc (3x). The combined organic layer was successively washed with water and brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 7:3) to give the desired
15 compound (200 mg, 28% yield) as a yellow solid.

e) 8-Bromo-5,11-dihydro-11-ethyl-5-methyl-2-(trifluoromethyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

To a solution of the 8-bromo-5,11-dihydro-11-ethyl-2-(trifluoromethyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (200 mg, 0.52 mmol) in DMF (2.6
20 mL) was added NaH (20 mg, 0.78 mmol), and the mixture was heated to 50 °C for 30 min. The reaction mixture was cooled to room temperature and treated with MeI (97 µL, 1.56 mmol). After 16 h, the reaction mixture was diluted with water. The resulting mixture was extracted with EtOAc (3x).
25 The combined organic layer was successively washed with water and brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 8.5:1.5) to give the title compound (110 mg 53% yield) as a white foam.

98
172
1.73

f) 5,11-Dihydro-11-ethyl-5-methyl-8-(2-propenyl)-2-(trifluoromethyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

Allyltributyltin (98 μ L, 0.32 mmol) and Pd(Ph₃P)₄ (16 mg, 0.02 mmol) were added to a degassed (N₂ through solution for 30 min) solution of 8-bromo-
5 5,11-dihydro-11-ethyl-5-methyl-2-(trifluoromethyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (11 mg, 0.27 mmol) in DMF (1.4 mL) at room temperature. The mixture was stirred at 90 °C for 1.5 h then was cooled to room temperature and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 8:2 to 7:3) to give the
10 title compound (101 mg, 99% yield).

g) 5,11-Dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-2-(trifluoromethyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

A stream of ozonised oxygen was bubbled through a cold (-78 °C) solution of
15 5,11-dihydro-11-ethyl-5-methyl-8-(2-propenyl)-2-(trifluoromethyl)-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (100 mg, 0.27 mmol) in CH₂Cl₂ (2.7 mL) and MeOH (2.7 mL) for 2.5 h. A stream of N₂ was next bubbled through the solution for 15 min and then solid NaBH₄ (52 mg, 1.4 mmol) was added to the solution. The reaction mixture was allowed to warm to room
20 temperature. After 1 h, aqueous saturated NH₄Cl (5 mL) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with CH₂Cl₂ and the aqueous layer was re-extracted with CH₂Cl₂. The combined organic layer was successively washed with water and brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The
25 residue was purified by flash chromatography (EtOAc:hexane: 75:25 to EtOAc 100%) to give the title compound (60 mg 59% yield) as a white solid.

h) 5,11-Dihydro-11-ethyl-5-methyl-8-[2-(4-quinolinylloxy)ethyl]-2-(trifluoromethyl)-6H-dipyrido[3,2b:2',3'-e] [1,4]diazepin-6-one

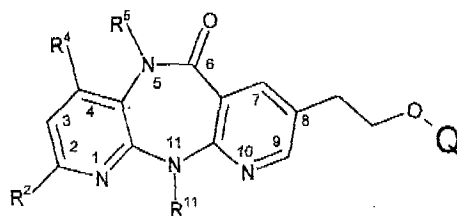
30 Diethyl azodicarboxylate (DEAD) (38 μ L, 0.24 mmol) was added dropwise to

95
123
174

- a solution of 5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-2-(trifluoromethyl)-6*H*-dipyrido[3,2-*b*:2',3'-*e*] [1,4]diazepin-6-one (58 mg, 0.16 mmol), 4-hydroxyquinoline (35 mg, 0.24 mmol) and Ph₃P (62 g, 0.24 mmol) in THF (1.6 mL) at room temperature. The mixture was stirred at room
- 5 temperature for 1h then was concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc:MeOH; 95:5) to give the title compound (24 mg, 31% yield) as a white solid: MS (ESI) *m/z* 494, 495 (MH)⁺.
- 10 i) 5,11-Dihydro-11-ethyl-5-methyl-8-{2-[(1-oxido-4-quinolinyl)oxy]ethyl}-2-(trifluoromethyl)-6*H*-dipyrido[3,2-*b*:2',3'-*e*] [1,4]diazepin-6-one
- Solid mCPBA (80-85%, *m*-chloroperbenzoic acid) (16 mg, 0.08 mmol) was added to a solution of 5,11-dihydro-11-ethyl-5-methyl-8-{2-(4-quinolinylloxy)ethyl}-2-(trifluoromethyl)-6*H*-dipyrido[3,2-*b*:2',3'-*e*]
- 15 [1,4]diazepin-6-one (22 mg, 0.04 mmol) in CH₂Cl₂ (0.5 mL) at room temperature. The mixture was stirred at room temperature for 2.5 h then diluted with CH₂Cl₂. The resulting solution was successively washed with aqueous 10% Na₂S₂O₃, aqueous saturated NaHCO₃ and brine, dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was
- 20 purified by flash chromatography (MeOH:CHCl₃, 5% to 10%) to give the title compound (11 mg, 52% yield) as a white solid: MS (ESI) *m/z* 511 (MH)⁺.

9A
175

51

TABLE 1
Compounds of formula I

I

Table 1 Entry #	R ²	R ⁴	R ⁵	R ¹¹	Q
1	Cl	Me	H	Et	
2	Cl	H	Me	Et	
3	H	Me	H	CycPr	
4	F	H	Me	Et	
5	F	Me	H	CycPr	
6	Cl	H	Me	CycPr	
7	F	Me	H	Et	

52

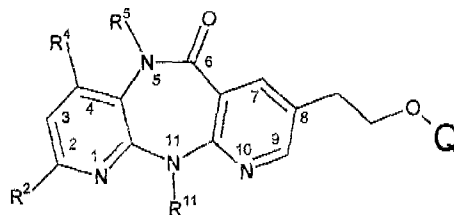


Table 1					
Entry #	R ²	R ⁴	R ⁵	R ¹¹	Q
8	H	Me	H	Et	
9	F	Me	H	Et	
10	Cl	H	Me	CycPr	
11	F	H	Me	Et	
12	H	Me	H	Et	
14	Cl	Me	H	Et	
15	H	Me	H	CycPr	

53

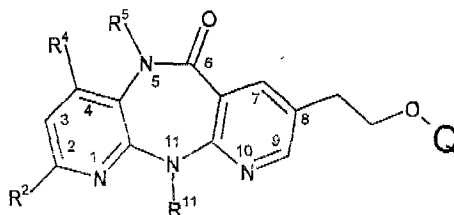


Table 1					
Entry #	R^2	R^4	R^5	R^{11}	Q
17	Cl	H	Me	Et	
18	F	H	Me	Et	
19	Cl	Me	H	Et	
20	Cl	H	Me	Et	
21	H	H	Me	Et	
22	Cl	H	H	Et	
23	F	Me	H	Et	

54

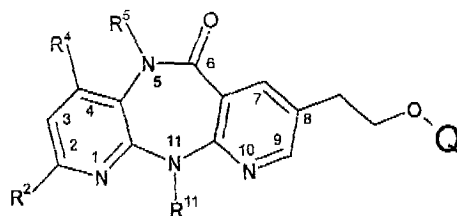
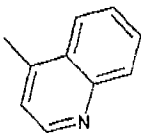
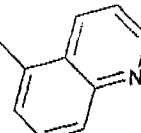
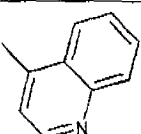
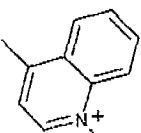
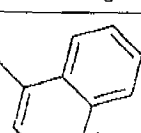
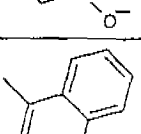


Table I					
Entry #	R^2	R^4	R^5	R^{11}	Q
24	H	H	Me	Et	
25	Cl	Me	H	Et	
26	H	H	Et	Et	
27	H	H	Et	Et	
28	Me	H	Me	Et	
29	CF ₃	H	H	Et	

REVERSE TRANSCRIPTASE (RT) ASSAYS

ASSAY THEORY:

- 5 Among the enzymes for which Human Immunodeficiency Virus (HIV-1) encodes is a reverse transcriptase (1), so-named because it transcribes a DNA copy from an RNA template. This activity can be quantitatively measured in a cell-free enzyme assay and is based upon the observation that reverse transcriptase is able to use a synthetic template poly r(C) primed with a
- 10 biotinylated oligo d(G) to transcribe a radio-labelled DNA strand utilising 3H-dGTP as a substrate. The assay described below utilises the wild-type enzyme (which is the predominant form of the enzyme observed in patients infected with HIV-1) and can also be used with mutant RT enzymes (for example, Y181C, prepared by site-directed mutagenesis in which the tyrosine
- 15 residue at codon 181 has been replaced by a cysteine residue) in analogous assay conditions. This assay allows compounds to be evaluated for their effectiveness at inhibiting the mutant enzymes.

MATERIALS:

- 20 a) Preparation of the enzyme
- Some HIV-1 IIIB clone BH10 RT mutants were provided by Dr. C.-K. Shih (Boehringer Ingelheim Pharmaceuticals Inc., U.S.A.) in the vector pKK233-2 (Pharmacia). Briefly an HIV RT clone pKRT2 containing only the RT p66
- 25 gene regulated by the lac operon/trc promoter was obtained from Dr. W. Summers (Yale University) (2). A variety of specific amino acid substitutions were introduced into the wild-type RT gene by site-directed mutagenesis. RT clones were subcloned into the pKK233-2 bacterial expression vector. Clones provided included wild-type, Val106Ala, Tyr181Cys, Tyr188Cys,

Tyr188Leu, Gly190Ala and Pro236Leu. Others were made in-house by site-directed mutagenesis of the pKK233-2 RT clones including Lys103Asn, Lys103Asn/Tyr181Cys, Lys103Asn/Leu100Ile, Lys103Asn/Pro225His, and Lys103Asn/Val108Ile.

5

b) Purification of Enzyme

Purification of recombinant reverse transcriptase was performed using a combination of methods previously described (3). A single colony from a fresh plate of transformed JM109 cells was used to initiate growth of a pre-culture grown o/n at 37°C. Two liters of growth medium were inoculated with this pre-culture. At OD₆₀₀ ~ 1.5 (5-6 h at 37°C), RT gene expression was induced with IPTG (1 mM final), and the fermentation was continued for a few more hours at 37 °C. After centrifugation, supernatants were discarded while cell pellets were pooled and stored at -80 °C until purification. Cells were thawed at 4 °C overnight and suspended in lysis buffer (MES 50mM pH 6, EDTA 1mM, 10% v/v glycerol, 0.02% w/v OBG, 0.02% w/v sodium azide). Lysozyme was added and the mixture was incubated on ice for 40 minutes. After homogenization using a Dounce in presence of lysozyme and sonication, the cells were centrifuged for 30 minutes. Supernatant (S1) was saved and stored at 4°C. The centrifuged pellet was resuspended in extraction buffer (MES 50mM pH 6, KPO₄ 50 mM pH 6, KCl 100mM, 10% v/v glycerol, 0.02% w/v OBG, 0.02% w/v sodium azide) and stirred for 30 minutes at 4°C. This second mixture was centrifuged again and the supernatant (S2) was saved. The above procedure was repeated 2 more times saving supernatants S3 and S4 and one last extraction was performed overnight (S5). Polymix P (0.005% final) was added to the combined supernatants to remove nucleic acids. This solution was stirred for 75 minutes at 4 °C and centrifuged for 1 h. The supernatant (SS1) was

10

15

20

25

99
180

precipitated on ice with 20% w/v ammonium sulfate and stirred for 1 h at 4 °C. The mixture was then centrifuged and the resulting supernatant (SS2) was precipitated with additional 40% w/v ammonium sulfate (60% total), stirred for 1 h and centrifuged again. The final pellet (P1) was stored overnight at 4 °C before undergoing purification the following day. All steps of the purification were performed at 4 °C unless otherwise stated.

Pellet (P1) was resuspended into MES 50mM pH 6, KPO₄ 10 mM pH 6, KCl 100mM, 10% v/v glycerol, 0.02% w/v OBG, 0.02% w/v sodium azide. The suspension was dialysed against the same buffer overnight using 12-14kD MWCO dialysis tubing. The dialysate was centrifuged and the supernatant was filtered through Millex-PF 0.8 µm filter units. The filtered sample was loaded on a Hydroxy Apatite column (30-mL bed volume) and washed with the same buffer. The bound enzyme was eluted with ~220 mL of a linear gradient of 10 to 300mM KPO₄ in the above buffer. The fractions containing p66/p51 heterodimer (as determined by SDS-PAGE 8% and Western blotting) were pooled for the next column. The RT containing fractions were diluted two-fold with Bis-Tris propane 50 mM pH 7.0, 0.02% w/v OBG, 10% v/v glycerol, 0.02% w/v sodium azide and loaded on a Hi-Trap Heparin Sepharose column (5-mL bed volume) and washed with the same buffer. The bound RT was then eluted with 75 mL of a linear gradient of 0 to 1 M ammonium sulfate in the same buffer. RT-containing fractions were pooled according to SDS-PAGE and Western blotting analyses. Protein concentration of this pool was determined by the Bradford method using BSA as standard. The final enzyme preparation was dialyzed in MES 50mM pH 6, KPO₄ 300 mM pH 6, KCl 175mM, 10% v/v glycerol, 0.02% w/v sodium azide and aliquoted and frozen at -80° C.

ASSAY PROCEDURE:

The radiometric enzyme assay has been adapted to a 96-well microtiter plate format and uses streptavidin scintillation proximity beads. The assay is
5 briefly described below. The HIV-1 RT enzyme was thawed and appropriately diluted into Tris/HCl 50 mM pH 7.8 containing NaCl 60mM, MgCl₂ hexahydrate 2mM, DTT 6mM, GSH 2mM and 0.02% w/v Chaps to give $\approx$ 3 nM enzyme. To 30 μ L of this enzyme solution was added 10 μ L of inhibitor solution (50 μ M to 2.5 nM inhibitor in same assay buffer as above
10 containing 15% v/v DMSO). The plate was pre-incubated for 15 minutes at room temperature before proceeding to the next step. In this pre-incubation step, the highest and lowest inhibitor concentrations were 12.5 μ M and 0.62 nM respectively and the concentration of DMSO was 3.75% v/v. Then the enzymatic reaction was initiated by addition of 10 μ L of substrates solution.
15 The final reaction mixture contained Tris/HCl 50 mM pH 7.8, NaCl 60 mM, MgCl₂•6H₂O 2 mM, DTT 6 mM, GSH 2 mM, Chaps 0.02% w/v DMSO 3% v/v, Poly rC 179 nM, Biotin dG₁₅ 18 nM, dGTP 288 nM, ³H-dGTP 71nM, and 1-2 nM enzyme.

In this incubation step, the highest and lowest inhibitor concentrations were
20 10 μ M and 0.5 nM respectively. After addition of substrates, the plate was covered with a plastic seal and incubated for 1 hour at 37°C in a dry incubator. Then the reaction was quenched by addition of 75 μ L of EDTA 0.5M containing 5mg/mL of streptavidin scintillation proximity beads.

The plate was shaken for 2 minutes at medium speed and incubated 1 hour
25 at room temperature. Then 75 μ L of cesium chloride 7 M solution was added, the plate was shaken for 2 minutes at medium speed and incubated again for 1 hour at room temperature. The plate was then covered with a plastic seal and counted using the TopCount-NXT™ Microplate Scintillation & Luminescence Counter, (Packard). Each well was counted for 60 seconds.

Each row contained at its extremities a blank and a control well.

The calculation for percent inhibition is as follows:

$$\% \cdot inhibition = \left(1 - \left[\frac{cpm \cdot well - cpm \cdot blank}{cpm \cdot control - cpm \cdot blank} \right] \right) * 100$$

Using the above assay, compounds of the invention were tested for
5 inhibition of RT wild-type (WT) and mutant enzymes. The results are listed
in Table 2, as IC₅₀ (nM).

To confirm the ability of these compounds to inhibit HIV replication, they
were also tested in the human T-Cell Culture (Syncytia) Assay described
10 below.

ELISA assay for assessment of activity in cell culture

Compounds of the invention were tested for their ability to inhibit HIV
15 replication in cell culture in a 96-well plate assay. Complete RPMI 1640,
consisting of RPMI 1640 + 10% fetal bovine serum, 10 µg/ml gentamycin and
10 µM β-mercaptoethanol was used for dilution of the compound as well as
cell growth medium. The T lymphocyte cell line C8166 was infected at a
multiplicity of infection of 0.001 with viruses coding for wild-type and
20 mutant reverse transcriptase. Cells were then incubated for three days in the
presence of serial dilutions of the compounds of the invention. The
supernatant was pooled from eight replica wells and the concentration of
extracellular p24 was determined using a commercially available HIV-1 p24
antigen assay kit (Beckman-Coulter®). The level of inhibition (% inhibition)
25 was calculated with the following equation:

$$\% \cdot inhibition = \left(1 - \left[\frac{p24 pg / mL \cdot inhibitor}{p24 pg / mL \cdot control} \right] \right) * 100$$

The results are listed in Table 3, as EC₅₀ (nM).

Specificity Assays

5

In order to assess the specificity of the enzyme inhibitory activity of the compounds provided by the invention, a few were tested, for their ability to inhibit other viral polymerases (such as Hepatitis C virus and Respiratory Syncytial virus RNA-dependent RNA polymerases) as well as mammalian polymerases (such as Calf Thymus RNA-dependent DNA polymerase and Human telomerase) using known assay methods. None of the compounds so tested was observed to possess any significant inhibitory activity against these enzymes. These results indicate that the enzyme inhibitory activity of the compounds provided by the invention is directed rather specifically against HIV-1 RT.

15

REFERENCES (INCORPORATED HEREIN BY REFERENCE)

1. Benn, S., et al. Science 230:949, 1985.
2. D'Aquila, R. T. and Summers, W. C. J. Acq. Imm. Def. Syn. 2:579, 1989.
- 20 3. a) Warren, T. C. et al. Protein Expression & Purification 3:479, 1992; b) Kohlstaedt, L. A. Science 256(5065): 1783, 1992.

