Industria y Comercio

SUPERINTENDENCIA

Delegatura de propiedad Industrial

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SOLICITUD

PATENTE DE INVENCIÓN

21. **EXPEDIENTE No.** 05-113041

54. **TÍTULO** Beta-carbolinas útiles para tratar enfermedades inflamatorias.

51. **CLASIFICACIÓN INTERNACIONAL** C 07D 471/04

71. **SOLICITANTE** Millenium Pharmaceuticals, Inc.

DOMICILIO Cambridge, Massachussets, Estados Unidos

74. **APODERADO** Luis Patiño Lopez

22. **BOGOTÁ, D. C.,** 4 de noviembre de 2005

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Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL 09 - Nov - 05

① **SOLICITUD DE :**

☒ Patente de Invención. ☐ Patente de Modelo de Utilidad.

☐ Capitulo I. ☒ Capitulo II.

② **SOLICITANTE (71)**	Nombre: **MILLENIUM PHARMACEUTICALS, INC.** Dirección: 40 Landsdowne Street, Cambridge, MA 02139 Estados Unidos Nacionalidad o Domicilio: Cambridge, Massachussets Estados Unidos Lugar de Constitución: Estados Unidos Teléfono: Fax: E-mail:	**IDENTIFICACION** C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número _____
③ **REPRESENTANTE O APODERADO**	Nombre: LUIS PATIÑO LEYVA Dirección Cra. 11ª No. 94-76 – Of. 308 Teléfono: 6161948 Fax: 6163976 E-mail:	**IDENTIFICACION** C.C. ☒ NIT ☐ C.E. ☐ Otro ☒ Cual _____ Número 2.930.616 TP 2698
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⑤ **PCT (86)**

Solicitud Internacional No. PCT/US2004/011080 Fecha: Abril 9 de 2004

Publicación Internacional No. WO 2004/092167 A1 Fecha Octubre 28 de 2004

⑥ **Titulo (54)** BETA-CARBOLINAS ÚTILES PARA TRATAR ENFERMEDADES INFLAMATORIAS.

⑦ **Clasificación Internacional (51)** C07D 471/04 ; A61K31/437

⑧ **PRIORIDAD:**	(33) País de Origen	(32) Fecha	(31) Numero de Solicitud
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⑨ **Comprobante de pago No. 05 – 83,688** **Fecha Noviembre 1 de 2005**

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

ANEXOS

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

(11) FIGURA CARACTERISTICA

(12) Solicito la concesión de la patente,

NOMBRE: LUIS PATIÑO LEYVA

FIRMA:

C.C. 2.930.616 Bogotá T.P. 2698

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

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Otros anexos que se acompañan a la solicitud.

Copia formato PCT/RO/101 correspondiente a la presentación de la solicitud internacional.

Copia formulario PCT/IB/304 correspondiente a la notificación relativa al envío o transmisión del documento de prioridad.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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GUSTAVO HOLLMAN	CONSIGNACION	BANCO POPULAR	050-00110-6	43140705	01/11/2005	1,329,000.00

C O N C E P T O

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	7 TRAMITES DE SOL. DE DISENOS INDUS	443,000.00
		TOTAL : $	443,000.00

SON: CUATROCIENTOS CUARENTA Y TRES MIL PESOS

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60/461,468 9 April 2003 (09.04.2003) US

(71) Applicant (*for all designated States except US*): MILLENIUM PHARMACEUTICALS, INC. [US/US]; 40 Landsdowne Street, Cambridge, MA 02139 (US).

(72) Inventors; and

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(74) Agents: SILVERMAN, Ian, Robert et al.; Millennium Pharmaceuticals, Inc., 40 Landsdowne Street, Cambridge, MA 02139 (US).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, 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, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

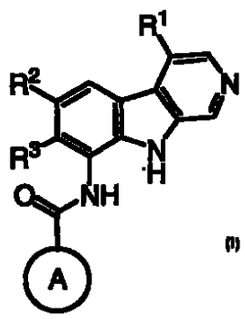
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WO 2004/092167 A1

(54) Title: BETA-CARBOLINES USEFUL FOR TREATING INFLAMMATORY DISEASE



(I)

(57) Abstract: This invention provides beta-carboline compounds of formula (I) wherein Ring A is a substituted pyridinyl, pyrimidinyl, morpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, pyranyl, tetrahydrofuranyl, cyclohexyl, cyclopentyl or thiomorpholinyl ring and R^1, R^2 and R^3 are as described in the specification. The compounds are IKK-2 inhibitors that are useful for treating IKK-2 mediated diseases such as inflammatory diseases and cancer.

BETA-CARBOLINES USEFUL FOR TREATING INFLAMMATORY DISEASE

Related Application

[0001] This application claims the benefit of US Provisional Patent Application 60/461468 filed April 9, 2003.

Field of the Invention

[0002] This invention relates to beta-carboline compounds, pharmaceutical compositions thereof, and methods of using the compositions for treating disease. The compounds are particularly useful for treating inflammatory disease and cancer.

Background of the Invention

[0003] The transcription (nuclear) factor NF-κB is a member of the Rel protein family, and is typically a heterodimer composed of p50 and p65 subunits. NF-κB is constitutively present in the cytosol, and is inactivated by its association with one of the IκB family of inhibitors. Palombella et al., WO 95/25533, teaches that the ubiquitin-proteasome pathway plays an essential role in the regulation of NF-κB activity, being responsible for the processing of p105 to p50 and the degradation of the inhibitor protein IκB-α. Chen et al., Cell 84:853 (1996), teaches that prior to degradation, IκB-α undergoes selectiv phosphorylation at serine residues 32 and 36 by the multisubunit IκB kinase complex (IKK). IκB-α is phosphorylated by IKK, which has two catalytic subunits, IKK-1 (IκB kinase α or IKK-α) and IKK-2 (IκB kinase β or

IKK-β). Once phosphorylated, IκB is targeted for ubiquitination and degradation by the 26S proteasome, allowing translocation of NF-κB into the nucleus, where it binds to specific DNA sequences in the promoters of target genes and stimulates their transcription. Inhibitors of IKK can block the phosphorylation of IKB and its further downstream effects, particularly those associated with NF-κB transcription factors.

[0004] The protein products of genes under the regulatory control of NF-κB include cytokines, chemokines, cell adhesion molecules, and proteins mediating cellular growth and control. Importantly, many of these proinflammatory proteins also are able to act, either in an autocrine or paracrine fashion, to further stimulate NF-κB activation. In addition, NF-κB plays a role in the growth of normal and malignant cells. Furthermore, NF-κB is a heterodimeric transcription factor which can activate a large number of genes which code, inter alia, for proinflammatory cytokines such as IL-1, IL-2, TNFα or IL-6. NF-κB is present in the cytosol of cells, building a complex with its naturally occurring inhibitor IκB. The stimulation of cells, for example by cytokines, leads to the phosphorylation and subsequent proteolytic degradation of IκB. This proteolytic degradation leads to the activation of NF-κB, which subsequently migrates into the nucleus of the cell and activates a large number of proinflammatory genes.

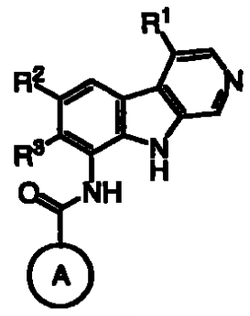
[0005] Rinehart et al., U.S. Pat. No. 4,631,149 (1986), discloses beta-carboline compounds useful as antiviral, antibacterial, and antitumor agents.

[0006] Ritzeler et al., WO 01/68648, discloses beta-carboline compounds with IKB kinase inhibitory activity for use in the treatment of inflammatory disorders (e.g., rheumatoid arthritis), osteoarthritis, asthma, cardiac infarct, Alzheimer's disease, carcinomatous disorders (potentiation of cytotoxic therapies) and atherosclerosis.

[0007] It would be beneficial to provide novel IKK inhibitors that possess good therapeutic properties, especially for the treatment of inflammatory disease.