TABLE 2
Inhibition of Wild-type and mutant strains of RT for compounds of formula I

compound no. (see Table 1)	IC ₅₀ WT RT (nM)	IC ₅₀ K103N (nM)	IC ₅₀ Y181C (nM)	IC ₅₀ V106A (nM)	IC ₅₀ P236L (nM)	IC ₅₀ Y188L (nM)	IC ₅₀ G190A (nM)	IC ₅₀ K103N/ Y181C (nM)	IC ₅₀ K103N/ P225H (nM)	IC ₅₀ Y188C (nM)	IC ₅₀ K103N/ V108I (nM)	IC ₅₀ K103N/ L100I (nM)
1	10.5	19	20	132		2740		52	31.2		29	28.5
2	5.5	28.5	25	148	50.5	258	14.5	24.5	27	7.1	31	46
3	16.5	45	73	79		3236		612	56		47	124
4	11	22.5	26	206		520		52	27		32	108
5	19	37.5	76	61		2541		536	38		38	98
6	25											
7	9.8	26	38	74		4318		199	46		45	98
8	10	37	43	190		7511		301	67		63	135
9	14	23	43	98		3395		175	25		58	88
10	41	67	206	1372		1422		710			255	229
11	9.5	18	27	336	56	661	19	55	15	6.6	55	102
12	12	31	38	121		>10000		212	38		75	124
14	15	19	28	77		2298		61	30		59	24
15	61	66	226	206		>10000		1522	93		215	270
17	14	25	29	319	60	198	25	48	23	8.1	83	40
18	11	37	41	737		1167		146	20		96	158
19	7	25	20	89		3938		91	16		56	27

135
135

compound no. (see Table 1)	IC ₅₀ WT RT (nM)	IC ₅₀ K103N (nM)	IC ₅₀ Y181C (nM)	IC ₅₀ V106A (nM)	IC ₅₀ P236L (nM)	IC ₅₀ Y188L (nM)	IC ₅₀ G190A (nM)	IC ₅₀ K103N/ Y181C (nM)	IC ₅₀ K103N/ P225H (nM)	IC ₅₀ Y188C (nM)	IC ₅₀ K103N/ V108I (nM)	IC ₅₀ K103N/ L100I (nM)
20	10	36	22	661		439		51	17		64	44
21	17	44	51	1106	129	1357	26	95	40	9.9	120	168
22	45			2493		2990		336				
23	6.1			105		5614		247				
24	5.6			344		627	25	70				169
25	8	19	17	84		2537		64	23		38	28
26	8.9	40	51	1745		5204		200	85		147	443
27	38			2271		5335		299				
28	19			605		1190	25	141	25	9.0	35	28
29	35			893		578		84				

WQ 01/00/38

63

PCT/CA01/00890

187
190
196
187

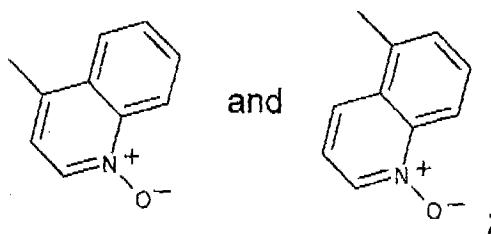
TABLE 3
Inhibition of wild type and mutant strains of HIV in cell cultures by compounds of formula I

compound no. (see Table I)	EC ₅₀ WT RT (nM)	EC ₅₀ K103N (nM)	EC ₅₀ Y181C (nM)	EC ₅₀ V106A (nM)	EC ₅₀ Y188L (nM)	EC ₅₀ K103N/ Y181C (nM)	EC ₅₀ K103N/ P225H (nM)	EC ₅₀ K103N/ V108I (nM)	EC ₅₀ K103N/ L100I (nM)
1	2.6								
2	2.2								
3	1.3								
4	0.98								
5	0.83								
6									
7	1.1								
8	0.93								
9	0.92								
10									
11	0.34	2		5					
12	0.26	2.8	4.1	118	7.6				
14	0.67		2.7	28					
15				12.4	52				
17	0.34	1.5			7.5	2.0	3.9		
18	0.34		2.8			1.5	5.0	3.2	
19	0.37			24				2.9	
				58					
					2.3				
					9.5	1.2			
					22		2.7	1.2	

66

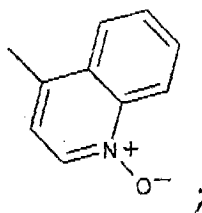
2. A compound according to claim 1, wherein R¹¹ is Et or cyclopropyl or a pharmaceutically acceptable salt thereof.

3. A compound according to claim 1, wherein Q is selected from the
5 group consisting of:



or a pharmaceutically acceptable salt thereof.

4. A compound according to claim 1, wherein Q is:



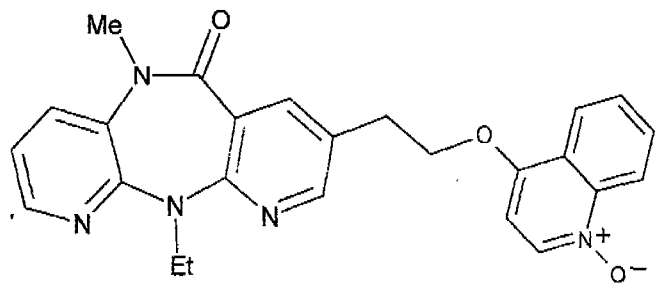
or a pharmaceutically acceptable salt thereof.

5. A compound according to claim 1, wherein R⁵ is Me, or a pharmaceutically acceptable salt thereof.

6. A compound according to claim 1, wherein R¹¹ is Et or a pharmaceutically acceptable salt thereof.

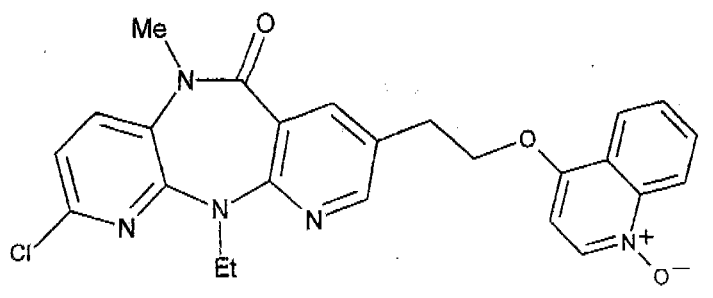
7. The compound:

67



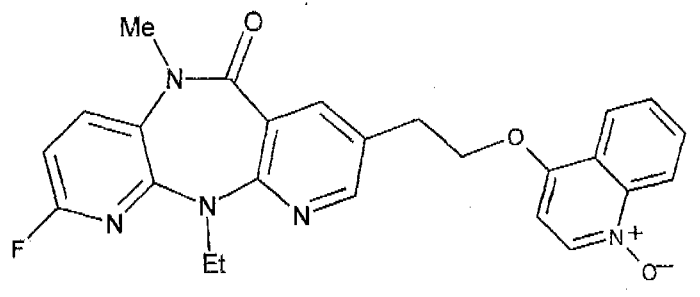
or a pharmaceutically acceptable salt thereof.

8. The compound:



5 or a pharmaceutically acceptable salt thereof.

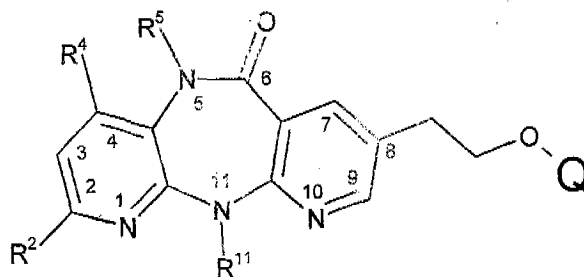
9. The compound:



or a pharmaceutically acceptable salt thereof.

10. An inhibitor of HIV replication of the general formula I:

68



I

wherein

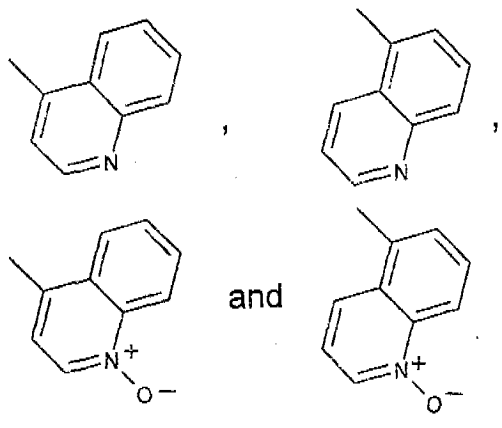
R² is selected from the group consisting of H, F, Cl, C₁₋₄ alkyl, C₃₋₄ cycloalkyl and CF₃;

5 R⁴ is H or Me;

R⁵ is H, Me or Et, with the proviso that R⁴ and R⁵ are not both Me, and if R⁴ is Me then R⁵ cannot be Et;

R¹¹ is Me, Et, cyclopropyl, propyl, isopropyl, or cyclobutyl;; and

Q is selected from the group consisting of:



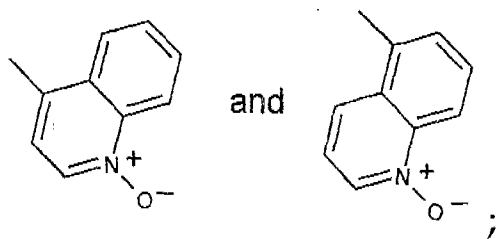
10

or a pharmaceutically acceptable salt thereof.

11. An inhibitor of HIV replication according to claim 10, wherein R¹¹ is Et or cyclopropyl or a pharmaceutically acceptable salt thereof.

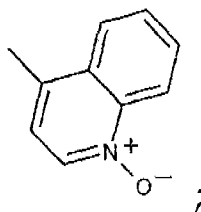
69

12. An inhibitor of HIV replication, according to claim 10, wherein Q is selected from the group consisting of:



5 or a pharmaceutically acceptable salt thereof.

13. An inhibitor of HIV replication, according to claim 10, wherein Q is:



or a pharmaceutically acceptable salt thereof.

10

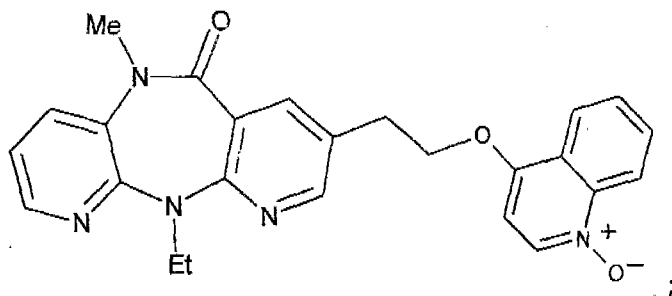
14. An inhibitor of HIV replication, according to claim 10, wherein R⁵ is Me.

15. An inhibitor of HIV replication, according to claim 10, wherein R¹¹

15 is Et.

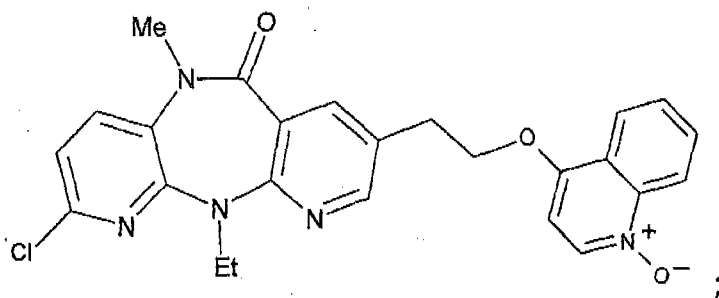
16. An inhibitor of HIV replication of the formula:

70



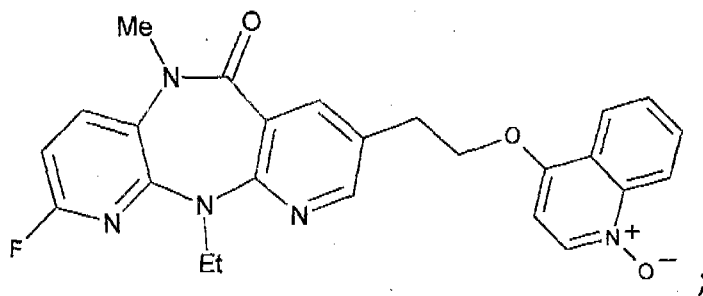
or a pharmaceutically acceptable salt thereof.

17. An inhibitor of HIV replication of the formula:



or a pharmaceutically acceptable salt thereof.

18. An inhibitor of HIV replication of the formula:



or a pharmaceutically acceptable salt thereof.

19. A method for the treatment or prevention of HIV infection, comprising administering to a patient an HIV inhibiting amount of a

compound of formula I according to claim 1, or a pharmaceutically acceptable salt thereof.

20. A pharmaceutical composition for the treatment or prevention of HIV
5 infection, comprising a compound of formula I according to claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

ANEXO 3



US006391919B1

(12) **United States Patent**
Kaltenbach et al.

(10) **Patent No.:** **US 6,391,919 B1**
(45) **Date of Patent:** **May 21, 2002**

(54) **BIS-AMINO ACID SULFONAMIDES
CONTAINING SUBSTITUTED BENZYL
AMINES HIV PROTEASE INHIBITORS**

WO 9405639 3/1994
WO 9506030 3/1995
WO 9628464 9/1996

(75) **Inventors:** **Robert F. Kaltenbach; George L.
Trainor**, both of Wilmington, DE (US)

Primary Examiner—Peter O'Sullivan

(73) **Assignee:** **Bristol-Myers Squibb Pharma
Company**

(57) **ABSTRACT**

(*) **Notice:** Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

This invention relates generally to bis-amino acid sulfona-
mides containing substituted benzyl amines of formula I:

(21) **Appl. No.:** **09/482,146**

(22) **Filed:** **Jan. 12, 2000**

(51) **Int. Cl.**⁷ **A61K 31/18; A61K 31/36;**
A61K 31/34

(52) **U.S. Cl.** **514/603; 514/18; 514/19;**
514/466; 514/469; 549/438; 549/462; 564/86

(58) **Field of Search** 514/18, 19, 466,
514/469, 603; 549/438, 462; 564/86

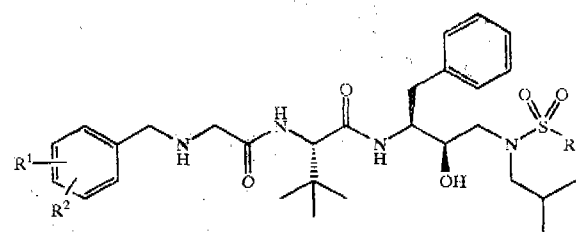
(56) **References Cited**

U.S. PATENT DOCUMENTS

6,150,556 A 11/2000 Getman et al. 564/92

FOREIGN PATENT DOCUMENTS

WO 9404492 3/1994



or stereoisomeric forms, stereoisomeric mixtures, or phar-
maceutically acceptable salt forms thereof, which are useful
as HIV protease inhibitors, pharmaceutical compositions
and diagnostic kits comprising the same, methods of using
the same for treating viral infection or as assay standards or
reagents, and intermediates and processes for making the
same.

24 Claims, No Drawings

1

BIS-AMINO ACID SULFONAMIDES CONTAINING SUBSTITUTED BENZYL AMINES HIV PROTEASE INHIBITORS

FIELD OF THE INVENTION

This invention relates generally to bis-amino acid sulfonamides containing substituted benzyl amines useful as HIV protease inhibitors, pharmaceutical compositions and diagnostic kits comprising the same, and methods of using the same for treating viral infection or as assay standards or reagents.

BACKGROUND OF THE INVENTION

Two distinct retroviruses, human immunodeficiency virus (HIV) type-1 (HIV-1) or type-2 (HIV-2), have been etiologically linked to the immunosuppressive disease, acquired immunodeficiency syndrome (AIDS). HIV seropositive individuals are initially asymptomatic but typically develop AIDS related complex (ARC) followed by AIDS. Affected individuals exhibit severe immunosuppression which predisposes them to debilitating and ultimately fatal opportunistic infections.

The disease AIDS is the end result of an HIV-1 or HIV-2 virus following its own complex life cycle. The virion life cycle begins with the virion attaching itself to the host human T-4 lymphocyte immune cell through the bonding of a glycoprotein on the surface of the virion's protective coat with the CD4 glycoprotein on the lymphocyte cell. Once attached, the virion sheds its glycoprotein coat, penetrates into the membrane of the host cell, and uncoats its RNA. The virion enzyme, reverse transcriptase, directs the process of transcribing the RNA into single-stranded DNA. The viral RNA is degraded and a second DNA strand is created. The now double-stranded DNA is integrated into the human cell's genes and those genes are used for virus reproduction.

At this point, RNA polymerase transcribes the integrated DNA into viral RNA. The viral RNA is translated into the precursor gag-pol fusion polypeptide. The polypeptide is then cleaved by the HIV protease enzyme to yield the mature viral proteins. Thus, HIV protease is responsible for regulating a cascade of cleavage events that lead to the virus particle's maturing into a virus that is capable of full infectivity.

The typical human immune system response, killing the invading virion, is taxed because the virus infects and kills the immune system's T cells. In addition, viral reverse transcriptase, the enzyme used in making a new virion particle, is not very specific, and causes transcription mistakes that result in continually changed glycoproteins on the surface of the viral protective coat. This lack of specificity decreases the immune system's effectiveness because antibodies specifically produced against one glycoprotein may be useless against another, hence reducing the number of antibodies available to fight the virus. The virus continues to reproduce while the immune response system continues to weaken. Eventually, the HIV largely holds free reign over the body's immune system, allowing opportunistic infections to set in and without the administration of antiviral agents, immunomodulators, or both, death may result.

There are at least three critical points in the virus's life cycle which have been identified as possible targets for antiviral drugs: (1) the initial attachment of the virion to the T-4 A lymphocyte or macrophage site, (2) the transcription of viral RNA to viral DNA (reverse transcriptase, RT), and (3) the processing of gag-pol protein by HIV protease.

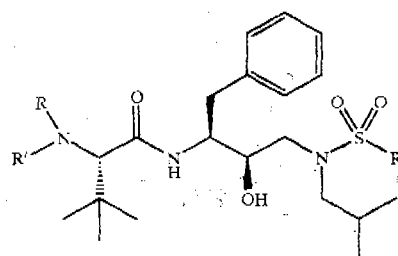
The genomes of retroviruses encode a protease that is responsible for the proteolytic processing of one or more

2

polyprotein precursors such as the pol and gag gene products. See Wellink, *Arch. Virol.* 98 1 (1988). Retroviral proteases most commonly process the gag precursor into the core proteins, and also process the pol precursor into reverse transcriptase and retroviral protease.

The correct processing of the precursor polyproteins by the retroviral protease is necessary for the assembly of the infectious virions. It has been shown that in vitro mutagenesis that produces protease-defective virus leads to the production of immature core forms which lack infectivity. See Crawford et al., *J. Virol.* 53 899 (1985); Katoh et al., *Virology* 145 280 (1985). Therefore, retroviral protease inhibition provides an attractive target for antiviral therapy. See Mitsuya, *Nature* 325 775 (1987).

As evidenced by the protease inhibitors presently marketed and in clinical trials, a wide variety of compounds have been studied as potential HIV protease inhibitors. One core, hydroxyethylamino-sulfonamides, has received significant attention. For example, PCT Applications WO94/05639, WO94/04492, WO95/06030, and WO96/28464 generically describe sulfonamides of the formula:



and methods of preparing them. Though some of the present compounds appear to fall within the generic descriptions of some of the above publications, they are not specifically disclosed, suggested, or claimed therein.

Even with the current success of protease inhibitors, it has been found that HIV patients can become resistant to a single protease inhibitor. Thus, it is desirable to develop additional protease inhibitors to further combat HIV infection.

SUMMARY OF THE INVENTION

Accordingly, one object of the present invention is to provide novel protease inhibitors.

It is another object of the present invention to provide a novel method for treating HIV infection which comprises administering to a host in need of such treatment a therapeutically effective amount of at least one of the compounds of the present invention or a pharmaceutically acceptable salt form thereof.

It is another object of the present invention to provide a novel method for treating HIV infection which comprises administering to a host in need thereof a therapeutically effective combination of (a) one of the compounds of the present invention and (b) one or more compounds selected from the group consisting of HIV reverse transcriptase inhibitors and HIV protease inhibitors.

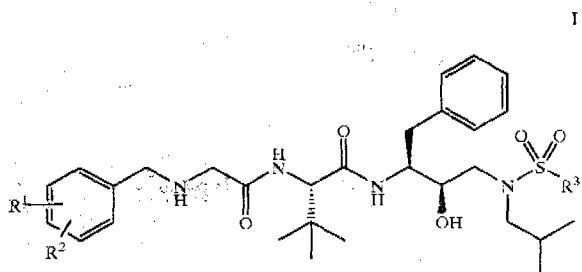
It is another object of the present invention to provide pharmaceutical compositions with protease inhibiting activity comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of at least one of the compounds of the present invention or a pharmaceutically acceptable salt form thereof.

3

It is another object of the present invention to provide a method of inhibiting HIV present in a body fluid sample which comprises treating the body fluid sample with an effective amount of a compound of the present invention.

It is another object of the present invention to provide a kit or container containing at least one of the compounds of the present invention in an amount effective for use as a standard or reagent in a test or assay for determining the ability of a potential pharmaceutical to inhibit HIV protease, HIV growth, or both.

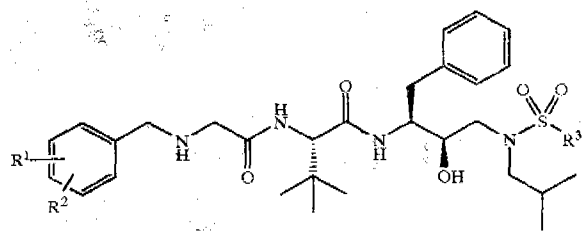
These and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that compounds of formula (I):



wherein R^1 , R^2 , and R^3 are defined below, stereoisomeric forms, mixtures of stereoisomeric forms, or pharmaceutically acceptable salt forms thereof, are effective protease inhibitors.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[1] Thus, in a first embodiment, the present invention provides a novel compound of Formula I:



or a pharmaceutically acceptable salt form thereof, wherein:

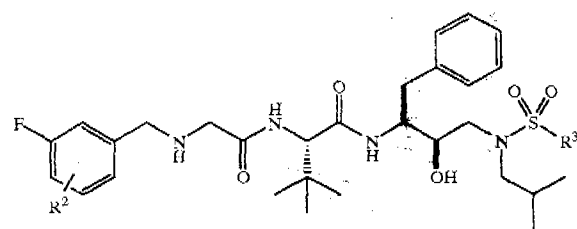
R^1 is F;

R^2 is F or H; and,

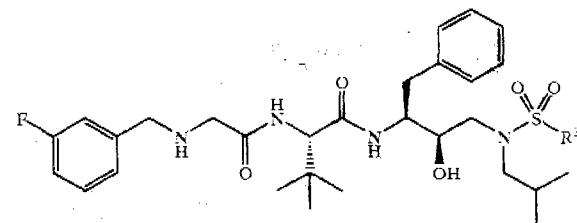
R^3 is selected from the group: 4-aminophenyl, 3-aminophenyl, 2,3-dihydrobenzofuran-5-yl, and 1,3-benzodioxol-5-yl.

[2] In a preferred embodiment, the present invention provides a novel compound of Formula II:

4



[3] In a more preferred embodiment, the present invention provides a novel compound of Formula IIa:



[4] In an even more preferred embodiment, the present invention provides a novel compound of Formula IIa, wherein:

R^3 is 3-aminophenyl.

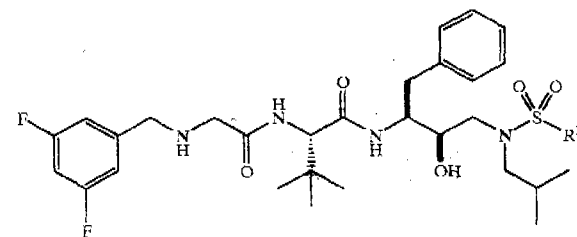
[5] In another even more preferred embodiment, the present invention provides a novel compound of Formula IIa, wherein:

R^3 is 4-aminophenyl.

[6] In another even more preferred embodiment, the present invention provides a novel compound of Formula IIa, wherein:

R^3 is 2,3-dihydrobenzofuran-5-yl or 1,3-benzodioxol-5-yl.

[7] In another more preferred embodiment, the present invention provides a novel compound of Formula IIb:



[8] In another even more preferred embodiment, the present invention provides a novel compound of Formula IIb, wherein:

R^3 is 3-aminophenyl.

[9] In another even more preferred embodiment, the present invention provides a novel compound of Formula IIb, wherein:

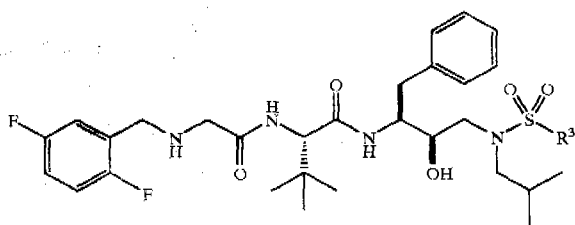
R^3 is 4-aminophenyl.

[10] In another even more preferred embodiment, the present invention provides a novel compound of Formula IIb, wherein:

5

R³ is 2,3-dihydrobenzofuran-5-yl or 1,3-benzodioxol-5-yl.

[11] In another more preferred embodiment, the present invention provides a novel compound of Formula IIc:



IIc

[12] In another even more preferred embodiment, the present invention provides a novel compound of Formula IIc, wherein:

R³ is 3-aminophenyl.

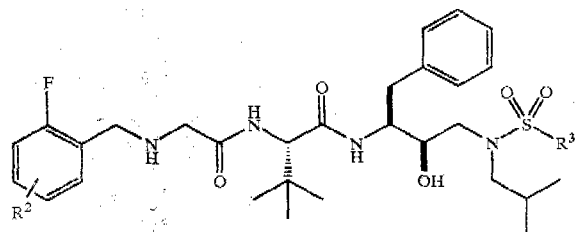
[13] In another even more preferred embodiment, the present invention provides a novel compound of Formula IIc, wherein:

R³ is 4-aminophenyl.

[14] In another even more preferred embodiment, the present invention provides a novel compound of Formula IIc, wherein:

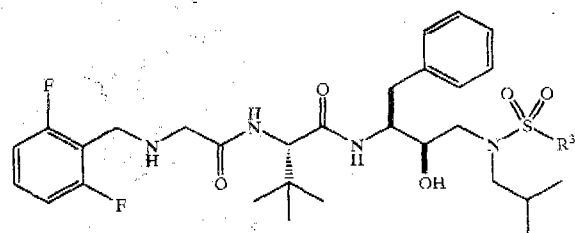
R³ is 2,3-dihydrobenzofuran-5-yl or 1,3-benzodioxol-5-yl.

[15] In another preferred embodiment, the present invention provides a novel compound of Formula III:



III

[16] In another more preferred embodiment, the present invention provides a novel compound of Formula IIIa:

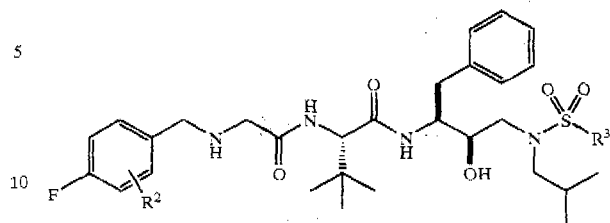


IIIa

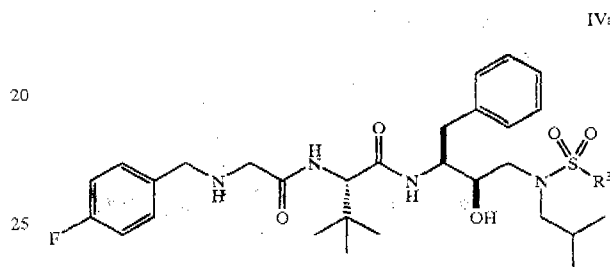
[17] In another preferred embodiment, the present invention provides a novel compound of Formula IV:

6

IV



[18] In another more preferred embodiment, the present invention provides a novel compound of Formula IVa:



IVa

In another embodiment, the present invention provides a novel pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound of formula I or pharmaceutically acceptable salt form thereof.

In another embodiment, the present invention provides a novel method for treating HIV infection which comprises administering to a host in need of such treatment a therapeutically effective amount of a compound of formula I or pharmaceutically acceptable salt form thereof.

In another embodiment, the present invention provides a novel method of treating HIV infection which comprises administering, in combination, to a host in need thereof a therapeutically effective amount of:

- a compound of formula I; and,
- at least one compound selected from the group consisting of HIV reverse transcriptase inhibitors and HIV protease inhibitors.

In another preferred embodiment, the reverse transcriptase inhibitor is selected from the group AZT, ddC, ddI, d4T, 3TC, delavirdine, efavirenz, nevirapine, Ro 18,893, trovirdine, MKC-442, HBY 097, ACT, UC-781, UC-782, RD4-2025, and MEN 10979, and the protease inhibitor is selected from the group saquinavir, ritonavir, indinavir, amprenavir, nelfinavir, zalcitabine, BMS-232623, GS3333, KNI-413, KNI-272, LG-71350, CGP-61755, PD 173606, PD 177298, PD 178390, PD 178392, U-140690, and ABI-378.

In an even more preferred embodiment, the reverse transcriptase inhibitor is selected from the group AZT, efavirenz, and 3TC and the protease inhibitor is selected from the group saquinavir, ritonavir, nelfinavir, and indinavir.

In a still further preferred embodiment, the reverse transcriptase inhibitor is AZT.

In another still further preferred embodiment, the protease inhibitor is ritonavir.

In another preferred embodiment, component (b) is a HIV reverse transcriptase inhibitor and a HIV protease inhibitor.

In another preferred embodiment, component (b) is two different HIV reverse transcriptase inhibitors.

In another embodiment, the present invention provides a pharmaceutical composition useful for the treatment of HIV infection, which comprises a therapeutically effective amount of:

- (a) a compound of formula I; and,
- (b) at least one compound selected from the group consisting of HIV reverse transcriptase inhibitors and HIV protease inhibitors, in one or more sterile containers.

In another embodiment, the present invention provides a novel method of inhibiting HIV present in a body fluid sample which comprises treating the body fluid sample with an effective amount of a compound of formula I.

In a ninth embodiment, the present invention provides a novel kit or container comprising a compound of formula I in an amount effective for use as a standard or reagent in a test or assay for determining the ability of a potential pharmaceutical to inhibit HIV protease, HIV growth, or both.

DEFINITIONS

As used herein, the following terms and expressions have the indicated meanings. It will be appreciated that the compounds of the present invention contain asymmetrically substituted carbon atoms, and may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis, from optically active starting materials. All chiral, diastereomeric, racemic forms and all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomer form is specifically indicated.

The processes of the present invention are contemplated to be practiced on at least a multigram scale, kilogram scale, multikilogram scale, or industrial scale. Multigram scale, as used herein, is preferably the scale wherein at least one starting material is present in 10 grams or more, more preferably at least 50 grams or more, even more preferably at least 100 grams or more. Multikilogram scale, as used herein, is intended to mean the scale wherein more than one kilogram of at least one starting material is used. Industrial scale as used herein is intended to mean a scale which is other than a laboratory scale and which is sufficient to supply product sufficient for either clinical tests or distribution to consumers.

The present invention is intended to include all isotopes of atoms occurring on the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include tritium and deuterium. Isotopes of carbon include C-13 and C-14.

As used herein, "HIV reverse transcriptase inhibitor" is intended to refer to both nucleoside and non-nucleoside inhibitors of HIV reverse transcriptase (RT). Examples of nucleoside RT inhibitors include, but are not limited to, AZT, ddC, ddI, d4T, and 3TC. Examples of non-nucleoside RT inhibitors include, but are not limited to, delavirdine (Pharmacia and Upjohn, U90152S), efavirenz (DuPont), nevirapine (Boehringer Ingelheim), Ro 18,893 (Roche), zalcitabine (Lilly), MKC-442 (Triangle), HBY 097 (Hoechst), HBY 1293 (Hoechst), ACT (Korean Research Institute), UC-781 (Rega Institute), UC-782 (Rega Institute), RD4-2025 (Tosoh Co. Ltd.), and MEN 10979 (Menarini Farmaceutici).

As used herein, "HIV protease inhibitor" is intended to refer to compounds which inhibit HIV protease. Examples include, but are not limited, saquinavir (Roche, Ro31-8959),

ritonavir (Abbott, ABT-538), indinavir (Merck, MK-639), amprenavir (Vertex/Glaxo Wellcome), nelfinavir (Agouron, AG-1343), zalcitabine (Boehringer Ingelheim), BMS-232623 (Bristol-Myers Squibb), GS3333 (Gilead Sciences), KNI-413 (Japan Energy), KNI-272 (Japan Energy), LG-71350 (LG Chemical), CGP-61755 (Ciba-Geigy), PD 173606 (Parke Davis), PD 177298 (Parke Davis), PD 178390 (Parke Davis), PD 178392 (Parke Davis), tipranavir (Pharmacia and Upjohn, U-1 40690), DMP-450 (DuPont) and ABT-378.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication commensurate with a reasonable benefit/risk ratio. "Prodrugs" are intended to include any covalently bonded carriers which release the active parent drug according to formula (I) or other formulas or compounds of the present invention in vivo when such prodrug is administered to a mammalian subject. Prodrugs of a compound of the present invention, for example formula (I), are prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent compound. Prodrugs include compounds of the present invention wherein the hydroxy or amino group is bonded to any group that, when the prodrug is administered to a mammalian subject, cleaves to form a free hydroxyl or free amino, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups in the compounds of the present invention, and the like.

"Stable compound" and "stable structure" are meant to indicate a compound that is sufficiently robust to survive

isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent. Only stable compounds are contemplated by the present invention.

"Substituted" is intended to indicate that one or more hydrogens on the atom indicated in the expression using "substituted" is replaced with a selection from the indicated group(s), provided that the indicated atom's normal valency is not exceeded, and that the substitution results in a stable compound. When a substituent is keto (i.e., =O) group, then 2 hydrogens on the atom are replaced.

"Therapeutically effective amount" is intended to include an amount of a compound of the present invention or an amount of the combination of compounds claimed effective to inhibit HIV infection or treat the symptoms of HIV infection in a host. The combination of compounds is preferably a synergistic combination. Synergy, as described for example by Chou and Talalay, *Adv. Enzyme Regul.* 22:27-55 (1984), occurs when the effect (in this case, inhibition of HIV replication) of the compounds when administered in combination is greater than the additive effect of the compounds when administered alone as a single agent. In general, a synergistic effect is most clearly demonstrated at suboptimal concentrations of the compounds. Synergy can be in terms of lower cytotoxicity, increased antiviral effect, or some other beneficial effect of the combination compared with the individual components.

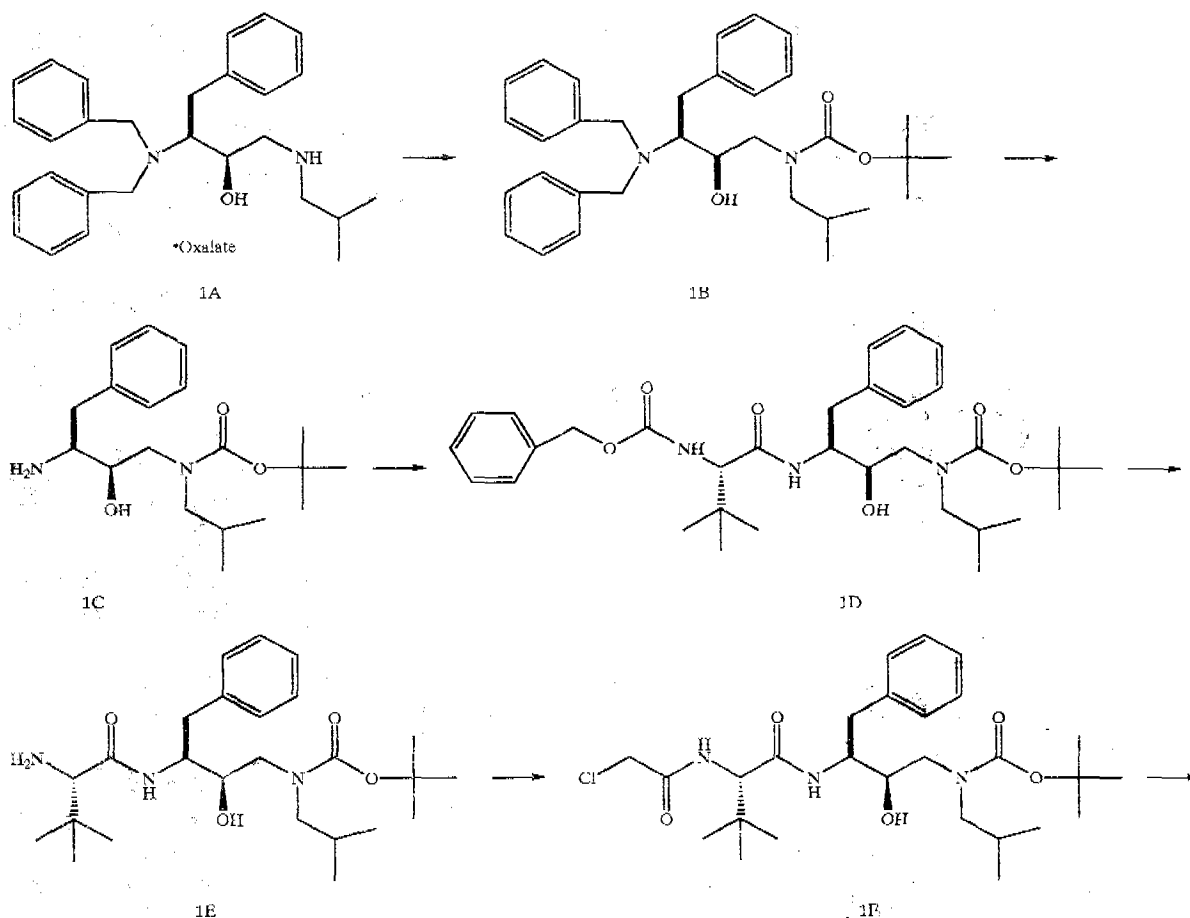
One diastereomer of a compound of Formula I may display superior activity compared with the other. When required, separation of the racemic material can be achieved by HPLC using a chiral column or by a resolution using a resolving agent such as camphonic chloride as in Thomas J. Tucker, et al, *J. Med. Chem.* 1994,37,2437-2444. A chiral compound of Formula I may also be directly synthesized using a chiral catalyst or a chiral ligand, e.g. Mark A. Huffman, et al, *J. Org. Chem.* 1995, 60, 1590-1594.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

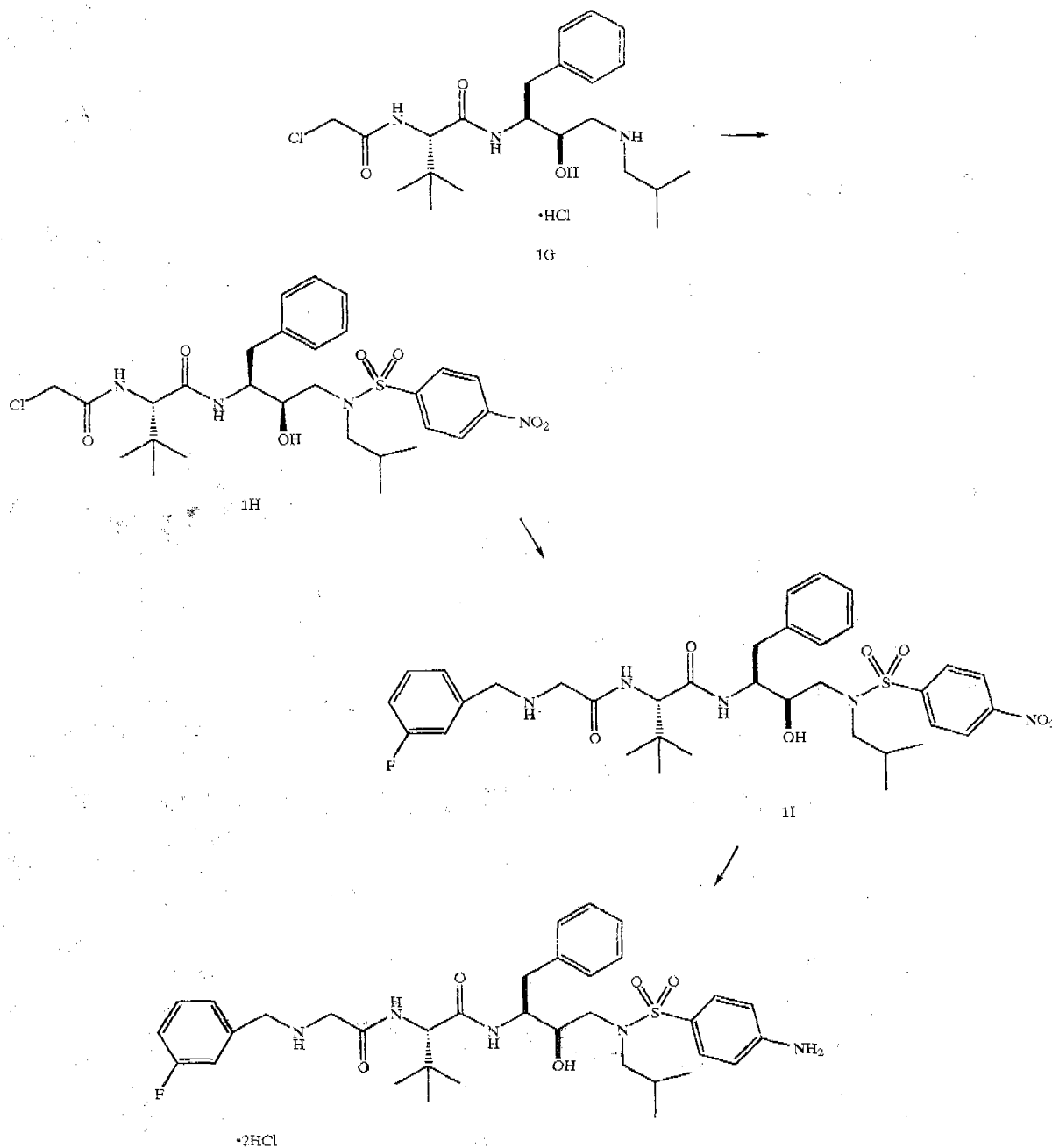
15

Abbreviations used in the Examples are defined as follows: "° C." for degrees Celsius, "d" for doublet, "dd" for doublet of doublets, "eq" for equivalent or equivalents, "g" for gram or grams, "mg" for milligram or milligrams, "mL" for milliliter or milliliters, "H" for hydrogen or hydrogens, "hr" for hour or hours, "m" for multiplet, "M" for molar, "min" for minute or minutes, "MHz" for megahertz, "MS" for mass spectroscopy, "nmr" or "NMR" for nuclear magnetic resonance spectroscopy, "t" for triplet, and "TLC" for thin layer chromatography.