Description of the Invention

[0008] This invention provides compounds that are useful for treating IKK-2-mediated diseases, such as inflammatory diseases and cancer. The compounds are represented by formula I:



I

wherein Ring A is selected from the group consisting of:

(a) a pyridinyl or pyrimidinyl ring that is substituted by (i) $-CH_2C(O)-G$ and 0-1 R^{6a} or (ii) 1-2 R^{6a}, and

(b) a morpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, pyranyl, tetrahydrofuranyl, cyclohexyl, cyclopentyl or thiomorpholinyl ring that is substituted by

(i) $-C(R^9)_3$, -W-G, or -G, (ii) 0-4 R^{6b} and (iii) 0-1 oxo groups on a ring carbon or 0-2 oxo groups on a ring sulfur;

each R^{6a} is independently selected from C_{1-6} aliphatic, halo, C_{1-6} alkoxy, or amino;

each R^{6b} is independently selected from C_{1-3} aliphatic or $-N(R^7)_2$, and two R^{6b} on the same or an adjacent carbon optionally are taken together with the intervening carbon(s) to form a 5-6 membered ring having 1-2 ring heteroatoms selected from N, O or S;

W is -Q-, -Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$, or $-C(R^9)_2-[C(R^9)(R^{12})]_2-$;

Q is $-C(R^9)_2-$ or $-C(R^9)_2C(R^9)_2-$;

G is -OH, $-NR^4R^5$, $-N(R^9)CONR^4R^5$, $-N(R^9)SO_2(C_{1-3}$ aliphatic), $-N(R^9)COCF_3$, $-N(R^9)CO(C_{1-6}$ aliphatic), $-N(R^9)CO$(heterocyclyl), $-N(R^9)CO$(heteroaryl), $-N(R^9)CO$(aryl), a 3-7 membered heterocyclyl ring, or a 5-6 membered heteroaryl, wherein each of the heteroaryl, aryl and heterocyclyl moieties of G is optionally substituted by 1-3 R^{10};

R^1 is hydrogen, halo, C_{1-3} aliphatic, amino, cyano, $(C_{1-3}$ alkyl$)_{1-2}$ amino, C_{1-3} alkoxy, $-CONH_2$, $-NHCOCF_3$, or $-CH_2NH_2$;

R^2 is hydrogen, halo, C_{1-3} aliphatic, $-CF_3$;

R^3 is hydrogen, halo, C_{1-6} aliphatic, C_{1-6} haloalkyl, C_{1-6} alkoxy, hydroxy, amino, cyano, or $(C_{1-6}$ alkyl$)_{1-2}$ amino;

R^4 is hydrogen, 3-7 membered heterocyclyl, or C_{1-6} aliphatic;

R^5 is hydrogen, C_{1-6} aliphatic group or a 3-7 membered heterocyclic ring having 1-2 ring heteroatoms selected from N, O, or S, wherein R^5 is optionally substituted by halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

each R^9 is independently selected from hydrogen or C_{1-3} aliphatic;

each R^{10} is independently selected from oxo, $-R^{11}$, $-T-R^{11}$, or $-V-T-R^{11}$;

each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain;

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$; and

R^{12} is hydrogen or an amino acid side chain.

[0009] The term "aliphatic" as used herein means straight-chain, branched or cyclic C_1-C_{12} hydrocarbons which are completely saturated or which contain one or more units of unsaturation but which are not aromatic. For example, suitable aliphatic groups include substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl. The terms "alkyl", "alkoxy", "hydroxyalkyl", "alkoxyalkyl", and "alkoxycarbonyl", used alone or as part of a larger moiety, include both straight and branched chains containing one to twelve carbon atoms. The terms "alkenyl" and "alkynyl", used alone or as part of a larger moiety, include both straight and branched chains containing two to twelve carbon atoms. The term

"cycloalkyl, used alone or as part of a larger moiety, includes cyclic C_3-C_{12} hydrocarbons which are completely saturated or which contain one or more units of unsaturation, but which are not aromatic.

[0010] The terms "haloalkyl", "haloalkenyl" and "haloalkoxy", mean alkyl, alkenyl or alkoxy, as the case may be, substituted with one or more halogen atoms. The term "halogen" means F, Cl, Br, or I.

[0011] The term "heteroatom" means nitrogen, oxygen, or sulfur and includes any oxidized form of nitrogen and sulfur, and the quaternized form of any basic nitrogen. Also the term "nitrogen" includes a substitutable nitrogen f a heterocyclic ring. As an example, in a saturated or partially unsaturated ring having 0-3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4-dihydro-2-pyrrolyl), NH (as in pyrrolidinyl) or NR^+ (as in N-substituted pyrrolidinyl).

[0012] The terms "carbocycle", "carbocyclyl", "carbocyclo", or "carbocyclic" as used herein means an aliphatic ring system having three to fourteen members. The terms "carbocycle", "carbocyclyl", "carbocyclo", or "carbocyclic" whether saturated or partially unsaturated, also refers to rings that are optionally substituted. The terms "carbocycle", "carbocyclyl", "carbocyclo", or "carbocyclic" also include aliphatic rings that are fused to one or more aromatic or nonaromatic rings, such as in a decahydronaphthyl or tetrahydronaphthyl, where the radical or point of attachment is on the aliphatic ring.

[0013] The term "aryl" used alone or as part of a larger moiety as in "aralkyl", "aralkoxy", or "aryloxyalkyl", refers to aromatic ring groups having five t fourteen

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

[0014] The term "heterocycle", "heterocyclyl", or "heterocyclic" as used herein includes non-aromatic ring systems having five to fourteen members, preferably five to ten, in which one or more ring carbons, preferably one to four, are each replaced by a heteroatom such as N, O, or S. Examples of heterocyclic rings include 3-1H-benzimidazol-2-one, (1-substituted)-2-oxo-benzimidazol-3-yl, 2-tetrahydrofuranyl, 3-tetrahydrofuranyl, 2-tetrahydropyranyl, 3-tetrahydropyranyl, 4-tetrahydropyranyl, [1,3]-dioxalanyl, [1,3]-dithiolanyl, [1,3]-dioxanyl, 2-tetrahydrothiophenyl, 3-tetrahydrothiophenyl, 2-morpholinyl, 3- morpholinyl, 4-morpholinyl, 2-thiomorpholinyl, 3-thiomorpholinyl, 4-thiomorpholinyl, 1-pyrrolidinyl, 2-pyrrolidinyl, 3-pyrrolidinyl, 1-piperazinyl, 2-piperazinyl, 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-piperidinyl, 4-thiazolidinyl, diazolonyl, N-substituted diazolonyl, 1-phthalimidinyl, benzoxanyl, benzopyrrolidinyl, benzopiperidinyl, benzoxolanyl, benzothiolanyl, and

benzothianyl. Also included within the scope of the term "heterocyclyl" or "heterocyclic", as it is used herein, is a group in which a non-aromatic heteroatom-containing ring is fused to one or more aromatic or non-aromatic rings, such as in an indolinyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl, where the radical or point of attachment is on the non-aromatic heteroatom-containing ring. The term "heterocycle", "heterocyclyl", or "heterocyclic" whether saturated or partially unsaturated, also refers to rings that are optionally substituted.

[0015] The term "heteroaryl", used alone or as part of a larger moiety as in "heteroaralkyl" or "heteroarylalkoxy", refers to heteroaromatic ring groups having five to fourteen members. Examples of heteroaryl rings include 2-furanyl, 3-furanyl, 3-furazanyl, N-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-oxadiazolyl, 5-oxadiazolyl, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 1-pyrazolyl, 2-pyrazolyl, 3-pyrazolyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-pyrimidyl, 3-pyridazinyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 5-tetrazolyl, 2-triazolyl, 5-triazolyl, 2-thienyl, 3-thienyl, carbazolyl, benzimidazolyl, benzothienyl, benzofuranyl, indolyl, quinolinyl, benzotriazolyl, benzothiazolyl, benzooxazolyl, benzimidazolyl, isoquinolinyl, indazolyl, isoindolyl, acridinyl, or benzoisoxazolyl. Also included within the scope of the term "heteroaryl", as it is used herein, is a group in which a heteroatomic ring is fused to one or more aromatic or nonaromatic rings where the radical or point of attachment is on the heteroaromatic ring. Examples include tetrahydroquinolinyl, tetrahydroisoquinolinyl, and

pyrido[3,4-d]pyrimidinyl. The term "heteroaryl" also refers to rings that are optionally substituted. The term "heteroaryl" may be used interchangeably with the term "heteroaryl ring" or the term "heteroaromatic".

[0016] An aryl (including aralkyl, aralkoxy, aryloxyalkyl and the like) or heteroaryl (including heteroaralkyl and heteroarylalkoxy and the like) group may contain one or more substituents. Examples of suitable substituents on the unsaturated carbon atom of an aryl, heteroaryl, aralkyl, or heteroaralkyl group include a halogen, $-R^{\circ}$, $-OR^{\circ}$, $-SR^{\circ}$, 1,2-methylene-dioxy, 1,2-ethylenedioxy, protected OH (such as acyloxy), phenyl (Ph), substituted Ph, $-O(Ph)$, substituted $-O(Ph)$, $-CH_2(Ph)$, substituted $-CH_2(Ph)$, $-CH_2CH_2(Ph)$, substituted $-CH_2CH_2(Ph)$, $-NO_2$, $-CN$, $-N(R^{\circ})_2$, $-NR^{\circ}C(O)R^{\circ}$, $-NR^{\circ}C(O)N(R^{\circ})_2$, $-NR^{\circ}CO_2R^{\circ}$, $-NR^{\circ}NR^{\circ}C(O)R^{\circ}$, $-NR^{\circ}NR^{\circ}C(O)N(R^{\circ})_2$, $-NR^{\circ}NR^{\circ}CO_2R^{\circ}$, $-C(O)C(O)R^{\circ}$, $-C(O)CH_2C(O)R^{\circ}$, $-CO_2R^{\circ}$, $-C(O)R^{\circ}$, $-C(O)N(R^{\circ})_2$, $-OC(O)N(R^{\circ})_2$, $-S(O)_2R^{\circ}$, $-SO_2N(R^{\circ})_2$, $-S(O)R^{\circ}$, $-NR^{\circ}SO_2N(R^{\circ})_2$, $-NR^{\circ}SO_2R^{\circ}$, $-C(=S)N(R^{\circ})_2$, $-C(=NH)-N(R^{\circ})_2$, $-(CH_2)_yNHC(O)R^{\circ}$, $-(CH_2)_yNHC(O)CH(V-R^{\circ})(R^{\circ})$; wherein each R° is independently selected from hydrogen, a substituted or unsubstituted aliphatic group, an unsubstituted heteroaryl or heterocyclic ring, phenyl (Ph), substituted Ph, $-O(Ph)$, substituted $-O(Ph)$, $-CH_2(Ph)$, or substituted $-CH_2(Ph)$; y is 0-6; and V is a linker group. Examples of substituents on the aliphatic group or the phenyl ring of R° include amino, alkylamino, dialkylamino, aminocarbonyl, halogen, alkyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylaminocarbonyloxy, dialkylaminocarbonyloxy, alkoxy, nitro, cyano, carboxy, alkoxycarbonyl, alkylcarbonyl, hydroxy, haloalkoxy, or haloalkyl.

[0017] An aliphatic group or a non-aromatic heterocyclic ring may contain one or more substituents. Examples of suitable substituents on the saturated carbon 20 of an aliphatic group or of a non-aromatic heterocyclic ring include those listed above for the unsaturated carbon of an aryl or heteroaryl group and the following: =O, =S, =NNHR*, $=NN(R^*)_2$, =N-, =NNHC(O)R*, $=NNHCO_2$(alkyl), $=NNHSO_2$(alkyl), or =NR*, where each R* is independently selected from hydrogen, an unsubstituted aliphatic group or a substituted aliphatic group. Examples of substituents on the aliphatic group include amino, alkylamino, dialkylamino, aminocarbonyl, halogen, alkyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylaminocarbonyloxy, dialkylaminocarbonyloxy, alkoxy, nitro, cyano, carboxy, alkoxycarbonyl, alkylcarbonyl, hydroxy, haloalkoxy, or haloalkyl.

[0018] Suitable substituents on the nitrogen of a non-aromatic heterocyclic ring include $-R^+$, $-N(R^+)2$, $-C(O)R^+$, $-CO_2R^+$, $-C(O)C(O)R^+$, $-C(O)CH_2C(O)R^+$, $-SO_2R^+$, $-SO_2N(R^+)_2$, $-C(=S)N(R^+)_2$, $-C(=NH)-N(R^+)_2$, and $-NR^+SO_2R^+$; wherein each R^+ is independently selected from hydrogen, an aliphatic group, a substituted aliphatic group, phenyl (Ph), substituted Ph, -O(Ph), substituted -O(Ph), CH_2(Ph), substituted CH_2(Ph), or an unsubstituted heteroaryl or heterocyclic ring. Examples of substituents on the aliphatic group or the phenyl ring include amino, alkylamino, dialkylamino, aminocarbonyl, halogen, alkyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylaminocarbonyloxy, dialkylaminocarbonyloxy, alkoxy, nitro, cyano, carboxy, alkoxycarbonyl, alkylcarbonyl, hydroxy, haloalkoxy, or haloalkyl.

[0019] The term "linker group" or "linker" means an organic moiety that connects two parts of a compound. Linkers are typically comprised of an atom such as oxygen or sulfur, a unit such as -NH-, -CH_2-, -C(O)-, -C(O)NH-, r a chain of atoms, such as an alkylidene chain. The molecular mass of a linker is typically in the range of about 14 to 200, preferably in the range of 14 to 96 with a length of up to about six atoms. Examples of linkers include a saturated or unsaturated C_{1-6} alkylidene chain which is optionally substituted, and wherein one or two saturated carbons of the chain are optionally replaced by -C(O)-, -C(O)C(O)-, -CONH-, -CONHNH-, -CO_2-, -OC(O)-, -$NHCO_2$-, -O-, -NHCONH-, -OC(O)NH-, -NHNH-, -NHCO-, -S-, -SO-, -SO_2-, -NH-, -SO_2NH-, or -$NHSO_2$-.

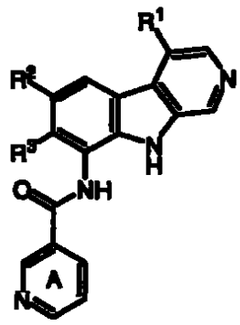
[0020] The term "alkylidene chain" or "alkylene chain" refers to an optionally substituted, straight or branched carbon chain that may be fully saturated or have one or more units of unsaturation. The optional substituents are as described above for an aliphatic group.

[0021] A combination of substituents or variables is permissible only if such a combination results in a stable or chemically feasible compound. A stable compound or chemically feasible compound is one in which the chemical structure is not substantially altered when kept at a temperature of 40 °C or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

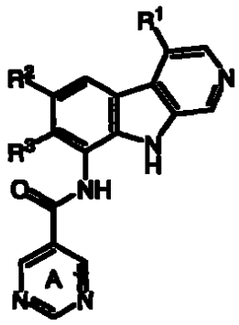
[0022] Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures

of the present compounds are within the scope of the invention. Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen atom by a deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention.

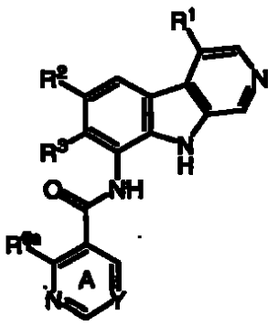
[0023] In one embodiment of the invention, Ring A is selected from a pyridinyl or a pyrimidinyl ring that is substituted by 1-2 R^{6a} groups. In this embodiment, preferred Ring A include a 3-pyridinyl or a 5-pyrimidinyl ring, shown below by the compounds of formula II-A and II-B, respectively.



II-A



II-B



II-C (Y = N or CH)

[0024] Preferably, R^{6a} is selected from halo or a C_{1-6} aliphatic, such as chloro or methyl. When R^{6a} is an aliphatic group such as methyl, a favorable position for the R^{6a} group is at the 2-position of the pyridinyl ring or the 4-position of the pyrimidinyl ring, as shown in II-C above. Particular Ring A moieties are 2-methyl-3-pyridinyl and 4-methyl-5-pyrimidinyl. It has been found that

compounds of formula II-C where R^{6a} is a methyl group are surprisingly more potent in biological testing for IKK inhibition than analogous compounds that have an unsubstituted Ring A pyridine, such as those described in the aforementioned Ritzeler et al. PCT application WO 01/68648.

[0025] Preferred R^1 groups are small groups such as hydrogen, methyl, amino and fluoro.

[0026] Preferred R^2 groups include hydrogen and halo. Chloro is a preferred R^2 halo group.

[0027] Preferred R^3 groups include hydrogen, halo (especially chloro) and alkoxy. Examples of suitable alkoxy groups include C_{1-4} alkoxy groups such as methoxy, ethoxy, propoxy and cyclopropylmethoxy.

[0028] In another embodiment, Ring A is selected from a 5-6 membered non-aromatic ring having 0-2 ring heteroatoms selected from nitrogen, oxygen and sulfur. These are designated generally as compounds of formula III. Examples of non-aromatic Ring A groups include a morpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, pyranyl, tetrahydrofuranyl, cyclohexyl, cyclopentyl and a thiomorpholinyl ring. Preferably, such non-aromatic rings are substituted by (i) $-C(R^9)_3$ or -W-G, (ii) 0-4 R^{6b}.and (iii) 0-1 oxo groups on a ring carbon or 0-2 oxo groups on a ring sulfur. More preferably, such non-aromatic rings are substituted by (i) -W-G, (ii) 0-2 R^{6b}.and (iii) 0-1 oxo groups on a ring carbon or 0-2 oxo groups on a ring sulfur.

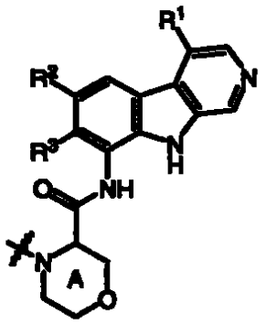
[0029] A preferred G is $-NR^4R^5$ or a 3-7 membered heterocyclyl ring. More preferably G is $-NR^4R^5$ or a 5-6 membered heterocyclyl ring, where G is substituted by 1-2 R^{10}.