Example 1



-continued



1B To a solution of N-[3(S)-[N,N-bis(phenylmethyl)amino]-
2(R)-hydroxy-4-phenylbutyl]-N-isobutylamine-oxalic
acid salt 1A (127.6 g, 251 mmol) in toluene (1 L), water
(500 mL) and CH_2Cl_2 (400 mL) was added NaOH (50%
aqueous, 44.5 g). After stirring 10 min the reaction
mixture was extracted with toluene. The combined
organic layers were washed with brine, dried (MgSO_4)
and the solvent was removed under reduced pressure. The
residue was taken up in THF (1 L), cooled to 0°C , and
was treated with triethylamine (28.15 g, 278 mmol) and
di-tert-butyl dicarbonate (55.23 g, 253 mmol). The solu-
tion was warmed to room temperature and was stirred
overnight. The solvent was removed under reduced pres-

sure and the residue was taken up in EtOAc (1 L), washed
with water, 5% citric acid, water, saturated NaHCO_3 ,
brine, and was dried (MgSO_4). The solvent was removed
under reduced pressure to give the carbamate 1B which
was used directly without further purification. CIMS
(NH_3) m/z : 517 ($\text{M}+\text{H}^+$, 100%)

1C To a solution of crude 1B (251 mmol possible) in
methanol (500 mL) was added palladium hydroxide on
carbon (20%, 10 g). The suspension was placed in a parr
bottle and was charged with hydrogen (55 psi). After
shaking overnight the reaction mixture was filtered
through Celite and the solvent was removed under
reduced pressure. The resulting solid was recrystallized

13

(EtOAc/hexane) to give the amine 1C as a white solid (56.6 g, 67% (2 steps)): CIMS (NH_3) m/z : 337 ($\text{M}+\text{H}^+$, 100%)

1D To a solution of N-carbobenzyloxy-L-tert-leucine (47.5 g, 179 mmol) in DMF (250 mL) at 0° C. was added N-hydroxybenzotriazole (38.6 g, 285 mmol) and EDC (35.7 g, 186 mmol). After stirring 1.5 hours the solution was added to a suspension of 1C (56.6 g, 167 mmol) and 4-methylmorpholine (52.9 g, 521 mmol) in DMF (200 mL). The reaction mixture was allowed to warm to room temperature. After stirring overnight N,N-dimethylethylenediamine (4 mL) was added, the solution was stirred 1.5 hours and the solvent was removed under reduced pressure. The residue was taken up in EtOAc (1 L), washed with water, 5% citric acid, water, saturated NaHCO_3 , brine, and was dried (MgSO_4). The solvent was removed under reduced pressure to give 1D (97.5 g, 100%) which was used without further purification. CIMS (NH_3) m/z : 584 ($\text{M}+\text{H}^+$, 100%)

1E To a solution of 1D (97.5 g, 167 mmol) in methanol (300 mL) was added palladium hydroxide on carbon (20%, 10 g). The suspension was placed in a parr bottle and was charged with hydrogen (55 psi). After shaking overnight the reaction mixture was filtered through Celite and the solvent was removed under reduced pressure. The resulting solid was recrystallized (EtOAc/hexane) to give the amine 1E as a white solid (72.8 g, 97%): CIMS (NH_3) m/z : 450 ($\text{M}+\text{H}^+$, 100%)

1F To a solution of amine 1E (43.8 g, 97.6 mmol) in EtOAc (400 mL) and water (270 mL) was added KHCO_3 (27.7 g, 276 mmol) and chloroacetyl chloride (12.4 g, 111 mmol). After stirring 3 hours, EtOAc (1 L) was added and the solution was washed with water, 5% citric acid, water, saturated NaHCO_3 , brine, and was dried (MgSO_4). The solvent was removed under reduced pressure to give 1F as a white solid (51.0 g, 99%): CIMS (NH_3) m/z : 526 ($\text{M}+\text{H}^+$, 100%)

1G To a solution of 1F (33.8 g, 64.2 mmol) in EtOAc (600 mL) was added 4N HCl in dioxane (80 mL, 320 mmol) and the reaction mixture was stirred 6 hours. The solvent

14

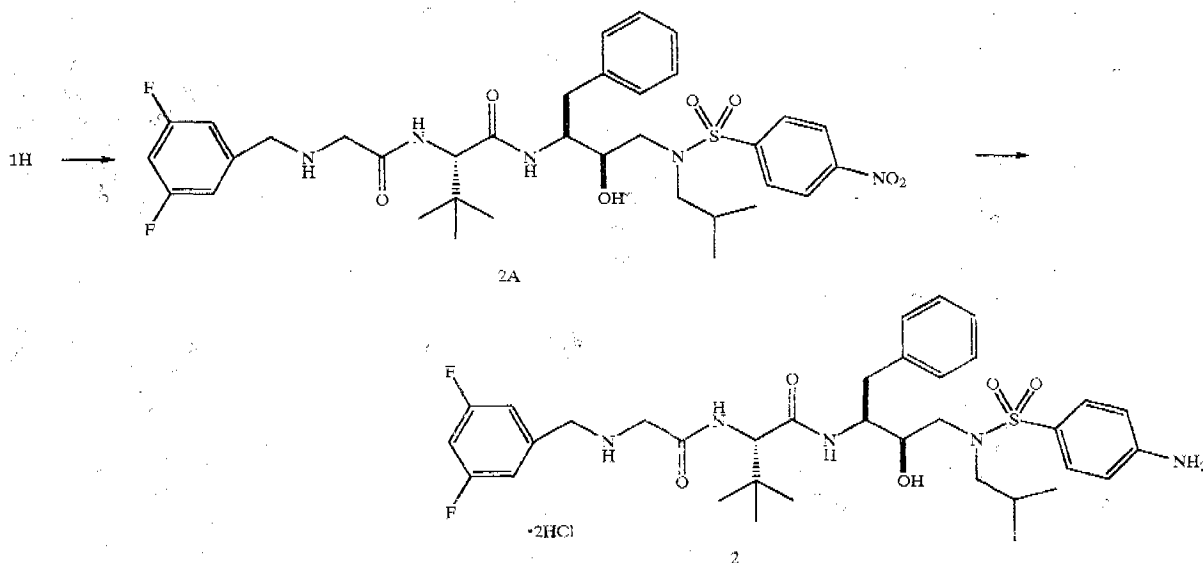
was removed under reduced pressure and the resulting solid was triturated with cold ether to give the hydrochloride salt 1G (28.75 g, 97%): CIMS (NH_3) m/z : 426 ($\text{M}+\text{H}^+$, 100%)

1H To a solution of the salt 1G (32.0 g, 69.2 mmol) in THF (350 mL) and water (450 mL) was added K_2CO_3 (56.7 g, 411 mmol) and 4-nitrobenzenesulfonyl chloride (16.9 g, 76.0 mmol). After stirring 4 hours, water was added and the suspension was extracted with EtOAc. The combined organic layers were washed with brine, 5% citric acid, water, saturated NaHCO_3 , brine, and was dried (MgSO_4). The solvent was removed under reduced pressure and the resulting solid was recrystallized (EtOAc/hexane) to give the sulfonamide 1H as a white solid (35.8 g, 85%): CIMS (NH_3) m/z : 611 ($\text{M}+\text{H}^+$, 100%).

1I To a solution of the chloride 1H (16.0 g, 26.1 mmol) in THF (200 mL) was added 3-fluorobenzylamine (20.0 g, 160 mmol) and the reaction mixture was refluxed overnight. The solvent was removed under reduced pressure and the residue was taken up in EtOAc and was washed with water, brine, and dried (MgSO_4). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 4% methanol/ CH_2Cl_2) to give the amine 1I as a white solid (16.3 g, 89%): CIMS (NH_3) m/z : 700 ($\text{M}+\text{H}^+$, 100%).

1 To a solution of 1I (14.6 g, 20.8 mmol) in methanol (500 mL) was added palladium hydroxide on carbon (20%, 1.5 g) and the reaction mixture was charged with hydrogen. After stirring 3 hours, the mixture was filtered through Celite and the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, 5% methanol/ CH_2Cl_2) to give the amine as a white solid (13.2 g, 95%). To a solution of the freebase (11.68 g, 17.4 mmol) in ether (300 mL) and EtOAc (100 mL) was added 1N HCl in ether (37 mL, 37 mmol). The resulting suspension was stirred 15 min and was filtered to give the bis-hydrochloride salt 1 as a white solid (12.5 g, 96%): CIMS (NH_3) m/z : 670 ($\text{M}+\text{H}^+$, 100%).

Example 2



15

2A To a solution of the chloride 1H (16.0 g, 26.1 mmol) in THF (200 mL) was added 3,5-difluorobenzylamine (25.0 g, 174 mmol) and the reaction mixture was refluxed overnight. The solvent was removed under reduced pressure and the residue was taken up in EtOAc and was washed with water, brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 4% methanol/CH₂Cl₂) to give the amine 2A as a white solid (15.2 g, 81%). CIMS (NH₃) m/z: 718 (M+H⁺, 100%).

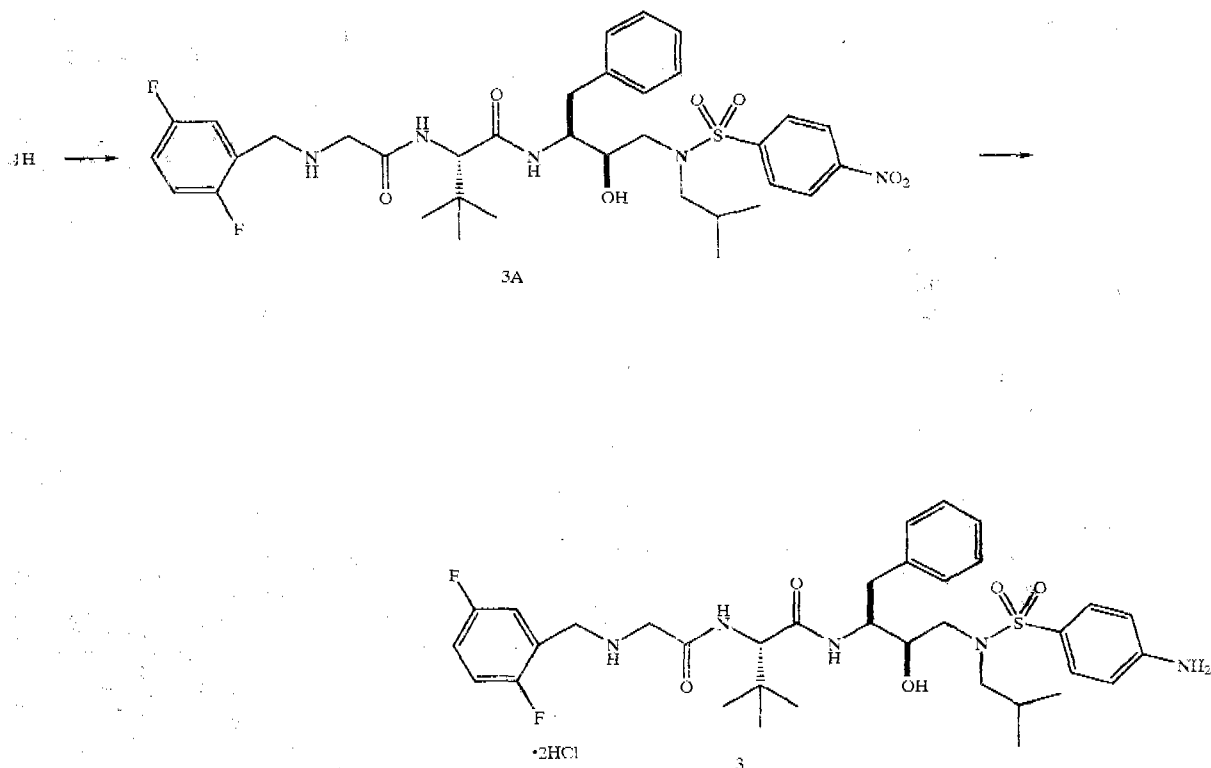
2 To a solution of 2A (15.2 g, 21.2 mmol) in methanol (500 mL) was added palladium hydroxide on carbon (20%, 1.5 g) and the reaction mixture was charged with hydrogen. After stirring 4 hours, the mixture was filtered through Celite and the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine as a white solid (10.3 g, 71%). To a solution of the freebase in ether (300 mL) and EtOAc (100 mL) was added 1N HCl in ether (32 mL, 32 mmol). The resulting suspension was stirred 15 min and was filtered to give the bis-hydrochloride salt 2 as a white solid: CIMS (NH₃) m/z: 688 (M+H⁺, 100%).

Example 3

16

3A To a solution of the chloride 1H (300 mg, 0.49 mmol) in THF (4 mL) was added 2,5-difluorobenzylamine (1.2 g, 8.5 mmol) and the reaction mixture was refluxed 4 hours. The reaction mixture was diluted with EtOAc and was washed with water, brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine 3A as a white solid (270 mg, 77%). CIMS (NH₃) m/z: 718 (M+H⁺, 100%).

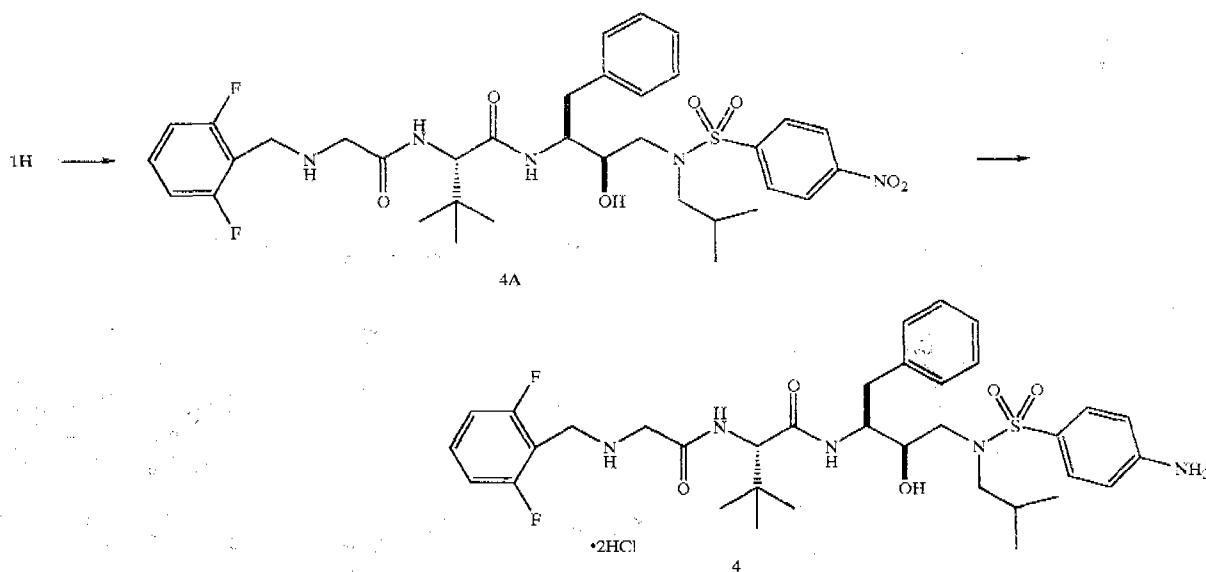
3 To a solution of 3A (260 mg, 0.36 mmol) in methanol (25 mL) was added palladium hydroxide on carbon (20%, 50 mg) and the reaction mixture was charged with hydrogen. After stirring 1 hour, the mixture was filtered through Celite and the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine as a white solid (226 mg, 91%). To a solution of the freebase in ether (30 mL) and EtOAc (10 mL) was added 4N HCl in dioxane (0.2 mL, 0.8 mmol). The resulting suspension was stirred 15 min and was filtered to give the bis-hydrochloride salt 3 as a white solid: CIMS (NH₃) m/z: 688 (M+H⁺, 100%).



17

Example 4

18

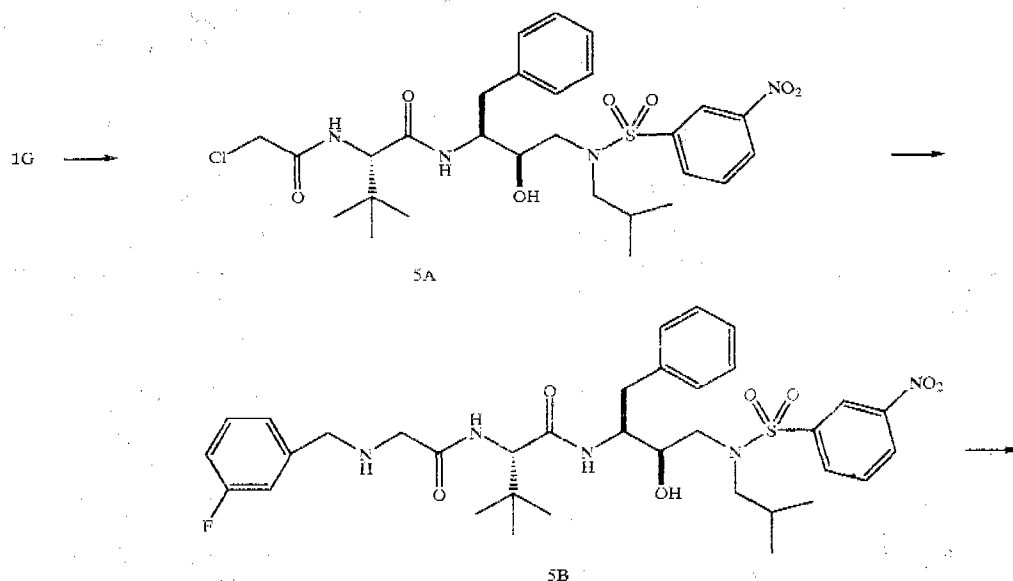


4A To a solution of the chloride 1H (300 mg, 0.49 mmol) in THF (4 mL) was added 2,6-difluorobenzylamine (1.2 g, 8.5 mmol) and the reaction mixture was refluxed 4 hours. The reaction mixture was diluted with EtOAc and was washed with water, brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine 4A as a white solid (306 mg, 87%). CIMS (NH₃) m/z: 718 (M+H⁺, 100%).

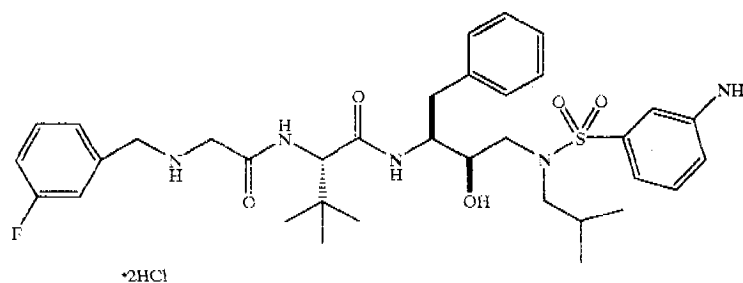
4 To a solution of 4A (295 mg, 0.41 mmol) in methanol (25 mL) was added palladium hydroxide on carbon (20%, 50 mg) and the reaction mixture was charged with hydrogen.