[0030] A preferred R^4 is a hydrogen, 5-6 membered heterocyclyl ring, or C_{1-6} aliphatic, more preferably hydrogen or C_{1-6} aliphatic. R^4 may also be a C_{1-6} alkoxy.

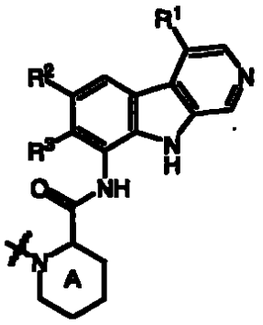
[0031] A preferred R^5 is a hydrogen, 5-6 membered heterocyclyl ring, or C_{1-6} aliphatic, more preferably hydrogen or C_{1-6} aliphatic.

[0032] Various formula III compounds where Ring A is a non-aromatic ring are shown in Table 2. For ease of viewing, substituents on these non-aromatic Ring A compounds, except for the oxo group in some cases, are not shown.

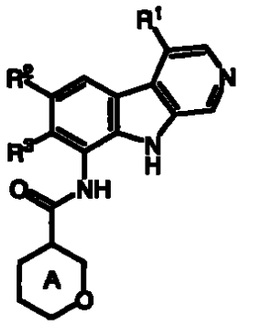
Table 2. Compounds where Ring A is Non-Aromatic



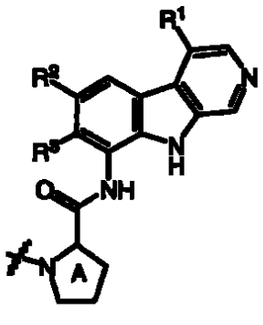
III-A



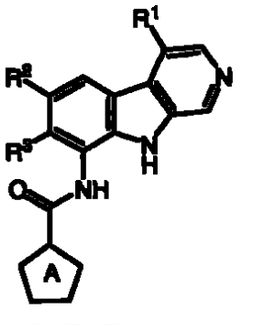
III-B



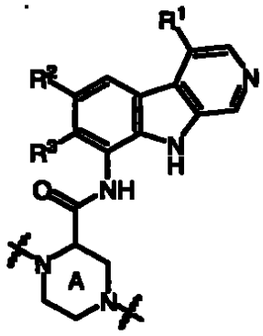
III-C



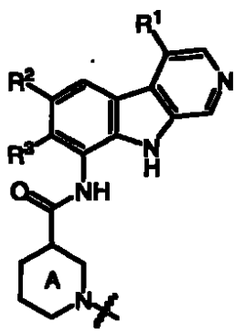
III-D



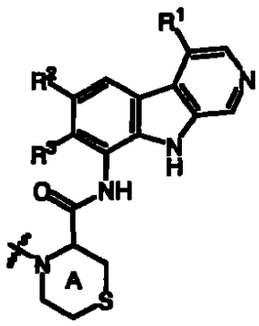
III-E



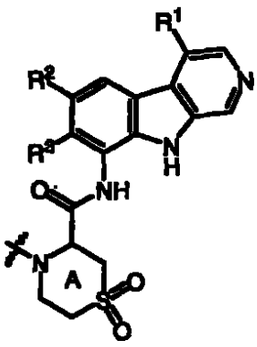
III-F



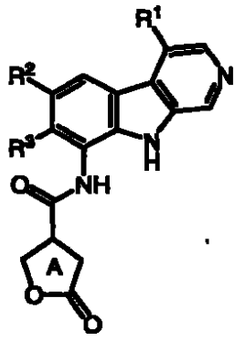
III-G



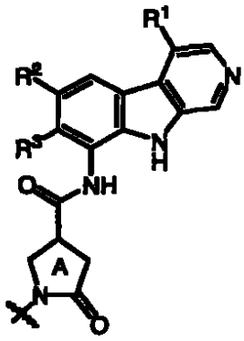
III-H



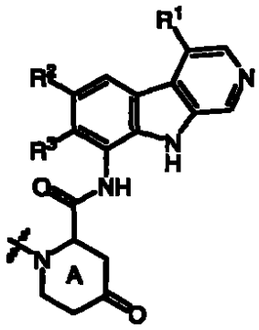
III-J



III-K



III-L



III-M

[0033] When Ring A is a non-aromatic, six-membered heterocylic ring, a favorable position for the -W-G and $-C(R^9)_3$ substituents on Ring A is ortho to the position where the beta-carboline portion is attached. For example, in compounds III-A, III-B, III-D, III-H, III-J and III-M, a preferred position for attachment of -W-G and $-C(R^9)_3$ is at the Ring A nitrogen or at N-1 in the case of compound III-F.

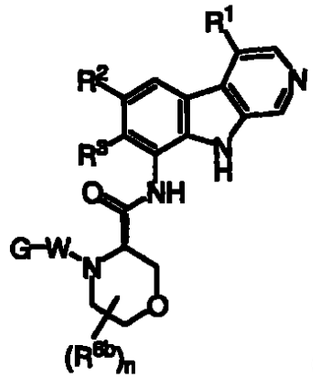
[0034] Preferably, W is -Q-, -Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$, or $-C(R^9)_2-[C(R^9)(R^{12})]_2-$ where R^9 is hydrogen. More preferably, W is -Q-, -Q-C(O)-, or $-C(R^9)_2-C(R^9)(R^{12})-$. R^{12} is hydrogen, C_{1-6} aliphatic, substituted or unsubstituted phenyl, substituted or unsubstituted benzyl or an amino acid side chain,

particularly the side chain of a natural amino acid. Examples of particular natural amino acids include alanine, phenylalanine, valine, leucine, isoleucine, serine, tyrosine, aspartic acid and glutamic acid.

[0035] In one embodiment, W is Q-C(O)-. In this embodiment, a preferred Q is $-CH_2-$ or $-CH_2-CH_2-$, more preferably $-CH_2-$.

[0036] In another embodiment, Ring A is substituted by 0-2 R^{6b}. A preferred R^{6b} group is methyl. When Ring A is a non-aromatic six membered ring, one embodiment provides compounds of formula III where there are two methyl groups on the Ring A position para to the position where the beta carboline portion is attached. An example of this embodiment is a compound where Ring A is a 6,6-dimethyl-morpholinyl ring. Preferably, such compounds are further substituted by -W-G as described above.

[0037] When Ring A is a morpholinyl ring, it has been found that compounds having the "S" stereochemistry at position 3 of the morpholine ring are preferred, as shown below by compounds of formula (*S*)-III-A.



(*S*)-III-A

where n is 0-4 and R^1, R^2, R^3, W, G and R^{6b} are as defined above. By anal gy, it is expected that "S" stereochemistry

is also preferred for other six-membered non-aromatic Ring A compounds of formula III.

[0038] One embodiment relates to compounds of formula III-A or (*S*)-III-A where R^1 is hydrogen, halo, methyl or amino;

R^2 is hydrogen, methyl or halo;

R^3 is hydrogen, halo, alkoxy, or (C_{1-6} aliphatic)$_2$ amino;

Ring A is substituted by 0-2 R^{6b};

R^{6b} is C_{1-3} aliphatic;

W is -Q-, -Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$, or $-C(R^9)_2-[C(R^9)(R^{12})]_2-$;

Q is $-C(R^9)_2-$ or $-C(R^9)_2C(R^9)_2-$;

G is $-NR^4R^5$, $-N(R^9)C(O)NR^4R^5$, $-N(R^9)SO_2$(C_{1-3} aliphatic), $-N(R^9)C(O)CF_3$, $-N(R^9)CO$(C_{1-6} aliphatic), and $-N(R^9)CO$(heterocyclyl), $-N(R^9)CO$(heteroaryl), $-N(R^9)CO$(aryl), a 5-6 membered heterocyclyl ring, or a 5-6 membered heteroaryl, wherein each of the heteroaryl, aryl and heterocyclyl moieties of G is optionally substituted by 1-3 R^{10};

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is hydrogen or a C_{1-6} aliphatic group that is optionally substituted by halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

R^9 is hydr gen;

each R^{10} is independently selected from oxo, R^{11}, T-R^{11}, r V-T-R^{11};

each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, -CN, $-SR^6$, $-S(O)_2R^6$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain;

V is -O-, $-N(R^7)-$, -S-, -S(O)-, $-S(O)_2-$, -C(O)-, or $-CO_2-$; and

R^{12} is hydrogen, C_{1-6} aliphatic, substituted or unsubstituted phenyl, or substituted or unsubstituted benzyl.