After stirring 1 hour, the mixture was filtered through Celite and the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine as a white solid (228 mg, 81%). To a solution of the freebase in ether (30 mL) and EtOAc (10 mL) was added 4N HCl in dioxane (0.2 mL, 0.8 mmol). The resulting suspension was stirred 15 min and was filtered to give the bis-hydrochloride salt 4 as a white solid: CIMS (NH₃) m/z: 688 (M+H⁺, 100%).

Example 5



-continued



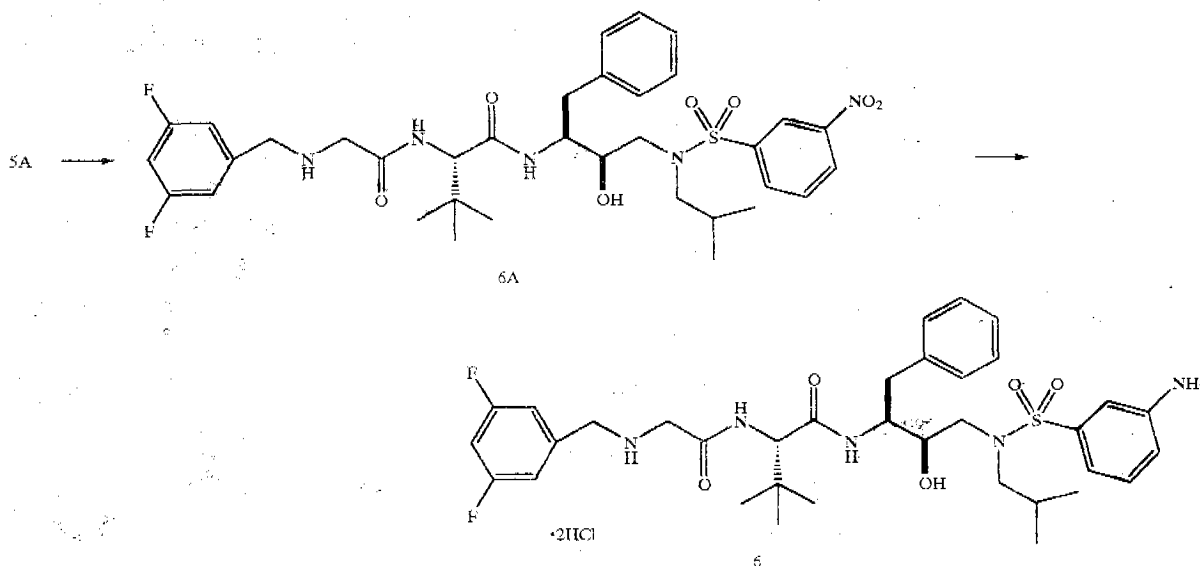
5

5A To a solution of the salt 1 G (28.8 g, 62.1 mmol) in THF (300 mL) and water (400 mL) was added K_2CO_3 (51.4 g, 370 mmol) and 3-nitrobenzenesulfonyl chloride (15.14 g, 68.3 mmol). After stirring 4 hours, water was added and the suspension was extracted with EtOAc. The combined organic layers were washed with brine, 5% citric acid, water, saturated $NaHCO_3$, brine, and was dried ($MgSO_4$). The solvent was removed under reduced pressure and the resulting solid was triturated with EtOAc and hexane to give the sulfonamide 5A as a white solid (32.1 g, 85%). CIMS (NH_3) m/z: 611 ($M+H^+$, 100%).

5B To a solution of the chloride 5A (16.0 g, 26.1 mmol) in THF (200 mL) was added 3-fluorobenzylamine (17.0 g, 135 mmol) and the reaction mixture was refluxed over-

reduced pressure. The residue was chromatographed (silica gel, 5% methanol/ CH_2Cl_2) to give the amine as a white solid (11.2 g, 97%). To a solution of the freebase in ether (400 mL) and EtOAc (75 mL) was added 1N HCl in ether (36 mL, 36 mmol). The resulting suspension was stirred 15 min and was filtered to give the bis-hydrochloride salt 5 as a white solid. CIMS (NH_3) m/z: 670 ($M+H^+$, 100%).

Example 6



night. The solvent was removed under reduced pressure and the residue was taken up in EtOAc and was washed with water, brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 4% methanol/ CH_2Cl_2) to give the amine 5B as a white solid (16.0 g, 87%). CIMS (NH_3) m/z: 700 ($M+H^+$, 100%).

5 To a solution of 5B (12.0 g, 17.22 mmol) in methanol (400 mL) was added palladium hydroxide on carbon (20%, 1.25 g) and the reaction mixture was charged with hydrogen. After stirring 3 hours, the mixture was filtered through Celite and the solvent was removed under

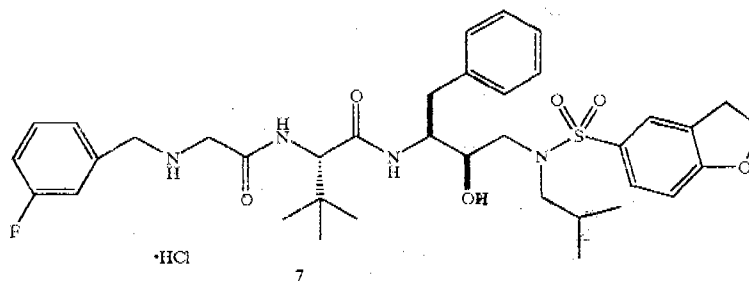
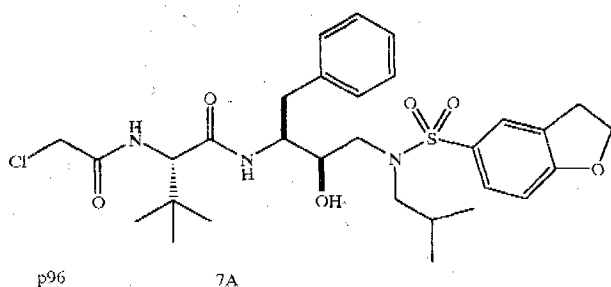
6 A To a solution of the chloride 5A (16.0 g, 26.1 mmol) in THF (200 mL) was added 3,5-difluorobenzylamine (25.0 g, 174 mmol) and the reaction mixture was stirred 2 hours and refluxed overnight. The solvent was removed under reduced pressure and the residue was taken up in EtOAc and was washed with water, brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 3.5% methanol/ CH_2Cl_2) to give the amine 6A as a white solid (15.6 g, 83%). CIMS (NH_3) m/z: 718 ($M+H^+$, 100%).

6 To a solution of 6A (14.4 g, 20.0 mmol) in methanol (500 mL) was added palladium hydroxide on carbon (20%, 1.5

21

g) and the reaction mixture was charged with hydrogen. After stirring 4 hours, the mixture was filtered through Celite and the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, 5% methanol/ CH_2Cl_2) to give the amine as a white solid (12.5 g, 91%). To a solution of the freebase (9.57 g, 13.9 mmol) in ether (300 mL) was added 1N HCl in ether (31 mL, 31 mmol). The resulting suspension was stirred 20 min and was filtered to give the bis-hydrochloride salt 6 as a white solid: (9.9 g, 94%). CIMS (NH_3) m/z : 688 ($\text{M}+\text{H}^+$, 100%).

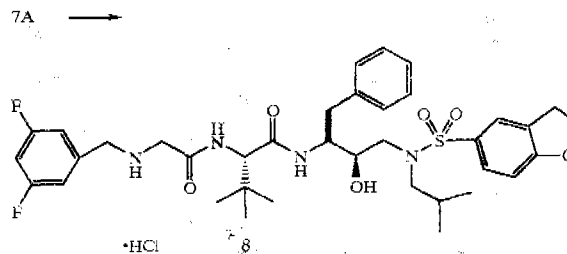
Example 7



40

7 To a solution of N-[2R-hydroxy-3-[(2,3-dihydro-2,3-dihydrobenzofuran-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-[(chloroacetyl)

Example 8



8 To a solution of 7A (100 mg, 0.16 mmol) in THF (2 mL) was added 3,5-difluorobenzylamine (605 mg, 4.2 mmol) and the reaction mixture was refluxed 6 hours. The reaction mixture was diluted with EtOAc and was washed with water (4x), brine, and dried (MgSO_4). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/ CH_2Cl_2) to give the amine as a white solid (83 mg, 70%). To a

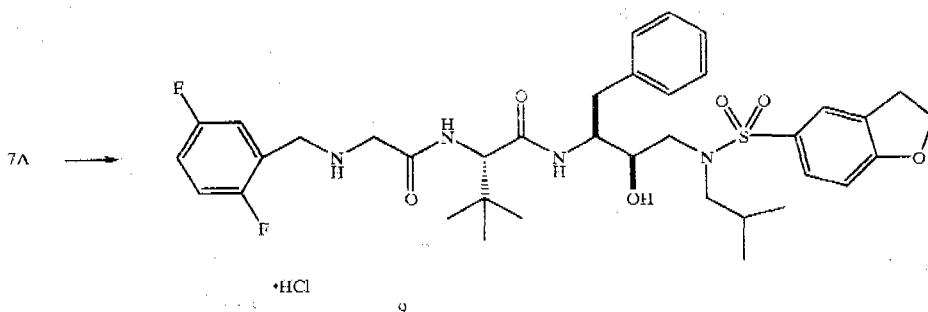
23

solution of the freebase (83 mg, 0.11 mmol) in ether (25 mL) was added 4N HCl in dioxane (0.05 mL, 0.20 mmol). After stirring 10 min the solvent was removed under reduced pressure and the resulting solid was triturated with ether and filtered to give the hydrochloride salt 8 as a white solid (65 mg, 75%): Anal. ($C_{37}H_{49}N_4O_6S_1F_2Cl_1$): Calc: C, 59.15; H, 6.45; N, 7.47. Found: C, 58.90; H, 6.51; N, 7.21.

Example 9

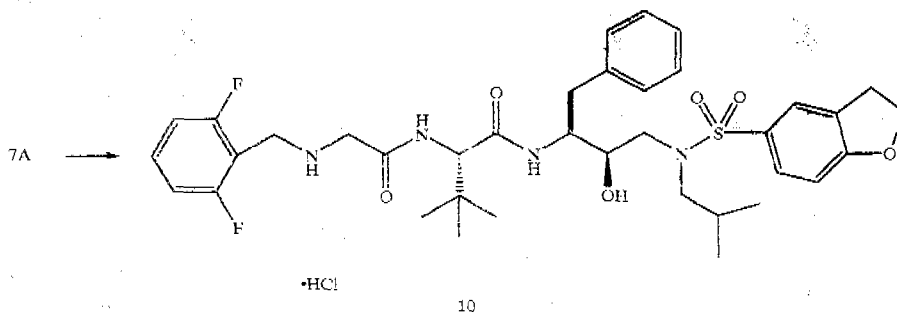
24

was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/ CH_2Cl_2) to give the amine as a white solid (103 mg, 87%). To a solution of the freebase (103 mg, 0.14 mmol) in ether (25 mL) was added 4N HCl in dioxane (0.05 mL, 0.20 mmol). After stirring 10 min the solvent was removed under reduced pressure and the resulting solid was triturated with ether and filtered to give the hydrochloride salt 10 as a white solid (82 mg, 76%): CIMS (NH_3) m/z: 715 ($M+H^+$, 100%).



9 To a solution of 7A (100 mg, 0.16 mmol) in THF (2 mL) was added 2,5-difluorobenzylamine (610 mg, 4.3 mmol) and the reaction mixture was refluxed 6 hours. The reaction mixture was diluted with EtOAc and was washed with water (4x), brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/ CH_2Cl_2) to give the amine as a white solid (110 mg, 93%). To a solution of the freebase (110 mg, 0.15 mmol) in ether (25 mL) was added 4N HCl in dioxane (0.05 mL, 0.20 mmol). After stirring 10 min the solvent was removed under reduced pressure and the resulting solid was triturated with ether and filtered to give the hydrochloride salt 9 as a white solid (76 mg, 66%): CIMS (NH_3) m/z: 715 ($M+H^+$, 100%).

Example 10

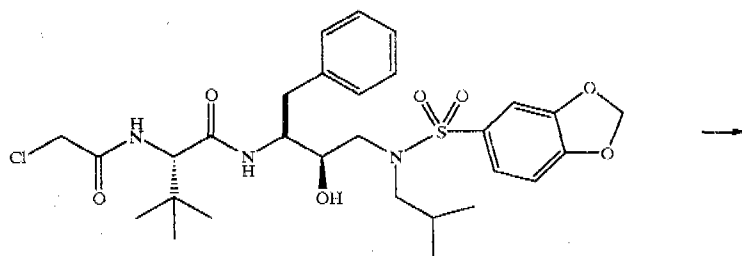


10 To a solution of 7A (100 mg, 0.16 mmol) in THF (2 mL) was added 2,6-difluorobenzylamine (600 mg, 4.2 mmol) and the reaction mixture was refluxed 6 hours. The reaction mixture was diluted with EtOAc and was washed with water (4x), brine, and dried ($MgSO_4$). The solvent

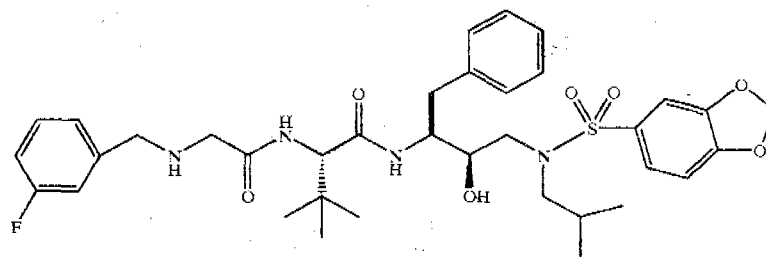
25

Example 11

26



11A



·HCl

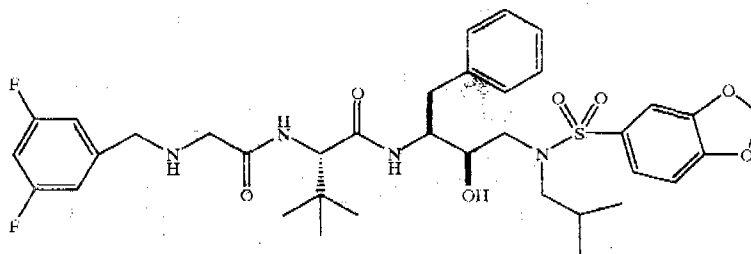
11

11 To a solution of **11A** (2R-hydroxy-3-[[[1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-[(chloroacetyl)amino]-3,3-dimethylbutanamide **11A** (750 mg, 1.23 mmol) in THF (2 mL) was added 3-fluorobenzylamine (1.1 g, 8.8 mmol) and the reaction mixture was stirred overnight. The reaction mixture was diluted with EtOAc and was washed with water (4x), brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to

give the amine as a white solid (532 mg, 62%). To a solution of the freebase (532 mg, 0.76 mmol) in ether (100 mL) was added 4N HCl in dioxane (0.22 mL, 0.88 mmol). After stirring 10 min the solvent was removed under reduced pressure and the resulting solid was triturated with ether and filtered to give the hydrochloride salt **11** as a white solid (417 mg, 75%); CIMS (NH₃) m/z: 699 (M+H⁺, 100%).

Example 12

11A

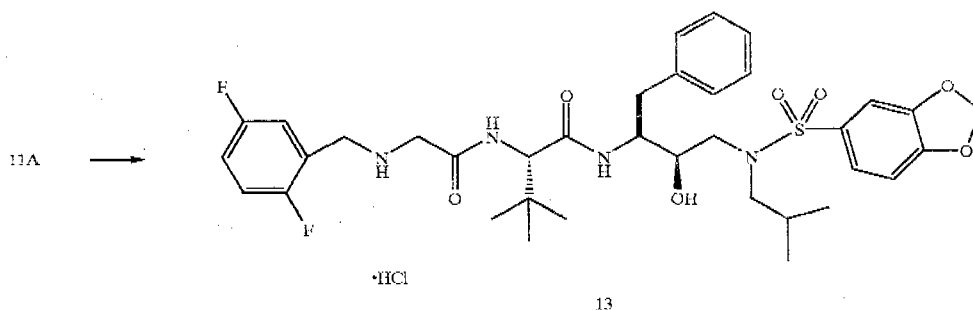


·HCl

12

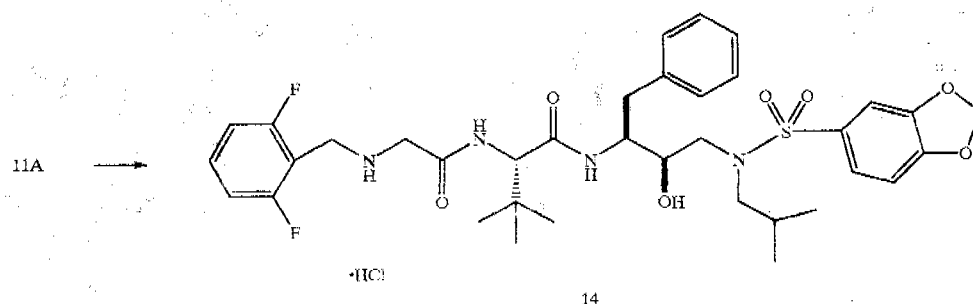
12 To a solution of **11A** (2.0 g, 3.27 mmol) in THF (7 mL) was added 3,5-difluorobenzylamine (2.42 g, 16.9 mmol) and the reaction mixture was refluxed 5 hours. The reaction mixture was diluted with EtOAc and was washed with water (4x), brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine as a white solid (2.26 g, 97%). To a

solution of the freebase (1.8 g, 2.51 mmol) in ether (100 mL) was added 4N HCl in dioxane (0.66 mL, 2.67 mmol). After stirring 10 min the solvent was removed under reduced pressure and the resulting solid was triturated with ether and filtered to give the benzylamine salt **12** as a white solid (1.65 g, 87%); CIMS (NH₃) m/z: 717 (M+H⁺, 100%). Anal. (C₃₆H₄₇N₄O₇S₁F₂Cl₁): Calc: C, 57.40; H, 6.17; N, 7.45. Found: C, 57.25; H, 6.25; N, 7.24.



13 To a solution of 11A (750 mg, 1.23 mmol) in THF (2 mL) was added 2,5-difluorobenzylamine (1.2 g, 8.5 mmol) and the reaction mixture was refluxed 6 hours. The reaction mixture was diluted with EtOAc and was washed with water (4 $\times$), brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine as a white solid (702 mg, 80%). To a solution of the freebase (702 mg, 0.98 mmol) in ether (100 mL) and EtOAc (25 mL) was added 4N HCl in dioxane (0.3 mmol, 1.2 mmol). After stirring 10 min the solvent was removed under reduced pressure and the resulting solid was triturated with ether and filtered to give the hydrochloride salt 13 as a white solid (586 mg, 79%); CIMS (NH₃) m/z: 717 (M+H⁺, 100%).

Example 14

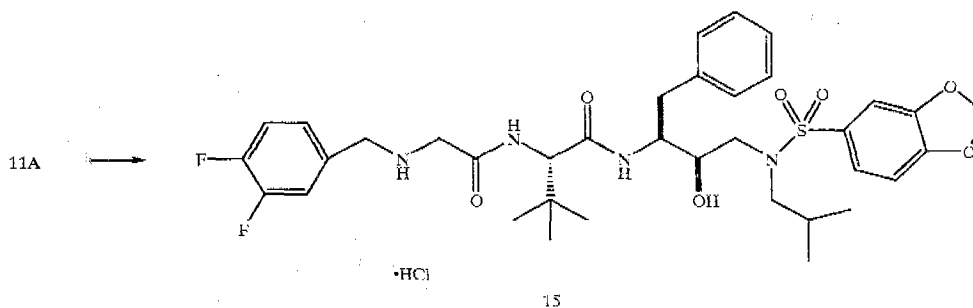


14 To a solution of 11A (750 mg, 1.23 mmol) in THF (2 mL) was added 2,6-difluorobenzylamine (1.2 g, 8.3 mmol) and the reaction mixture was refluxed 6 hours. The reaction mixture was diluted with EtOAc and was washed with water (4 $\times$), brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine as a white solid (717 mg, 81%). To a solution of the freebase (717 mg, 1.00 mmol) in ether (100 mL) was added 4N HCl in dioxane (0.3 mL, 1.2 mmol). After stirring 10 min the solvent was removed under reduced pressure and the resulting solid was triturated with ether and filtered to give the hydrochloride salt 14 as a white solid (663 mg, 88%); CIMS (NH₃) m/z: 717 (M+H⁺, 100%).

29

Example 15

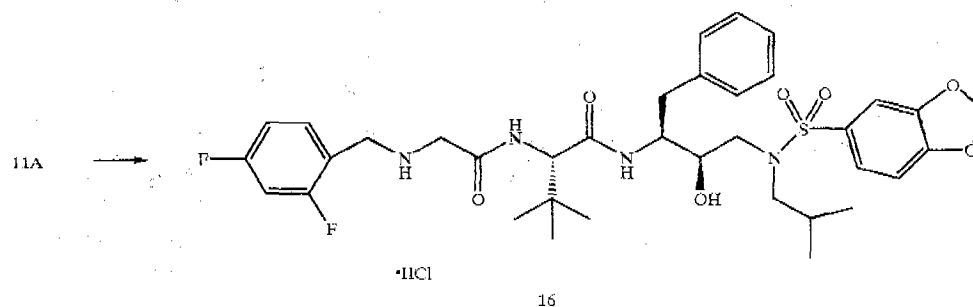
30



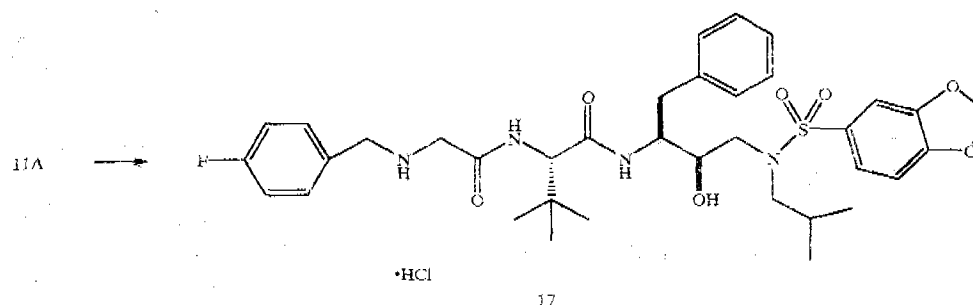
15 To a solution of 11A (750 mg, 1.23 mmol) in THF (2 mL) was added 3,4-difluorobenzylamine (1.2 g, 8.3 mmol) and the reaction mixture was refluxed 3 hours. The reaction mixture was diluted with EtOAc and was washed with water (4x), brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine as a white solid (760 mg, 86%). To a solution of the freebase (760 mg, 1.06 mmol) in ether (100 mL) was added 4N HCl in dioxane (0.3 mL, 1.2 mmol). After stirring 10 min the resulting solid was filtered to give the hydrochloride salt 15 as a white solid (730 mg, 91%); CIMS (NH₃) m/z: 717 (M+H⁺, 100%).

16 To a solution of 11A (750 mg, 1.23 mmol) in THF (2 mL) was added 2,4-difluorobenzylamine (1.2 g, 8.3 mmol) and the reaction mixture was refluxed 6 hours. The reaction mixture was diluted with EtOAc and was washed with water (4x), brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine as a white solid (693 mg, 79%). To a solution of the freebase (693 mg, 0.97 mmol) in ether (100 mL) was added 4N HCl in dioxane (0.3 mL, 1.2 mmol). After stirring 10 min the resulting solid was filtered to give the hydrochloride salt 16 as a white solid (638 mg, 88%); CIMS (NH₃) m/z: 717 (M+H⁺, 100%).

Example 16



Example 17



31

17 To a solution of 11A (500 mg, 0.82 mmol) in THF (2 mL) was added 4-fluorobenzylamine (1.0 g, 8.0 mmol) and the reaction mixture was stirred overnight. The reaction mixture was diluted with EtOAc and was washed with water (4x), brine, and dried (MgSO₄). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH₂Cl₂) to give the amine as a white solid (470 mg, 82%). To a solution of the freebase (400 mg, 0.57 mmol) in ether (30 mL) was added 4N HCl in dioxane (0.18 mL, 0.7 mmol). After stirring 15 min the resulting solid was filtered to give the hydrochloride salt 17 as a white solid (413 mg, 98%); CIMS (NH₄)⁺ m/z: 699 (M+H⁺, 100%).

Utility

The compounds of formula I possess HIV protease inhibitory activity and are therefore useful as antiviral agents for the treatment of HIV infection and associated diseases. The compounds of formula I possess HIV protease inhibitory activity and are effective as inhibitors of HIV growth. The ability of the compounds of the present invention to inhibit viral growth or infectivity is demonstrated in standard assay of viral growth or infectivity, for example, using the assay described below.

The compounds of formula I of the present invention are also useful for the inhibition of HIV in an ex vivo sample containing HIV or expected to be exposed to HIV. Thus, the compounds of the present invention may be used to inhibit HIV present in a body fluid sample (for example, a serum or semen sample) which contains or is suspected to contain or be exposed to HIV.

The compounds provided by this invention are also useful as standard or reference compounds for use in tests or assays for determining the ability of an agent to inhibit viral clone replication and/or HIV protease, for example in a pharmaceutical research program. Thus, the compounds of the present invention may be used as a control or reference compound in such assays and as a quality control standard. The compounds of the present invention may be provided in a commercial kit or container for use as such standard or reference compound.

Since the compounds of the present invention exhibit specificity for HIV protease, the compounds of the present invention may also be useful as diagnostic reagents in diagnostic assays for the detection of HIV protease. Thus, inhibition of the protease activity in an assay (such as the assays described herein) by a compound of the present invention would be indicative of the presence of HIV protease and HIV virus.

As used herein "μg" denotes microgram, "mg" denotes milligram, "g" denotes gram, "μL" denotes microliter, "mL" denotes milliliter, "L" denotes liter, "nM" denotes nanomolar, "μM" denotes micromolar, "mM" denotes millimolar, "M" denotes molar and "nM" denotes nanomolar. "Sigma" stands for the Sigma-Aldrich Corp. of St. Louis, Mo.

p HIV RNA Assay

DNA Plasmids and in Vitro RNA Transcripts

Plasmid pDAB 72 containing both gag and pol sequences of BH 10 (bp 113-1816) cloned into PTZ 19R was prepared according to Erickson-Viitanen et al. *AIDS Research and Human Retroviruses* 1989, 5, 577. The plasmid was linearized with Bam HI prior to the generation of in vitro RNA transcripts using the Riboprobe Gemini system 11 kit (Promega) with T7 RNA polymerase. Synthesized RNA was

32

purified by treatment with RNase free DNase (Promega), phenol-chloroform extraction, and ethanol precipitation. RNA transcripts were dissolved in water, and stored at -70° C. The concentration of RNA was determined from the A₂₆₀.

Probes

Biotinylated capture probes were purified by HPLC after synthesis on an Applied Biosystems (Foster City, Calif.) DNA synthesizer by addition of biotin to the 5' terminal end of the oligonucleotide, using the biotin-phosphoramidite reagent of Cocuzza, *Tet. Lett.* 1989, 30, 6287. The gag biotinylated capture probe (5'-biotin-CTAGCTCCCTGCTTGCCCCATACTA 3') was complementary to nucleotides 889-912 of HXB2 and the pol biotinylated capture probe (5'-biotin-CCCTATCATTTTGGTTTCCAT 3') was complementary to nucleotides 23742395 of HXB2. Alkaline phosphatase conjugated oligonucleotides used as reporter probes were prepared by Syngene (San Diego, Calif.) The pol reporter probe (5'-CTGTCCTACTTTGATAAAACCTC 3') was complementary to nucleotides 2403-2425 of HXB2. The gag reporter probe (5'-CCCAGTATTTGTCTACAGCCCTCT 3') was complementary to nucleotides 950-973 of HXB2. All nucleotide positions are those of the GenBank Genetic Sequence Data Bank as accessed through the Genetics Computer Group Inc. Sequence Analysis Software Package (Devereau Nucleic Acids Research 1984, 12, 387). The reporter probes were prepared as 0.5 μM stocks in 2xSSC (0.3 M NaCl, 0.03 M sodium citrate), 0.05 M Tris pH 8.8, 1 mg/mL BSA. The biotinylated capture probes were prepared as 100 μM stocks in water. Streptavidin coated plates:

Streptavidin coated plates were obtained from Du Pont Biotechnology Systems (Boston, Mass.).

Cells and Virus Stocks

MT-2 and MTA cells were maintained in RPM 1640 supplemented with 5% fetal calf serum (FCS) for MT-2 cells or 10% FCS for MTA cells, 2 mM L-glutamine and 50 μg/mL gentamycin, all from Gibco. HIV-RF was propagated in MT4 cells in the same medium. Virus stocks were prepared approximately 10 days after acute infection of MT-4 cells and stored as aliquots at -70° C. Infectious titers of HIV-1 (RF) stocks were 1-3x10⁷ PFU (plaque forming units)/mL as measured by plaque assay on MT-2 cells (see below). Each aliquot of virus stock used for infection was thawed only once.

For evaluation of antiviral efficacy, cells to be infected were subcultured one day prior to infection. On the day of infection, cells were resuspended at 5x10⁵ cells/mL in RPMI 1640, 5% FCS for bulk infections or at 2x10⁶/mL in Dulbecco's modified Eagles medium with 5% FCS for infection in microtiter plates. Virus was added and culture continued for 3 days at 37° C.

HIV RNA Assay

Cell lysates or purified RNA in 3 M or 5 M GED were mixed with 5 M GED and capture probe to a final guanidinium isothiocyanate concentration of 3 M and a final biotin oligonucleotide concentration of 30 nM. Hybridization was carried out in sealed U bottom 96 well tissue culture plates (Nunc or Costar) for 16-20 hours at 37° C. RNA hybridization reactions were diluted three-fold with deionized water to a final guanidinium isothiocyanate concentration of 1 M and aliquots (150 μL) were transferred to streptavidin coated microtiter plates wells. Binding of capture probe and capture probe-RNA hybrid to the immobilized streptavidin was allowed to proceed for 2 hours at room temperature, after which the plates were washed 6 times with DuPont ELISA plate wash buffer (phosphate buffered saline (PBS), 0.05% Tween 20.) A second hybridization of reporter probe

to the immobilized complex of capture probe and hybridized target RNA was carried out in the washed streptavidin coated well by addition of 120 μ L of a hybridization cocktail containing 4 $\times$ SSC, 0.66% Triton $\times$ 100, 6.66% deionized formamide, 1 mg/mL BSA and 5 nM reporter probe. After hybridization for one hour at 37 $^{\circ}$ C., the plate was again washed 6 times. Immobilized alkaline phosphatase activity was detected by addition of 100 μ L of 0.2 mM 4-methylumbelliferyl phosphate (MUBP, JBL Scientific) in buffer δ (2.5 M diethanolamine pH 8.9 (JBL Scientific), 10 mM MgCl₂, 5 mM zinc acetate dihydrate and 5 mM N-hydroxyethyl-ethylene-diamine-triacetic acid). The plates were incubated at 37 $^{\circ}$ C. Fluorescence at 450 nM was measured using a microplate fluorometer (Dynateck) exciting at 365 nM.

Microplate Based Compound Evaluation in HIV-1 Infected MT-2 Cells

Compounds to be evaluated were dissolved in DMSO and diluted in culture medium to twice the highest concentration to be tested and a maximum DMSO concentration of 2%. Further three-fold serial dilutions of the compound in culture medium were performed directly in U bottom microtiter plates (Nunc). After compound dilution, MT-2 cells (50 μ L) were added to a final concentration of 5×10^5 per mL (1×10^5 per well). Cells were incubated with compounds for 30 minutes at 37 $^{\circ}$ C. in a CO₂ incubator. For evaluation of antiviral potency, an appropriate dilution of HIV-1 (RF) virus stock (50 μ L) was added to culture wells containing cells and dilutions of the test compounds. The final volume in each well was 200 μ L. Eight wells per plate were left uninfected with 50 μ L of medium added in place of virus, while eight wells were infected in the absence of any antiviral compound. For evaluation of compound toxicity, parallel plates were cultured without virus infection.

After 3 days of culture at 37 $^{\circ}$ C. in a humidified chamber inside a CO₂ incubator, all but 25 μ L of medium/well was removed from the HIV infected plates. Thirty seven μ L of 5 M GED containing biotinylated capture probe was added to the settled cells and remaining medium in each well to a final concentration of 3 M GED and 30 nM capture probe. Hybridization of the capture probe to HIV RNA in the cell lysate was carried out in the same microplate well used for virus culture by sealing the plate with a plate sealer (Costar), and incubating for 16–20 hrs in a 37 $^{\circ}$ C. incubator. Distilled water was then added to each well to dilute the hybridization reaction three-fold and 150 μ L of this diluted mixture was transferred to a streptavidin coated microtiter plate. HIV RNA was quantitated as described above. A standard curve, prepared by adding known amounts of pDAB 72 in vitro RNA transcript to wells containing lysed uninfected cells, was run on each microtiter plate in order to determine the amount of viral RNA made during the infection.

In order to standardize the virus inoculum used in the evaluation of compounds for antiviral activity, dilutions of virus were selected which resulted in an IC₅₀ value (concentration of compound required to reduce the HIV RNA level by 90%) for dideoxycytidine (ddC) of 0.2 μ g/mL. IC₅₀ values of other antiviral compounds, both more and less potent than ddC, were reproducible using several stocks of HIV-1 (RF) when this procedure was followed. This concentration of virus corresponded to $\sim 3 \times 10^5$ PFU (measured by plaque assay on MT-2 cells) per assay well and typically produced approximately 75% of the maximum viral RNA level achievable at any virus inoculum. For the HIV RNA assay, IC₅₀ values were determined from the percent reduction of net signal (signal from infected cell samples minus signal from uninfected cell samples) in the RNA assay

relative to the net signal from infected, untreated cells on the same culture plate (average of eight wells). Valid performance of individual infection and RNA assay tests was judged according to three criteria. It was required that the virus infection should result in an RNA assay signal equal to or greater than the signal generated from 2 ng of pDAB 72 in vitro RNA transcript. The IC₅₀ for ddC, determined in each assay run, should be between 0.1 and 0.3 μ g/mL. Finally, the plateau level of viral RNA produced by an effective protease inhibitor should be less than 10% of the level achieved in an uninhibited infection. A compound was considered active if its IC₅₀ was found to be less than 1 μ M.

For antiviral potency tests, all manipulations in microtiter plates, following the initial addition of 2 $\times$ concentrated compound solution to a single row of wells, were performed using a Perkin Elmer/Cetus ProPette.

Dosage and Formulation

The antiviral compounds of this invention can be administered as treatment for viral infections by any means that produces contact of the active agent with the agent's site of action, i.e., the viral protease, in the body of a mammal. They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination of therapeutic agents. They can be administered alone, but preferably are administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

The dosage administered will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the age, health, and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; and the effect desired. A daily dosage of active ingredient can be expected to be about 0.001 to about 1000 milligrams per kilogram of body weight, with the preferred dose being about 0.1 to about 30 mg/kg.

Dosage forms of compositions suitable for administration contain from about 1 mg to about 100 mg of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5–95% by weight based on the total weight of the composition. The active ingredient can be administered orally in solid dosage forms, such as capsules, tablets and powders, or in liquid dosage forms, such as elixirs, syrups and suspensions. It can also be administered parenterally, in sterile liquid dosage forms.

Gelatin capsules contain the active ingredient and powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium

bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts, and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben and chlorobutanol. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, supra, a standard reference text in this field.

Useful pharmaceutical dosage-forms for administration of the compounds of this invention can be illustrated as follows:

Capsules

A large number of unit capsules can be prepared by filling standard two-piece hard gelatin capsules each with 100 mg of powdered active ingredient, 150 mg of lactose, 50 mg of cellulose, and 6 mg magnesium stearic.

Soft Gelatin Capsules

A mixture of active ingredient in a digestible oil such as soybean oil, cottonseed oil or olive oil can be prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules containing 100 mg of the active ingredient. The capsules should then be washed and dried.

Tablets

A large number of tablets can be prepared by conventional procedures so that the dosage unit is 100 mg of active ingredient, 0.2 mg of colloidal silicon dioxide, 5 milligrams of magnesium stearate, 275 mg of microcrystalline cellulose, 11 mg of starch and 98.8 mg of lactose. Appropriate coatings may be applied to increase palatability or delay absorption.

Suspension

An aqueous suspension can be prepared for oral administration so that each 5 mL contain 25 mg of finely divided active ingredient, 200 mg of sodium carboxymethyl cellulose, 5 mg of sodium benzoate, 1.0 g of sorbitol solution, U.S.P., and 0.025 mg of vanillin.

Injectable

A parenteral composition suitable for administration by injection can be prepared by stirring 1.5% by weight of active ingredient in 10% by volume propylene glycol and water. The solution is sterilized by commonly used techniques.

Combination of Components (a) and (b)

Each therapeutic agent component of this invention can independently be in any dosage form, such as those described above, and can also be administered in various ways, as described above. In the following description component (b) is to be understood to represent one or more agents as described previously. Thus, if components (a) and (b) are to be treated the same or independently, each agent of component (b) may also be treated the same or independently.

Components (a) and (b) of the present invention may be formulated together, in a single dosage unit (that is, combined together in one capsule, tablet, powder, or liquid, etc.) as a combination product. When component (a) and (b) are not formulated together in a single dosage unit, the component (a) may be administered at the same time as component (b) or in any order, for example component (a) of this invention may be administered first, followed by administration of component (b), or they may be administered in the reverse order. If component (b) contains more than one agent, e.g., one RT inhibitor and one protease inhibitor, these agents may be administered together or in any order. When not administered at the same time, preferably the administration of component (a) and (b) occurs less than about one

hour apart. Preferably, the route of administration of component (a) and (b) is oral. The terms oral agent, oral inhibitor, oral compound, or the like, as used herein, denote compounds which may be orally administered. Although it is preferable that component (a) and component (b) both be administered by the same route (that is, for example, both orally) or dosage form, if desired, they may each be administered by different routes (that is, for example, one component of the combination product may be administered orally, and another component may be administered intravenously) or dosage forms.

As is appreciated by a medical practitioner skilled in the art, the dosage of the combination therapy of the invention may vary depending upon various factors such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the kind of concurrent treatment, the frequency of treatment, and the effect desired, as described above.

The proper dosage of components (a) and (b) of the present invention will be readily ascertainable by a medical practitioner skilled in the art, based upon the present disclosure. By way of general guidance, typically a daily dosage may be about 100 milligrams to about 1.5 grams of each component. If component (b) represents more than one compound, then typically a daily dosage may be about 100 milligrams to about 1.5 grams of each agent of component (b). By way of general guidance, when the compounds of component (a) and component (b) are administered in combination, the dosage amount of each component may be reduced by about 70-80% relative to the usual dosage of the component when it is administered alone as a single agent for the treatment of HIV infection, in view of the synergistic effect of the combination.

The combination products of this invention may be formulated such that, although the active ingredients are combined in a single dosage unit, the physical contact between the active ingredients is minimized. In order to minimize contact, for example, where the product is orally administered, one active ingredient may be enteric coated. By enteric coating one of the active ingredients, it is possible not only to minimize the contact between the combined active ingredients, but also, it is possible to control the release of one of these components in the gastrointestinal tract such that one of these components is not released in the stomach but rather is released in the intestines. Another embodiment of this invention where oral administration is desired provides for a combination product wherein one of the active ingredients is coated with a sustained-release material which effects a sustained-release throughout the gastrointestinal tract and also serves to minimize physical contact between the combined active ingredients. Furthermore, the sustained-released component can be additionally enteric coated such that the release of this component occurs only in the intestine. Still another approach would involve the formulation of a combination product in which the one component is coated with a sustained and/or enteric release polymer, and the other component is also coated with a polymer such as a lowviscosity grade of

134
204
215

hydroxypropyl methylcellulose or other appropriate materials as known in the art, in order to further separate the active components. The polymer coating serves to form an additional barrier to interaction with the other component. In each formulation wherein contact is prevented between components (a) and (b) via a coating or some other material, contact may also be prevented between the individual agents of component (b).