[0039] Another embodiment relates to compounds of formula III-A or (*S*)-III-A where:

R^1 is hydrogen, methyl, fluoro or amino;

R^2 is chloro;

R^3 is hydrogen or alkoxy;

Ring A is substituted by -W-G and 0-2 R^{6b};

R^{6b} is methyl;

W is -Q-, -Q-C(O)- or $-C(R^9)_2-C(R^9)(R^{12})-$;

Q is $-C(R^9)_2-$ or $-C(R^9)_2C(R^9)_2-$;

G is $-NR^4R^5$, $-N(R^9)C(O)NR^4R^5$, $-N(R^9)C(O)CF_3$, $-N(R^9)CO(C_{1-6}$ aliphatic), and $-N(R^9)CO$(heterocyclyl), $-N(R^9)CO$(heteroaryl), a 5-6 membered heterocyclyl ring, r a 5-6 membered heteroaryl, wherein each of the heteroaryl and heterocyclyl moieties of G is optionally substituted by 1-3 R^{10};

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is hydrogen or C_{1-6} aliphatic;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken

together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

R^9 is hydrogen;

each R^{10} is independently selected from oxo, R^{11}, $T-R^{11}$, or $V-T-R^{11}$;

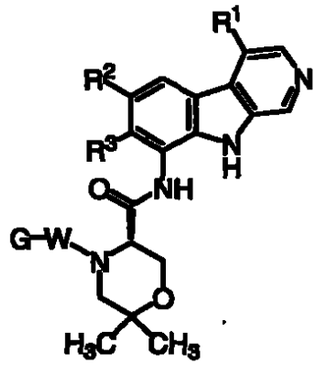
each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain;

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$; and

R^{12} is hydrogen, C_{1-6} aliphatic, phenyl, or benzyl.

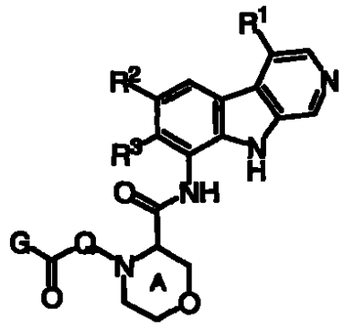
[0040] Preferred compounds of formula III-A are the compounds of formula (*S*)-III-A':



(*S*)-III-A'

where R^1, R^2, R^3, W and G are as defined above for (*S*)-III-A.

[0041] Another embodiment relates to compounds of formula III-A-a:



III-A-a

or a pharmaceutically acceptable salt thereof, wherein:

Q is $-CH_2-$, $-CH(R^9)-$, or $-C(R^9)_2-$;

G is $-NR^4R^5$ or a 3-7 membered heterocyclyl or heteroaryl ring that is optionally substituted by 1-2 R^{10};

R^1 is hydrogen, halo, C_{1-3} aliphatic, amino, cyano, $(C_{1-3}$ alkyl$)_{1-2}$ amino, C_{1-3} alkoxy, $(C_{1-3}$ aliphatic$)-C(O)-$, $(C_{1-6}$ aliphatic$)-CO_2-$, or $(C_{1-3}$ aliphatic$)-C(O)NH-$;

R^2 is hydrogen, halo, C_{1-3} aliphatic, C_{1-3} alkoxy, C_{1-3} haloalkoxy, or C_{1-3} haloalkyl;

R^3 is hydrogen, halo, C_{1-6} aliphatic, C_{1-6} haloalkyl, C_{1-6} alkoxy, hydroxy, amino, cyano, or $(C_{1-6}$ alkyl$)_{1-2}$ amino;

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is a C_{1-6} aliphatic group that is optionally substituted by halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

Ring A is substituted by 0-4 R^{6b};

each R^{6b} is independently selected from a C_{1-6} aliphatic group;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

each R^9 is independently selected from a C_{1-3} aliphatic;

each R^{10} is independently selected from R^{11}, $T-R^{11}$, or $V-T-R^{11}$;

each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain; and

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$.

[0042] One embodiment relates to compounds of formula III-A-a where:

Q is $-CH_2-$, or $-CH(R^9)-$;

G is $-NR^4R^5$ or a 5-6 membered heterocyclyl or heteroaryl ring that is optionally substituted by 1-2 R^{10};

R^1 is hydrogen, halo, C_{1-2} alkyl, amino, or (C_{1-2} alkyl)$_{1-2}$ amino;

R^2 is hydrogen, halo, C_{1-2} aliphatic, C_{1-2} alkoxy, or C_{1-2} haloalkyl;

R^3 is hydrogen, halo, C_{1-2} aliphatic, C_{1-2} alkoxy, or C_{1-2} haloalkyl;

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is a C_{1-6} aliphatic group that is optionally substituted by halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

Ring A is substituted by 0-2 R^{6b};

each R^{6b} is independently selected from a C_{1-3} aliphatic group;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

R^8 is C_{1-4} aliphatic;

R^9 is independently selected from a C_{1-3} aliphatic;

each R^{10} is independently selected from R^{11}, $T-R^{11}$, or $V-T-R^{11}$;

each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain; and

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$.

[0043] Another embodiment relates to compounds of formula III-A-a where:

Q is $-CH_2-$, or $-CH(R^9)-$;

G is $-NR^4R^5$ or a 5-6 membered heterocyclyl ring, having 1-2 ring heteroatoms selected from oxygen or nitrogen, that is optionally substituted by 1-2 R^{10};

R^1 is hydrogen, halo, methyl, amino, or $(C_{1-2}$ alkyl$)_{1-2}$ amino;

R^2 is hydrogen, halo, C_{1-2} aliphatic, or C_{1-2} haloalkyl;

R^3 is hydrogen, halo, or C_{1-2} aliphatic;

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is a C_{1-6} aliphatic group that is optionally substituted by halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

Ring A is substituted by zero or two R^{6b};

each R^{6b} is independently selected from a C_{1-3} aliphatic group;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

R^8 is C_{1-4} aliphatic;

R^9 is independently selected from a C_{1-3} aliphatic;

each R^{10} is independently selected from R^{11}, $T-R^{11}$, or $V-T-R^{11}$;

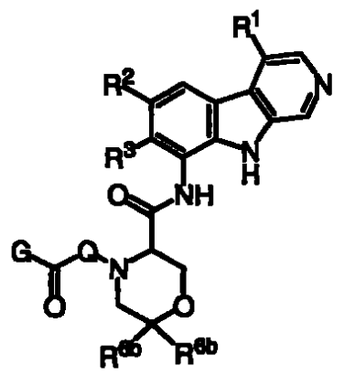
each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain; and

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$.

[0044] In preferred compounds of formula III-A-a, Q is $-CH_2-$; G is selected from an optionally substituted piperidinyl, piperazinyl, morpholinyl, pyrrolidinyl, or $-NR^4R^5$; R^4 is hydrogen or C_{1-6} aliphatic; and R^5 is C_{1-6} aliphatic, 5-6 membered heterocyclyl, or C_{1-6} hydroxyalkyl. More preferred are compounds where G is unsubstituted or substituted by 1-2 groups independently selected from the group consisting of: C_{1-3} alkyl, HO-alkyl, alkoxycarbonyl, mono- or dialkylaminocarbonyl, and HO_2C-alkyl. For compounds of formula III-A-a in each of the above embodiments, the (*S*) stereochemistry at the morpholine 3-position is preferred.

[0045] Another embodiment relates to compounds of formula III-A-aa:



III-A-aa

or a pharmaceutically acceptable salt thereof wherein,

Q is $-CH_2-$, or $-CH(R^9)-$;

G is $-NR^4R^5$ or a 3-7 membered heterocyclyl ring that is optionally substituted by 1-2 R^{10};

R^1 is hydrogen, halo, C_{1-2} alkyl, amino, or $(C_{1-2}$ alkyl$)_{1-2}$ amino;

R^2 is hydrogen, halo, C_{1-2} aliphatic, C_{1-2} alkoxy, or C_{1-2} haloalkyl;

R^3 is hydrogen, halo, C_{1-2} aliphatic, C_{1-2} alkoxy, or C_{1-2} haloalkyl;

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is a C_{1-6} aliphatic group that is optionally substituted by halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

each R^{6b} is independently selected from hydrogen or a C_{1-6} aliphatic;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

R^8 is C_{1-4} aliphatic;

R^9 is independently selected from a C_{1-3} aliphatic;

each R^{10} is independently selected from R^{11}, $T-R^{11}$, or $V-T-R^{11}$;

each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, r $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain; and

V is -O-, -N(R^7)-, -S-, -S(O)-, -S$(O)_2$-, -C(O)-, or -CO_2-.

[0046] Another embodiment relates to compounds of formula III-A-aa where:

Q is -CH_2-;

G is -NR^4R^5 or a 5-6 membered heterocyclyl ring that is optionally substituted by 1-2 R^{10};

R^1 is hydrogen, halo or methyl;

R^2 is hydrogen, halo, C_{1-2} aliphatic, C_{1-2} alkoxy, or C_{1-2} haloalkyl;

R^3 is hydrogen;

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is a C_{1-6} aliphatic group that is optionally substituted by halo, -OR^7, -CN, -SR^8, -S$(O)_2R^8$, -C(O)R^7, -CO_2R^7, -N$(R^7)_2$, -C(O)N$(R^7)_2$, -N(R^7)C(O)R^7, -N(R^7)CO_2R^8, or -N(R^7)C(O)N$(R^7)_2$;

each R^{6b} is independently selected from hydrogen or a C_{1-6} aliphatic;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

R^8 is C_{1-4} aliphatic;

each R^{10} is independently selected from R^{11}, T-R^{11}, or V-T-R^{11};

each R^{11} is independently selected from C_{1-6} aliphatic, halo, -OR^7, -CN, -SR^8, -S$(O)_2R^8$, -C(O)R^7, -CO_2R^7, -N$(R^7)_2$, -C(O)N$(R^7)_2$, -N(R^7)C(O)R^7, -N(R^7)CO_2R^7, or -N(R^7)C(O)N$(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain; and

V is -O-, -N(R^7)-, -S-, -S(O)-, -S$(O)_2$-, -C(O)-, or -CO_2-.

[0047] Preferred compounds of III-A-aa are compounds where:

Q is $-CH_2-$;

G is selected from an optionally substituted piperidinyl, piperazinyl, morpholinyl, pyrrolidinyl, or $-NR^4R^5$;

R^1 is hydrogen, halo or methyl;

R^2 is halo;

R^3 is hydrogen;

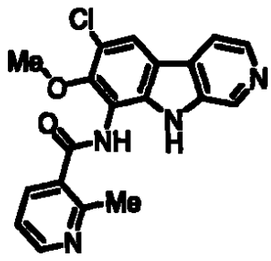
R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is C_{1-6} alkoxy, C_{1-6} aliphatic, or C_{1-6} hydroxyalkyl;

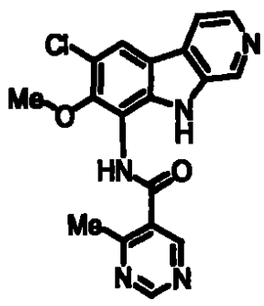
each R^{6b} is independently selected from hydrogen or a C_{1-3} aliphatic. Preferably, each R^{6b} is hydrogen or methyl. For compounds of formula III-A-aa in each of the above embodiments, the (*S*) stereochemistry is preferred at the three position of the Ring A morpholine.

[0048] Examples of specific formula I compounds are shown in Tables 3 and 4 below.

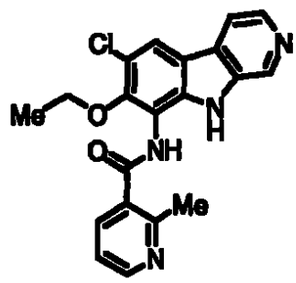
Table 3. Specific examples of formula I compounds