Dosage forms of the combination products of the present invention wherein one active ingredient is enteric coated can be in the form of tablets such that the enteric coated component and the other active ingredient are blended together and then compressed into a tablet or such that the enteric coated component is compressed into one tablet layer and the other active ingredient is compressed into an additional layer. Optionally, in order to further separate the two layers, one or more placebo layers may be present such that the placebo layer is between the layers of active ingredients. In addition, dosage forms of the present invention can be in the form of capsules wherein one active ingredient is compressed into a tablet or in the form of a plurality of microtablets, particles, granules or non-perils, which are then enteric coated. These enteric coated microtablets, particles, granules or non-perils are then placed into a capsule or compressed into a capsule along with a granulation of the other active ingredient.

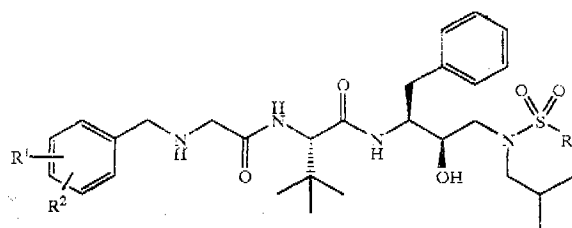
These as well as other ways of minimizing contact between the components of combination products of the present invention, whether administered in a single dosage form or administered in separate forms but at the same time or concurrently by the same manner, will be readily apparent to those skilled in the art, based on the present disclosure.

Pharmaceutical kits useful for the treatment of HIV infection, which comprise a therapeutically effective amount of a pharmaceutical composition comprising a compound of component (a) and one or more compounds of component (b), in one or more sterile containers, are also within the ambit of the present invention. Sterilization of the container may be carried out using conventional sterilization methodology well known to those skilled in the art. Component (a) and component (b) may be in the same sterile container or in separate sterile containers. The sterile containers of materials may comprise separate containers, or one or more multi-part containers, as desired. Component (a) and component (b), may be separate, or physically combined into a single dosage form or unit as described above. Such kits may further include, if desired, one or more of various conventional pharmaceutical kit components, such as for example, one or more pharmaceutically acceptable carriers, additional vials for mixing the components, etc., as will be readily apparent to those skilled in the art. Instructions, either as inserts or as labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, may also be included in the kit.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

What is claimed:

1. A compound of formula I:



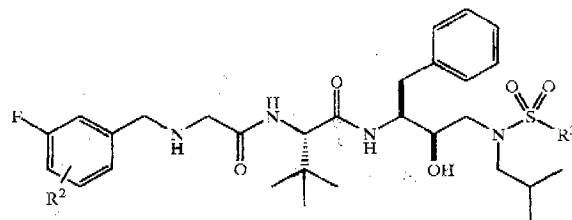
or a pharmaceutically acceptable salt form thereof, wherein:

R¹ is F;

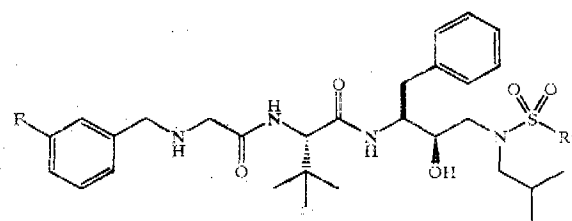
R² is F or H; and,

R³ is selected from the group: 4-aminophenyl, 3-aminophenyl, 2,3-dihydrobenzofuran-5-yl, and 1,3-benzodioxol-5-yl.

2. A compound according to claim 1, wherein the compound is of Formula II:



3. A compound according to claim 2, wherein the compound is of Formula IIa:



4. A compound according to claim 3, wherein:

R³ is 3-aminophenyl.

5. A compound according to claim 3, wherein:

R³ is 4-aminophenyl.

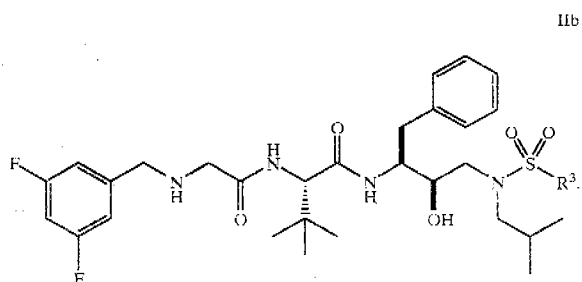
6. A compound according to claim 3, wherein:

R³ is 2,3-dihydrobenzofuran-5-yl or 1,3-benzodioxol-5-yl.

7. A compound according to claim 2, wherein the compound is of Formula IIb:

39

40



8. A compound according to claim 7, wherein:

R³ is 3-aminophenyl.

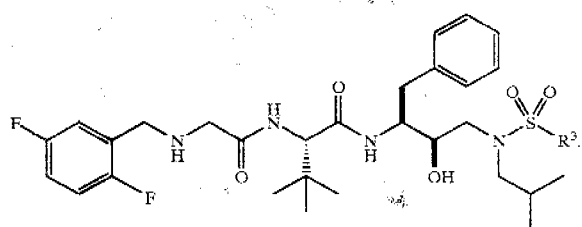
9. A compound according to claim 7, wherein:

R³ is 4-aminophenyl.

10. A compound according to claim 7, wherein:

R³ is 2,3-dihydrobenzofuran-5-yl or 1,3-benzodioxol-5-yl.

11. A compound according to claim 2, wherein the compound is of Formula IIc:



12. A compound according to claim 11, wherein:

R³ is 3-aminophenyl.

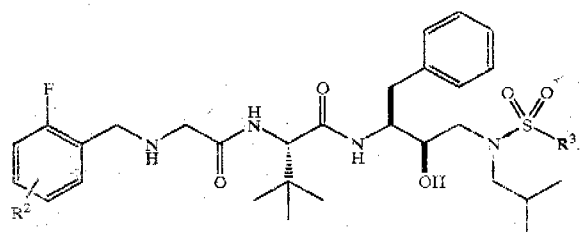
13. A compound according to claim 11, wherein:

R³ is 4-aminophenyl.

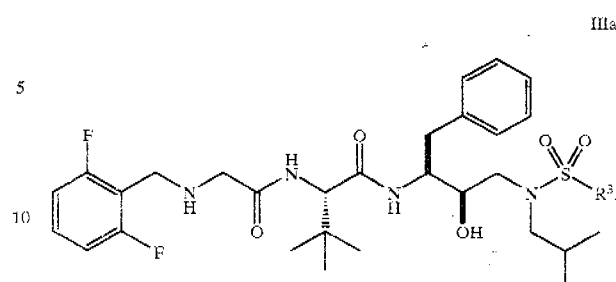
14. A compound according to claim 11, wherein:

R³ is 2,3-dihydrobenzofuran-5-yl or 1,3-benzodioxol-5-yl.

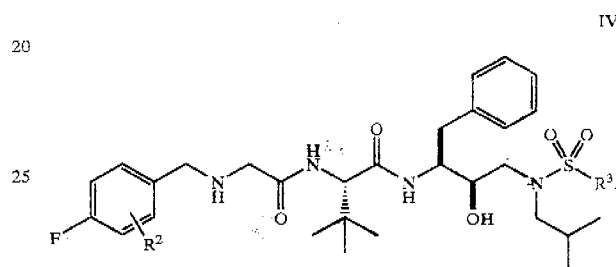
15. A compound according to claim 1, wherein the compound is of Formula III:



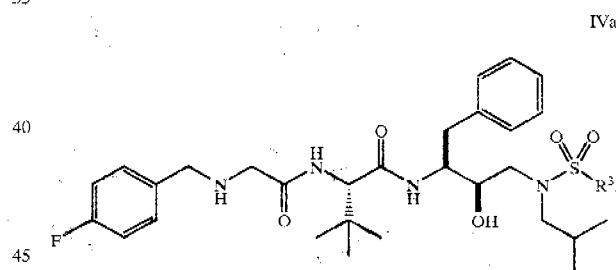
16. A compound according to claim 15, wherein the compound is of Formula IIIa:



17. A compound according to claim 1, wherein the compound is of Formula IV:



18. A compound according to claim 17, wherein the compound is of Formula IVa:



19. A pharmaceutical composition, comprising: a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound according to claim 1.

20. A method for treating HIV infection, comprising: administering to a host in need of such treatment a therapeutically effective amount of a compound according to claim 1, or a pharmaceutically acceptable salt form thereof.

21. A method of treating HIV infection which comprises administering, in combination, to a host in need thereof a therapeutically effective amount of:

(a) a compound according to claim 1 or stereoisomeric forms, mixtures of stereoisomeric forms, or pharmaceutically acceptable salts thereof; and,

(b) at least one compound selected from the group consisting of HIV reverse transcriptase inhibitors and HIV protease inhibitors.

137
218217

US 6,391,919 B1

41

22. A method according to claim 21, wherein the reverse transcriptase inhibitor is selected from the group AZT, ddC, ddI, d4T, 3TC, delavirdine, efavirenz, nevirapine, Ro 18,893, zalcitabine, MKC-442, HBV 097, ACT, UC-781, UC-782, RD4-2025, and MEN 10979 and the protease inhibitor is selected from the group saquinavir, ritonavir, indinavir, amprenavir, nelfinavir, zalcitabine, BMS-232623, GS3333, KNI-413, KNI-272, LG-71350, CGP-61755, PD 173606, PD 177298, PD 178390, PD 178392, U-140690, and ABT-378.

42

23. A method according to claim 22, wherein the reverse transcriptase inhibitor is selected from the group AZT, efavirenz, and 3TC and the protease inhibitor is selected from the group saquinavir, ritonavir, nelfinavir, and indinavir.

24. A method according to claim 21, wherein compound (b) is ritonavir.

* * * * *

138
118
214

SECRETARIA DE INDUSTRIA Y COMERCIO



Nº 05-070000-00000000

RECEPCION DE OBSERVACIONES
RECEPCION DE OBSERVACIONES
RECEPCION DE OBSERVACIONES

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RIT : 000170002/2

RECIBO OFICIAL DE CASH : 07 - 51,776
FECHA : JUNIO 27 DE 2007

***** CONSIGNACION *****

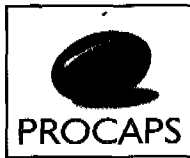
DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	Nº. PAGO	FECHA PGO	V.L. PAGO
LAD. PROCARPS	CONSIGNACION	BANCO POPULAR	050 00110-0	50770404	27/06/2007	1,200,000.00

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

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005 01-04	PRESENTACION DE OBSERVACIONES	
		12 MARCAS Y LEHAS COMERCIALES	256,000.00
		TOTAL :	256,000.00

CON: DOSCIENTOS CINCUENTA Y SEIS MIL PESOS

RESPONSABLE : _____



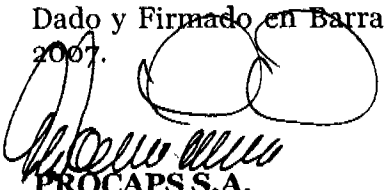
PODER ESPECIAL

Yo, **WALTER TANAKA TATEKAWA**, mayor de edad y vecino de la ciudad de Barranquilla (Colombia), identificado como aparece al pie de mi firma, quien en el presente documento actúa en calidad de Representante Legal de la sociedad **PROCAPS S.A.** sociedad legalmente constituida y domiciliada en la ciudad de Barranquilla (Colombia), por el presente documento otorgo a la doctora **LAURA CATALINA SILVA BARRERA**, abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 147399, poder especial, amplio y suficiente, para formular oposiciones u observaciones a la solicitud de patente No. 05070211, titulada **"USO DE UNA ASOCIACION QUE CONTIENE UN INHIBIDOR NO NUCLEOSIDO DE LA TRANSCRIPTASA INVERSA (NNRTI) CON UN INHIBIDOR DE CITOCROMO P450, TALES COMO LOS INHIBIDORES DE LA PROTEASA"** de **BOEHRINGER INGELHEIM INTERNACIONAL GMBH**, publicada en la gaceta No. 575 del 30 de Abril de 2007.

Nuestro apoderado queda facultado, de manera expresa, para realizar todo tipo de actos necesarios para llevar a cabo el trámite de la oposición, incluyendo, sin limitación, preparar y presentar solicitudes, hacer declaraciones, pagar tasas, contribuciones e impuestos, recabar los títulos o certificados. Asimismo quedan autorizados para impugnar administrativa y judicialmente todo lo que fuere resuelto en el expediente a que se hace expresa referencia en el presente poder, con todas las facultades generales y específicas que fuera requerido para ella.

De la misma manera, además de las facultades que le confiere la naturaleza del presente mandato, se encuentran facultados para recibir, desistir, transigir, sustituir, y reasumir el presente poder, incoar acciones, interponer recursos y demás facultades conferidas por la ley.

Dado y Firmado en Barranquilla (Colombia) a los 16 días del mes de Julio de 2007.


PROCAPS S.A.

WALTER TANAKA TATEKAWA

C.C. N° 16.251.923

Representante Legal

Acepto:



LAURA CATALINA SILVA BARRERA

C.C. 52'455.803 de Bogotá

T.P.A. 147399 del C.S. de la J.

[Handwritten signature]

AUTENTICACION DE FIRMA
 COMPARECENCIA
 C.O.C. mod. Decr. 2282 de 1999, art. 1 mod. 36)
 El Notario Cuarenta y Uno (41) del círculo
 de Notarías con, compareció personalmente:
 SILVA Barrera Laura C.
 C.I. 52455803
 Bta. 1 17399 del Consejo
 de la Abogacía, con documento di-
 S1 C
 que va en este escrito es
 Santa Catalina Slob
 25 JUL 2007
 ALPORDÉ GETA DILIGENCIA EL SUSCRITO



***** Pag. 1
C A M A R A D E C O M E R C I O
D E B A R R A N Q U I L L A
10*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

EL SUSCRITO SECRETARIO DE LA CAMARA DE COMERCIO DE BARRANQUILLA,

C E R T I F I C A

Que por Escritura Pública No. 1,190 del 22 de Julio de 1976, otorgada en la Notaria Tercera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 28 de Julio de 1976 bajo el No. 6,070 del libro respectivo, fue constituida la sociedad----- limitada denominada "PRODUCTORA DE CAPSULAS DE GELATINAS LIMITADA "PROCAPS LIMITADA".-----

C E R T I F I C A

Que por Escritura Pública No. 1,307 del 28 de Junio de 1979, otorgada en la Notaria Tercera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 23 de Julio de 1979 bajo el No. 10,039 del libro respectivo, la sociedad antes mencionada----- se transformo en sociedad anonima bajo la denominacion social de "PRODUCTORA DE CAPSULAS DE GELATINA S.A." PROCAPS S.A.".-----

C E R T I F I C A

Que por Escritura Pública No. 3,810 del 31 de Dic/bre de 1980, otorgada en la Notaria Primera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 30 de Enero de 1981 bajo el No. 12,705 del libro respectivo, la sociedad antes mencionada----- se transformo en sociedad de responsabilidad limitada bajo al razon social de "PRODUCTORA DE CAPSULAS DE GELATINA LIMITADA "PROCAPS LI - MITADA".-----

C E R T I F I C A

Que por Escritura Pública No. 3,755 del 10 de Nov/bre de 1983, otorgada en la Notaria Unica de Soledad, inscrito(as) en esta Cámara de Comercio, el 22 de Nov/bre de 1983 bajo el No. 17,922 del libro respectivo, la sociedad antes mencionada----- se transformo en sociedad anonima bajo la denominacion social de "PRODUCTORA DE CAPSULAS DE GELATINA S.A." PROCAPS S.A.".-----

C E R T I F I C A

Que por Escritura Pública No. 3,615 del 06 de Dic/bre de 1996, otorgada en la Notaria 2a. de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 20 de Dic/bre de 1996 bajo el No. 67,218 del libro respectivo, la sociedad antes mencionada-----

***** C O N T I N U A *****

RECIBIDA EN LA NOTARIA PRIMERA DE BARRANQUILLA

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

cambio de razon social, por la denominacion LABORATORIOS PROCAPS S.A

C E R T I F I C A

Que por Escritura Pública No. 3,775 del 18 de Dic/bre de 1996, otorgada en la Notaria 2a. de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 23 de Enero de 1997 bajo el No. 67,704 del libro respectivo, la sociedad antes mencionada-----
cambio de razon social, por la denominacion PROCAPS S.A. -----

C E R T I F I C A

Que dicha sociedad ha sido reformada por las siguientes escrituras y/o documentos privados:

Numero	aaaa/mm/dd	Notaria	No. Insc o Reg	aaaa/mm/dd
39	1979/01/18	Notaria 3a. de Barranquilla.	9,452	1979/01/24
992	1981/04/30	Notaria 1a. de Barranquilla.	13,112	1981/05/06
1,464	1982/08/27	Notaria Unica de Soledad	15,533	1982/09/08
2,744	1983/08/18	Notaria Unica de Soledad	17,416	1983/08/26
3,826	1984/07/16	Notaria Unica de Soledad	19,554	1984/07/27
3,835	1985/08/26	Notaria Unica de Soledad	22,230	1985/09/04
2,260	1986/09/10	Notaria Unica de Soledad.	24,995	1986/09/15
1,849	1989/07/11	Notaria 2a. de Barranquilla.	33,909	1989/07/13
444	1990/02/21	Notaria 2a. de Barranquilla.	36,101	1990/03/16
3,090	1991/12/17	Notaria 2a. de Barranquilla	43,993	1992/01/28
269	1993/02/01	Notaria 2a. de Barranquilla	48,428	1993/02/16
2,342	1993/07/28	Notaria 2a. de Barranquilla	50,582	1993/08/10
3,652	1993/11/09	Notaria 2a. de Barranquilla	51,798	1993/11/22
3,615	1996/12/06	Notaria 2a. de Barranquilla	67,218	1996/12/20
89	1997/01/16	Notaria 2a. de Barranquilla	67,704	1997/01/23
2,070	1997/07/17	Notaria 2a. de Barranquilla	70,627	1997/07/31
2,281	1997/08/04	Notaria 2a. de Barranquilla	70,813	1997/08/14
765	1999/04/22	Notaria 3a. de Barranquilla	80,628	1999/04/23
459	2001/02/21	Notaria 2a. de Barranquilla	91,502	2001/02/23
2,608	2001/10/05	Notaria 2a. de Barranquilla	95,771	2001/11/08
1,730	2004/07/29	Notaria 2. de Barranquilla	112,999	2004/08/26
416	2005/03/03	Notaria 2. de Barranquilla	116,364	2005/03/10
1,613	2005/07/28	Notaria 2. de Barranquilla	119,322	2005/08/18

C E R T I F I C A

Que de acuerdo con la(s) escritura(s) arriba citada(s), la sociedad se rige por las siguientes disposiciones:

DENOMINACION O RAZON SOCIAL:

PROCAPS S.A.-----

SIGLA: PROCAPS.

DOMICILIO PRINCIPAL: Barranquilla.

NIT No: 890.106.527-5.

MATRICULA MERCANTIL: 24,802.

C E R T I F I C A

***** C O N T I N U A *****



***** Pag. 2
C A M A R A D E C O M E R C I O
D E B A R R A N Q U I L L A
10*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

Direccion para notificaciones judiciales:

CL 80 No 78 B - 201.

De la ciudad de Barranquilla.

C E R T I F I C A

DURACION: El término de duración de la sociedad se fijó hasta el 18 de Agosto de 2073.