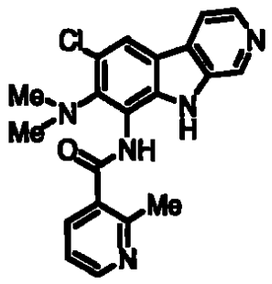
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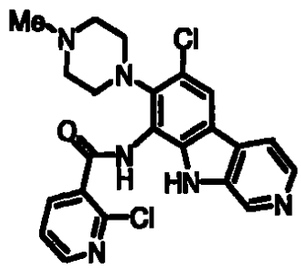
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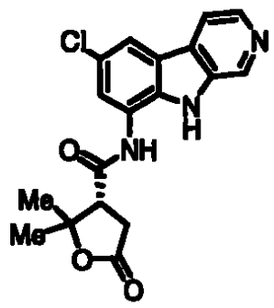
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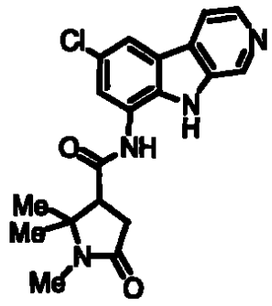
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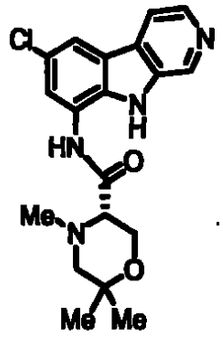
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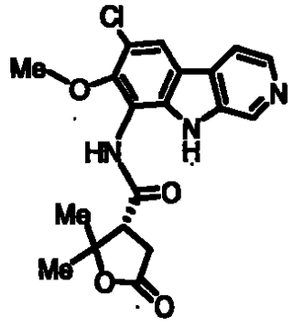
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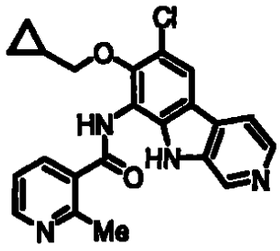
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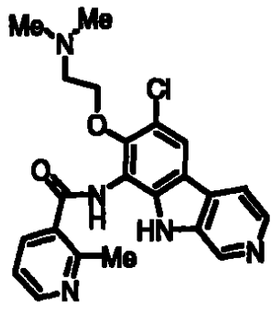
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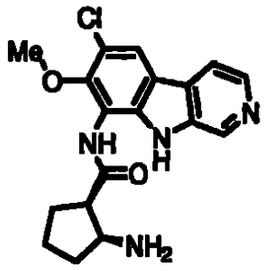
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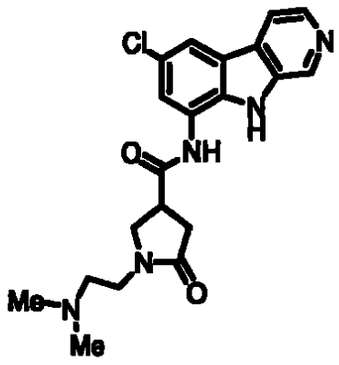
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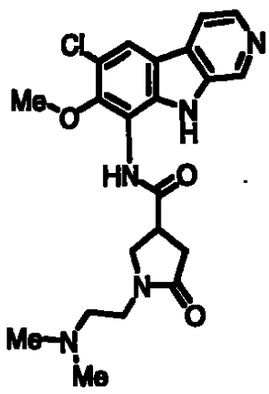
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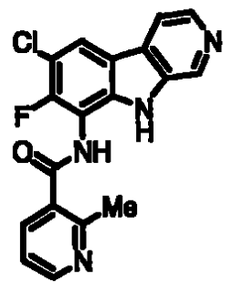
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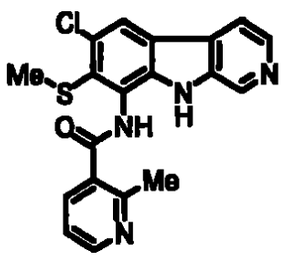
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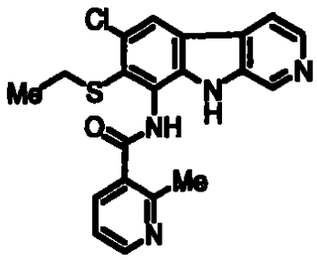
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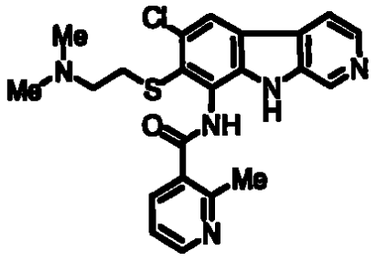
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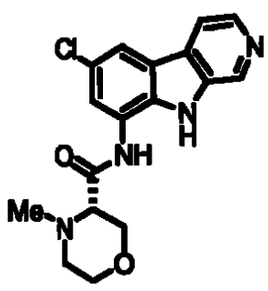
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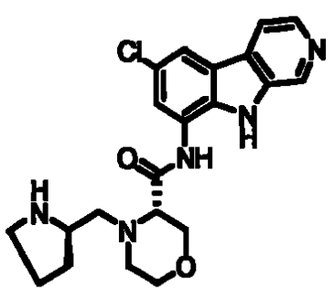
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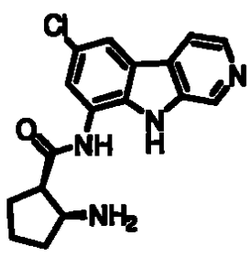
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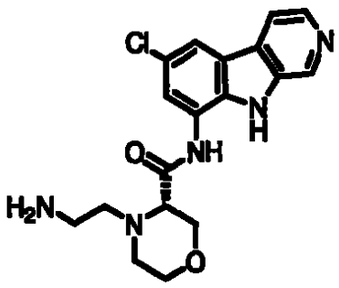
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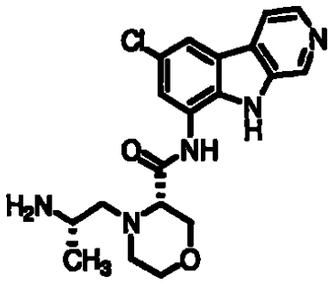
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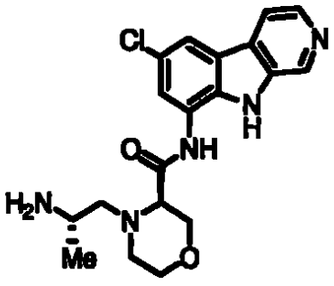
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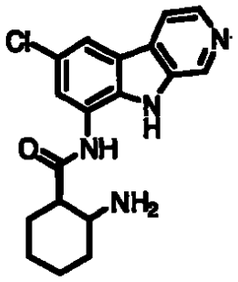
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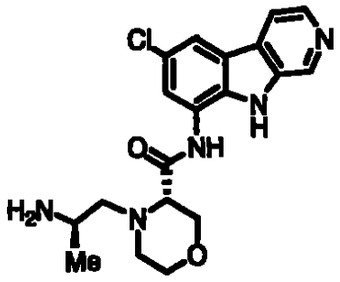
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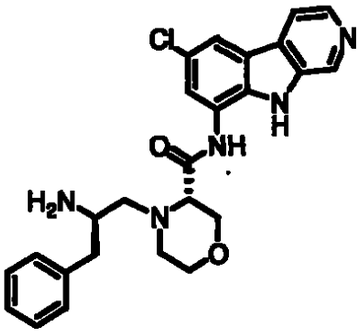
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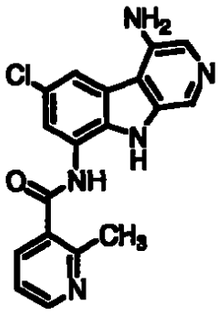
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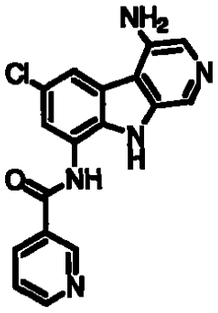
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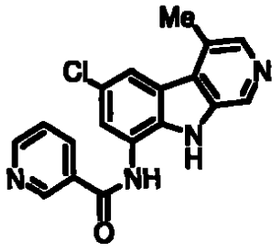
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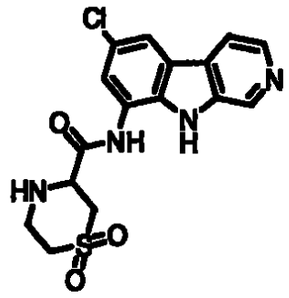
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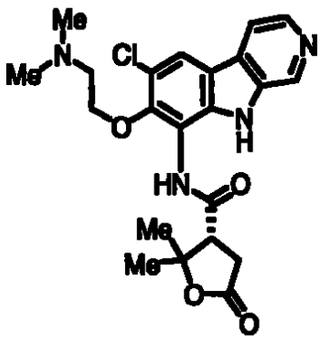
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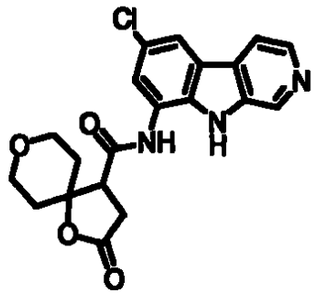
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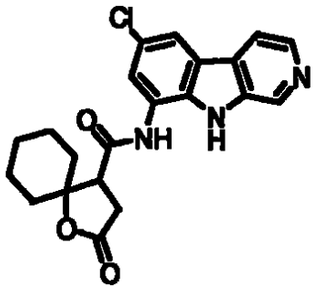
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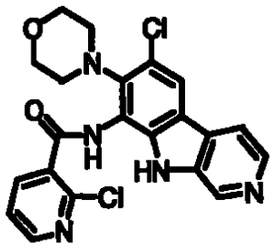
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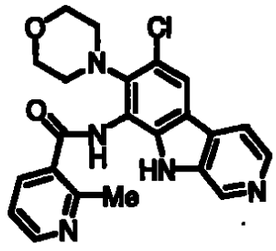
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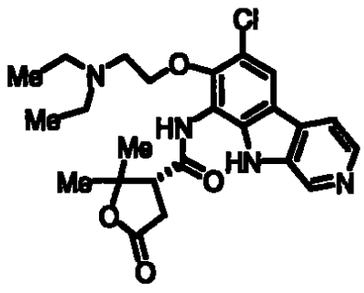
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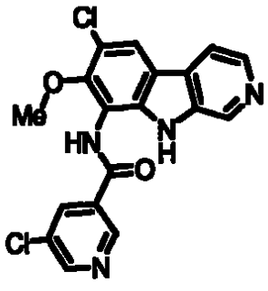