C E R T I F I C A

OBJETO SOCIAL: El objeto principal de la sociedad sera: a)-La fabricacion de capsulas y otros productos similares a partir de materiales tales como gelatinas u otros de distinta naturaleza, que puedan ser utilizados como envases o recipientes de productos y sustancias quimicas, farmaceuticas y cosmeticas, para uso y consumo humano y veterinario; b)-La investigacion, desarrollo y fabricacion de toda clase de productos y sustancias quimicas, farmaceuticas y cosmeticas para uso y consumo humano, animal y vegetal; c)-La compra y venta de materias primas, insumos y productos intermedios necesarios para los procesos de fabricacion de sustancias quimicas, farmaceuticas, alimenticias y cosmeticas, asi como la comercializacion interna y externa de los productos terminados, d) La compra, venta, importacion, exportacion y distribucion de toda clase de elementos instrumentales y equipos medicos, quirurgicos, odontologicos, y hospitalarios para la salud humana y animal. e) La compra, venta, exportacion y distribucion de dulces, confites, galletas y todo tipo de golosinas o alimentos para el consumo humano. f) Actuar como representante o agente de personas naturales y juridicas, nacionales y extranjeras en la realizacion de operaciones o transacciones relacionadas con la industria farmaceutica y en especial con cualquiera de las actividades descritas en los literales a), b), c). d) y e) antes descritos; g) Prestar los servicios de asesoria y consultoria en las areas administrativas, de mercadeo, analisis clinico, asistencia tecnica, investigacion, costos y produccion, exportaciones, importaciones, ventas, relacionadas con iguales o similares actividades a las que constituyen el objeto de PROCAPS S.A., asi como los de publicidad y promocion de productos quimicos, cosmeticos y farmaceuticos para uso humano, animal o industrial; h)-Adquirir, operar, administrar y explotar, a cualquier titulo, empresa o establecimientos dedicados a la investigacion, desarrollo, fabricacion o comercializacion de productos quimicos, farmaceuticos, hospitalarios o cosmeticos. En desarrollo de su objeto principal, la sociedad podra tomar interes social como accionista, socio o fundador de empresas que tengan igual o similar objeto, com-

***** C O N T I N U A *****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.

NIT: 890.106.527-5.

prar, vender, importar y exportar maquinarias, insumos y repuestos requeridos para el desarrollo de sus actividades; tomar dinero en prestamo, financiar y garantizar las operaciones comerciales que celebre con terceros relativas a su objeto; celebrar y ejecutar todo tipo de actos y contratos necesarios para desarrollar sus operaciones; girar, aceptar, otorgar y protestar todo tipo de titulos valores e instrumentos negociables; comprar, vender y constituir gravámenes sobre bienes muebles e inmuebles; designar representantes o apoderados en el pais y en el exterior y, en general, realizar cualquier acto o negocio que sea necesario o conveniente para el desarrollo de su objeto social y para la mejor proteccion de sus intereses corporativos o la de sus socios. La sociedad podra garantizar el cumplimiento de obligaciones adquiridas por terceros o por sus socios, para lo cual podra inclusive constituir gravámenes reales sobre sus bienes de cualquier naturaleza.

C E R T I F I C A

CAPITAL	Nro Acciones	Valor Acción
Autorizado		
\$*****2,000,000,000	*****2,000,000	*****1,000
Suscrito		
\$*****1,224,500,000	*****1,224,500	*****1,000
Pagado		
\$*****1,224,500,000	*****1,224,500	*****1,000

C E R T I F I C A

ADMINISTRACION: La direccion y administracion de la sociedad correspondera a la Asamblea General de Accionistas, a la Junta Directiva, al Presidente, al Vicepresidente y a un Gerente General. Corresponde a la Junta Directiva las siguientes funciones y atribuciones entre otras: Autorizar cualquier acto o contrato cuya cuantia sea superior al equivalente en pesos Colombianos a doscientos cincuenta mil dolares de los Estados Unidos de America (US\$250.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la transacion. Cuando se trate de operaciones en virtud de las cuales la sociedad garantice obligaciones de terceros o de los socios, se requerira que la operacion sea aprobada con el voto de por lo menos 2/3 partes de los integrantes de la Junta Directiva. La sociedad tendra un Presidente, quien ejercera la representacion legal de la sociedad y tendra las siguientes atribuciones entre otras: Ejecutar las decisiones de la Asamblea General de Accionistas y de la Junta Directiva. Ejercer la Representacion Legal de la sociedad en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Constituir apoderados judiciales y extrajudiciales, recibir notificaciones, absolver interrogatorios de parte, confesar obligaciones de la sociedad, transigir, disistir y allanar a la sociedad en cualquier asunto judicial. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos colombianos a doscientos cincuenta mil dolares de los

***** C O N T I N U A *****



CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----
NIT: 890.106.527-5.

Estados Unidos de America (US\$250.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la transacion o celebracion del acto. Las demas funciones que le correspondan como Representante Legal por disposicion de la ley, de estos estatutos o de la Junta Directiva. El Presidente tendra un suplente, designado por la Junta Directiva, quien reemplazara al Presidente en sus faltas temporales, absolutas o meramente accidentales, con entidades facultades y sin que sea necesario para ello acreditar la ausencia del principal. La sociedad tendra un Vicepresidente designado por la Junta Directiva, quien tambien ejercera la representacion legal de la sociedad, pudiendo ejercerla conjunta o separadamente con el Presidente. El vicepresidente tendra las siguientes facultades entre otras. Ejecutar dentro de su competencia las decisiones de la Asamblea General de Accionistas, de la Junta Directiva y del Presidente. Ejercer la representacion legal de la sociedad en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Constituir apoderados judiciales y extrajudiciales, recibir notificaciones, absolver interrogatorios de parte, confesar obligaciones de la sociedad, transigir, desistir y allanar a la sociedad en cualquier asunto judicial. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos colombianos a ciento cincuenta mil dolares de los Estados Unidos de America (US\$150.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion o celebracion del acto, siempre que no se trate de enajenar bienes inmuebles o cualquier otro activo fijo de la sociedad, inclusive de naturaleza muebles, acciones, cuotas o partes de interes inversiones y todo tipo de derechos de la sociedad, o de constituir gravámenes sobre cualquiera de los anteriores, facultades que radican exclusiva y privativamente en cabeza del Presidente. Las demas funciones que le corresponda como Representante legal por disposicion de la ley, de estos estatutos o de la Junta Directiva. El Vicepresidente tendra (1) suplente, designado por la Junta Directiva, quien lo reemplazara conjunta o separadamente en sus faltas temporales, absolutas o meramente accidentales, con identicas facultades y sin que sea necesario para ello acreditar la ausencia del principal. La sociedad tendra un (1) Gerente General, con su respectivo Suplente, designados por la Junta Directiva. El Suplente reemplazara al Gerente General, en sus faltas temporales, absolutas o meramente accidentales, quien junto con el gerente general, llevaran la representacion legal de la sociedad, con identicas facultades, pudiendolas ejercer conjunta o separadamente con el Presidente y Vicepresidente, con identicas facultades que el Gerente General y sin que sea necesario para ello acreditar

***** C O N T I N U A *****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

la ausencia del titular. El Gerente General tendra las siguientes facultades entre otras: Ejercer la representacion legal de la sociedad, en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos Colombianos a Cincuenta Mil dolares de los Estados Unidos de America (US\$50.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion o celebracion del acto, siempre que no se trate de enajenar a cualquier titulo bienes inmuebles o cualquier otro activo fijo de la sociedad, inclusive de naturaleza mueble, acciones, cuotas o partes de interes, inversiones y todo tipo de derechos de la sociedad o de constituir gravámenes sobre cualquiera de las anteriores facultades que radican exclusiva y privativamente en cabeza del Presidente.

C E R T I F I C A

Que según Acta No. 116 del 27 de Junio de 1997 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad:

PROCAPS S.A.-----

cuya parte pertinente se inscribió en esta Cámara de Comercio, el 15 de Agosto de 1997 bajo el No. 70,838 del libro respectivo, y según Acta No. 127 del 22 de Marzo de 2000 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad:

PROCAPS S.A.-----

cuya parte pertinente se inscribió en esta Cámara de Comercio, el 29 de Mayo de 2000 bajo el No. 87,250 del libro respectivo, fueron hechos los siguientes nombramientos:

CLASE:

JUNTA DIRECTIVA

Principales

1. Minski Gontovnik Ruben
2. Minski Gontovnik Meyer
3. Minski Gontovnik Jose

CC.*****7,463,982
CC.*****7,461,324
CC.*****8,671,834

Suplentes

1. Minski Zilberblum Elliot
2. Minski Deitel Daniel
3. Minski Sharon

CC.*****72,219,541

PA.*990,212,082,805

C E R T I F I C A

Que según Acta No. 116 del 27 de Junio de 1997 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 15 de Agosto de 1997 bajo el No. 70,838 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre
Presidente.

Minski Gontovnik Ruben

Identificación

CC.*****7,463,982

***** C O N T I N U A *****



***** Pag. 4
CAMARA DE COMERCIO
DE BARRANQUILLA
10*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

Suplente del Presidente

Minski Minski Abraham

CC.*****802,575

Vicepresidente

Minski Gontovnik Meyer

CC.*****7,461,324

Suplente del vicepresidente

Minski Gontovnik Jose

CC.*****8,671,834

C E R T I F I C A

Que según Acta No. 11 del 01 de Febrero de 2001 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 12 de Febrero de 2001 bajo el No. 91,273 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre

Identificación

Gerente General.

Uribe Ochoa Guillermo Luis

CC.*****70,073,754

C E R T I F I C A

Que según Acta No. 406 del 02 de Sep/bre de 2004 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 09 de Sep/bre de 2004 bajo el No. 113,263 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre

Identificación

Suplente del Gerente.

Tanaka Walter

CC.*****16,251,923

C E R T I F I C A

Que según Acta No. 124 del 29 de Marzo de 1999 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 04 de Agosto de 1999 bajo el No. 82,226 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre

Identificación

Revisor Fiscal Ppal.

Vargas Pertuz Ana Isabel

CC.*****32,696,645

C E R T I F I C A

Que según Acta No. 127 del 22 de Marzo de 2000 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio,

***** C O N T I N U A *****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

el 29 de Mayo de 2000 bajo el No. 87,250 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Suplente del Revisor Fiscal.	
Lozada Villareal Milena	CC.*****32,748,111

C E R T I F I C A

Que por escritura publica No. 2.608 del 5 de Octubre del 2.001, otorgada en la Notaria 2a. de Barranquilla, inscrita en esta Camara de Comercio el 8 de Noviembre del 2.001, bajo los Nos. 95.771, 2.056, 2.057, 2.058, 2.059, 2.060, 2.061, 2.062 y 2.064 del libro respectivo, se reforma totalmente el articulo 65, contenido en la escritura publica No. 3.705 de Noviembre 6 de 1.998, de la Notaria Segunda del Circuito de Barranquilla, y se constituyen los siguientes Apoderados Generales, quienes tendran las funciones que a continuacion se indican en razon de su cargo en la sociedad: 1.-Se confiere Poder Especial, Amplio y Suficiente a los senores: WALTER TANAKA TATEKAWA, C.C. No. 16.251.923; WILLIAM ALBERTO BARROS CERRA, C.C. No. 8.715.941, para que cada uno en forma independiente en nombre y representacion de PROCAPS S.A., suscriba todas las declaraciones tributarias, aduaneras y cambiarias a que este obligada la sociedad, asi como para que responda todo tipo de requerimiento o solicitudes de informacion de las autoridades tributarias, aduaneras o cambiarias, en los ordenes Distrital, Departamental y Nacional. 2.-Se confiere Poder Especial, amplio y suficiente, a los siguientes senores que conforman el Grupo A y el Grupo B para que conjuntamente suscriban contratos de suministros de mercancías y servicios con entidades oficiales, hasta por la suma de US\$800.000.00 (OCHOCIENTOS MIL DOLARES DE LOS ESTADOS UNIDOS DE AMERICA), liquidados a la tasa representativa del mercado vigente en el dia que se realice la respectiva transaccion. GUPO A: CLAUDIA TANGARIFE CASTILLO C.C. No. 41.660.293. BRUPO B: WALTER TANAKA TATEKAWA C.C. No. 16.251.923, CARMEN ALICIA FLOREZ C.C. No. 22.434.044, CARMEN ALICIA HERRERA DIAZGRANADOS C.C. No. 22.377.540. Para el ejercicio de estas facultades se requerira por lo menos la firma de uno (1) de los apoderados especiales del Grupo A y uno de los apoderados especiales del Grupo B; 3.-Se otorga Poder General, Amplio y Suficiente a la Doctora JOSEFINA MIELES BUELVAS C.C. No. 33.138.497, a la Doctora MARCELA CARVAJALINO PAGANO C.C. No. 22.579.748, y al Doctor DANIEL MORENO VILLALBA, C.C. No. 19.113.210; para que cada uno representen a PROCAPS S.A., ante todo tipo de autoridades administrativas y judiciales, en asuntos de naturaleza cambiaria y fiscal, tales como: Contestar e interponer demandas y requerimientos, comparecer en juicios, pedir la practica de pruebas, recibir notificaciones, interponer recursos y ejercer acciones contencioso administrativas y de tutela, transigir, conciliar, recibir en nombre de la sociedad desistir, sustituir y reasumir este mandato. Para la celebracion de cualquier acuerdo conciliatorio o transaccional, judicial o extrajudicial, cualquiera que sea su cuantia, los Apoderados deberan aportar la autorizacion escrita del Representante Legal de la sociedad. Los Apoderados podran presentar solicitudes de devolucion y/o compensacion de impuestos y recibir los pagos por devoluciones de

***** C O N T I N U A *****



CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----
NIT: 890.106.527-5.

impuestos, firmar, notificarse y realizar todo acto necesario para cumplir con el mandato encomendado.-----

C E R T I F I C A

Que segun documento privado de fecha 7 de junio de 2000, inscrito en esta Camara de Comercio el 8 de junio de 2000, bajo el No.1.786 del libro respectivo, consta que RUBEN MINSKI GONTOVNIK, C.C. No. 7.463.982 de Barranquilla, actuando como Presidente y Representante Legal de la sociedad PROCAPS S.A, confiere poder especial, amplio y suficiente a KAREN FIGUEROA GARCIA, C.C.No.32.732.070 de Barranquilla, para que en su nombre y representacion de la sociedad suscriba todas declaraciones cambiarias a que este obligada PROCAPS S.A, en el giro normal de sus negocios y operaciones sociales.--

C E R T I F I C A

Que segun Documento Privado del 13 de diciembre del 2.002, inscrito en esta Camara de Comercio el 15 de enero del 2.003 bajo el No.2.290 del libro respectivo, el señor RUBEN MINSKI GONTOVNIK, C.C.No.7.463.982, en calidad de REPRESENTANTE LEGAL, y en condicion de PRESIDENTE de PROCAPS S.A., mediante el presente documento otorgo PODER ESPECIAL al Sr. MARLON TATIS PAREDES, C.C.No.72.176.874, para que en representacion de PROCAPS S.A., suscriba declaraciones, reportes y cualquier tipo de documentos relacionados con tramites o registros de Comercio Exterior ante el Ministerio de Comercio Exterior y/o cualquier entidad del Estado que haga sus veces. En consecuencia, el Sr. MARLON TATIS PAREDES, podra suscribir en representacion de PROCAPS S.A. declaraciones, reportes y cualquier tipo de documentos relacionados con tramites o registros de Comercio Exterior ante el Ministerio de Comercio Exterior y/o cualquier entidad del Estado que haga sus veces.-----

C E R T I F I C A

Que segun documento privado del 10 de noviembre del 2004, inscrito en esta Camara de Comercio el 1 de diciembre del 2.004, bajo el No. 2.753 del libro respectivo consta que RUBEN MINSNSKI GONTOVNIK, C.C. 7.463.982 de Barranquilla, actuando como Presidente y Representante Legal de la sociedad PROCAPS S.A, confiere poder especial, amplio y suficiente a ANTONIO VARELA DIAZ, C.C.No.72.004.669 de Barranquilla, para que en nombre y representacion de la sociedad firme las declaraciones cambiarias a que este obligada PROCAPS S.A., a suscribir en el giro normal de sus negocios y operaciones sociales. Por lo anterior, el señor ANTONIO VARELA DIAZ, queda facultado

***** C O N T I N U A *****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.

NIT: 890.106.527-5.

para firmar las declaraciones comabiarías y demás documentos relacionados con las declaraciones, notificaciones, aportar y solicitar las pruebas que sean necesarias, desistir en general a realizar todo acto necesario de tal forma que pueda cumplir con el mandato dado.-

C E R T I F I C A

Que según documento privado del 22 de Febrero de 2005, inscrito en esta Cámara de Comercio el 28 de Marzo de 2.005, bajo el No. 2.820 del libro respectivo, consta que WALTER TANAKA TATEKAWA C.C. 16.251-923 de Palmira, quien actúa como representante legal de la sociedad PROCAPS S.A., otorga Poder Especial, Amplio y Suficiente al señor MARLON TATIS PAREDES C.C. No. 72.176.874, para que en representación de PROCAPS S.A., suscriba declaraciones aduaneras, reportes y cualquier tipo de documentos relacionados con los trámites o registros de comercio exterior ante la dirección de impuestos y aduanas nacionales y/o cualquier entidad que haga sus veces, y para que responda todo tipo de requerimientos y/o solicitudes de información de las autoridades aduaneras en los ordenes distrital, departamental y nacional.

C E R T I F I C A

Que por Escritura Pública No. 2,011 del 10 de Octubre de 2006, otorgada en la Notaría 2 a. de Barranquilla cuya parte pertinente se inscribió en esta Cámara de Comercio, el 15 de Dic/bre de 2006 bajo el No. 3,183 del libro respectivo,-----
Consta que RUBEN MINSKI GONTOVNIK CC.No.7.463.928, quien manifiesto que en su condición de Presidente y Representante Legal de la ----- sociedad PROCAPS S.A., confiere poder especial amplio y suficiente a los siguientes señores que conforman el Grupo A y el Grupo B, para que conjuntamente suscriban contratos de suministro de mercancías y servicios con entidades oficiales, hasta por la suma de ochocientos mil dólares de los Estados Unidos de America (US\$800.000.00), ---- liquidados a la tasa representativa del mercado vigente en el día que se realice la respectiva transacción. Grupo A: IVAN VILLAREAL CAMACHO CC.No.91.201.749. FERNANDO RINCON RIVERA CC.No.79.484.187. CLAUDIA TANGARIFE CASTILLO CC.No.41.660.293. Grupo B. WALTER TANAKA TATEKAWA CC.No.16.251.923. CARMEN ALICIA FLOREZ DE LA ROTA ----- CC.No.22.434.044. RICARDO DORADO HURTADO CC.No.79.715.772.-----

C E R T I F I C A

Que en esta Cámara de Comercio no aparecen inscripciones posteriores de documentos referentes a reforma, disolución, liquidación o nombramientos de representantes legales de la expresada sociedad.

C E R T I F I C A

Que su última Renovación fue el: 20 de Marzo de 2007.

La información sobre embargos de establecimiento se suministra en Certificados de Matrícula, la de contratos sujetos a registro, en

***** C O N T I N U A *****

03-12007678 024802 PROCAPS S.A.
Barranquilla, 17 de Julio de 2007

Hr:09:52:49

No. 11263221



***** Pag. 6
C A M A R A D E C O M E R C I O
D E B A R R A N Q U I L L A
10*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE
DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

Certificados Especiales.

m. Rayson

2020
Bogotá, D.C.,

Doctor:
Emilio Ferrero
Apoderado

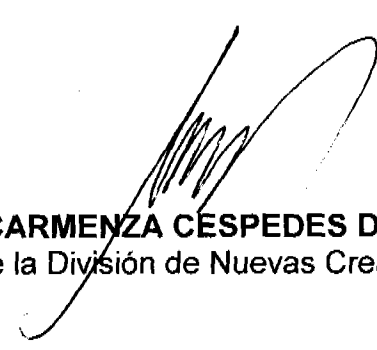
Oficio N° 011334

ASUNTO:	Radicación PCT N°	05-70211
	Trámite	011
	Evento	379
	Actuación	440
	Folios	010

Con fundamento en lo previsto por el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica que la oposición presentada por la doctora Laura Catalina Silva Barrera, en su calidad de apoderada de Procaps S.A., reúne los requisitos formales exigidos por la ley al momento de su presentación.

En cumplimiento de lo dispuesto en la citada norma, se le concede el término de sesenta (60) días hábiles siguientes a la fecha de notificación del presente oficio, para que haga valer sus argumentaciones y presente pruebas.

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 oficio fue notificado por fijación en lista de fecha, 14 SEP 2007.

202021