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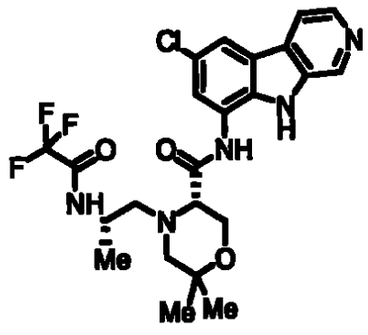
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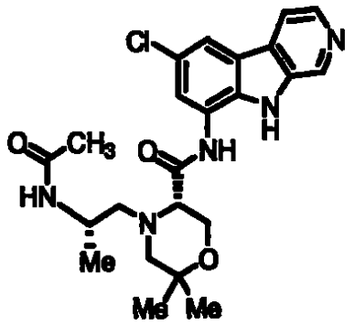
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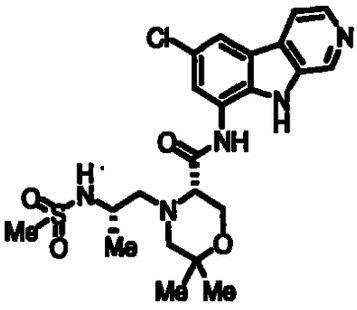
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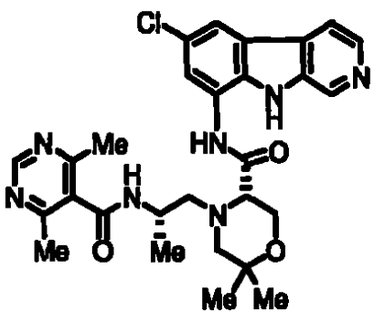
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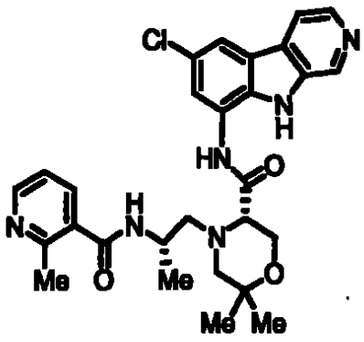
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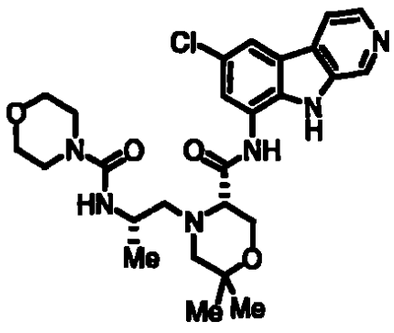
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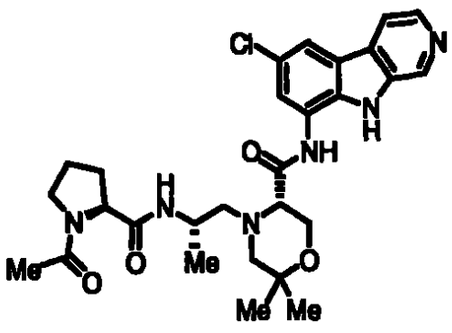
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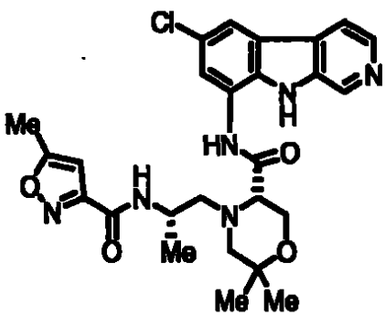
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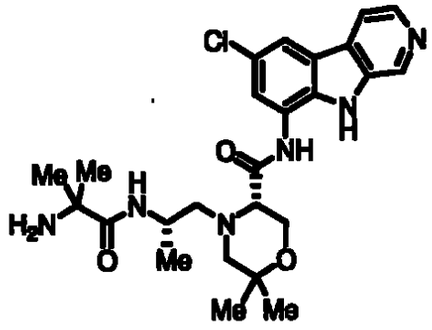
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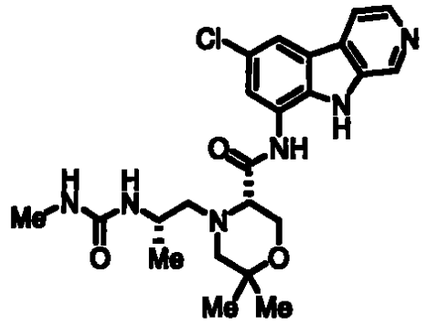
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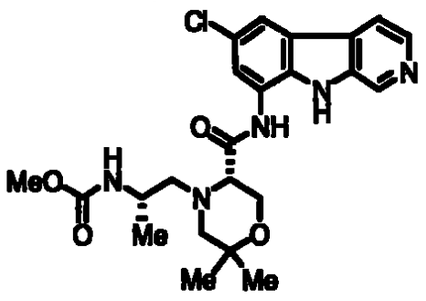
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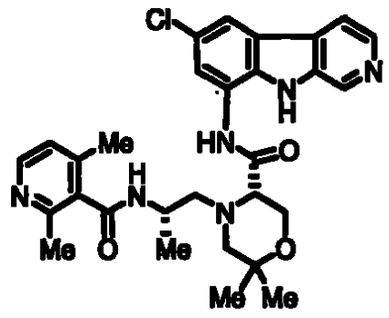
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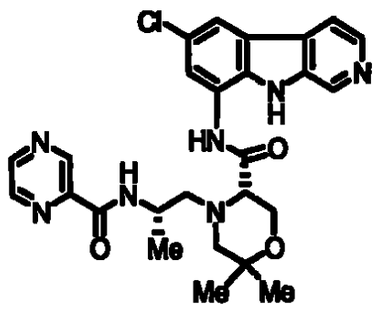
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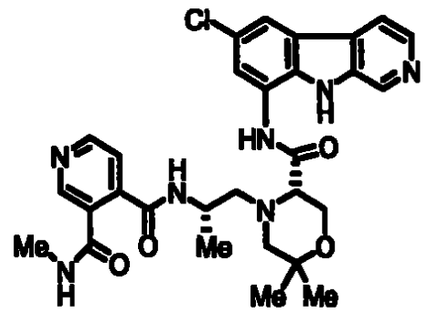
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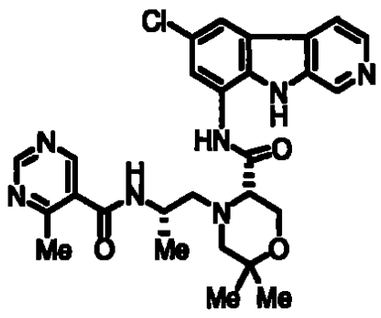
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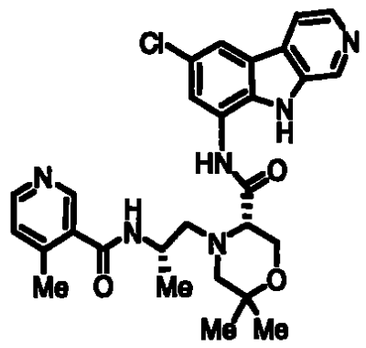
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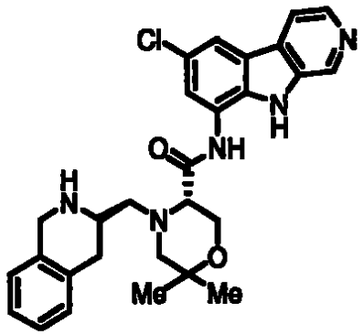
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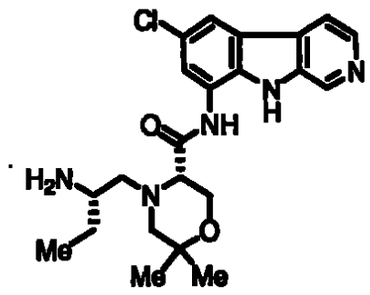
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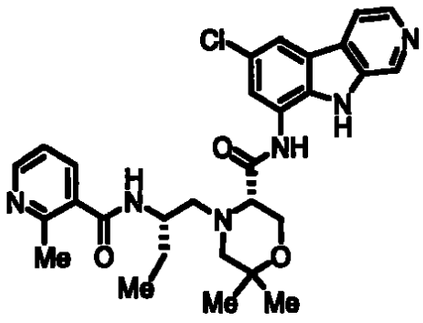
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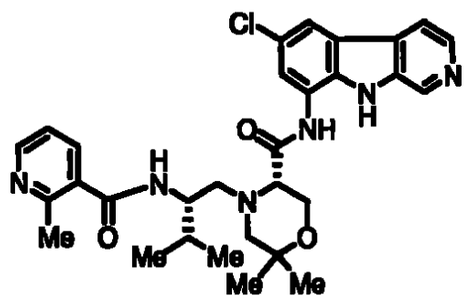
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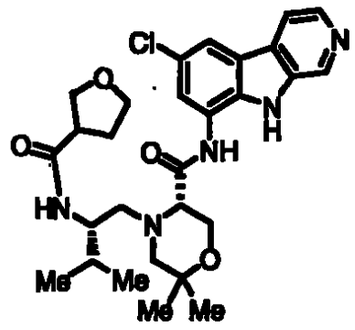
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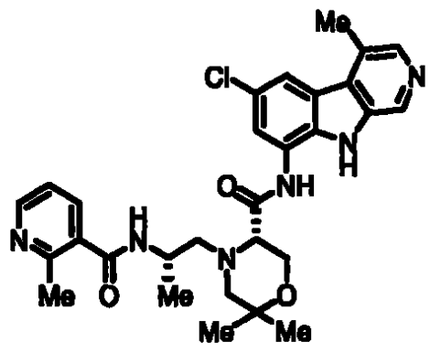
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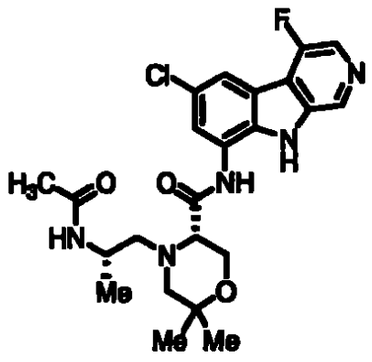
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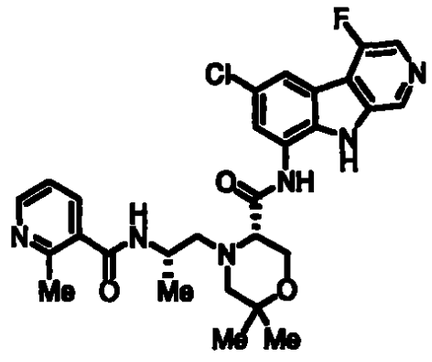
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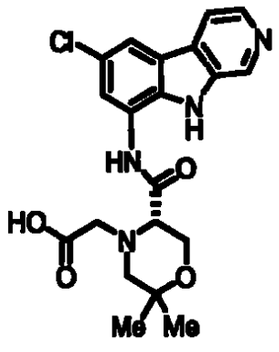
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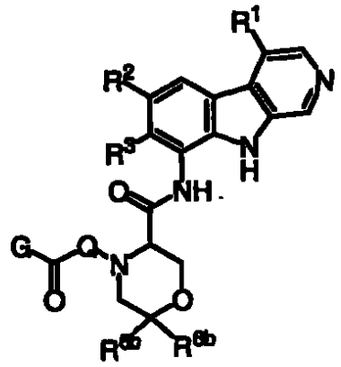
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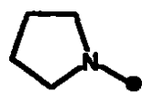
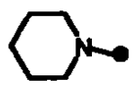
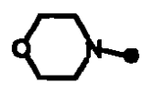
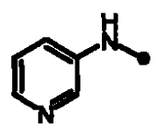
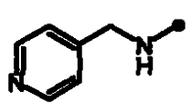
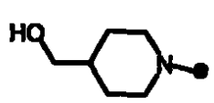
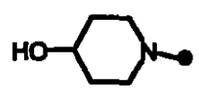
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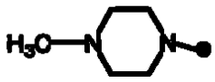
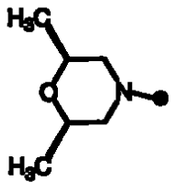
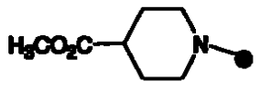
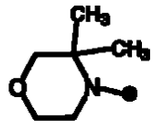
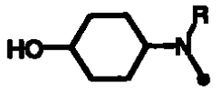
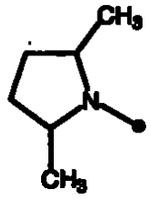
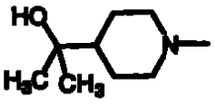
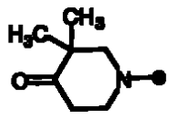
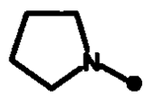
[0049] Table 4 below shows specific examples of III-A-aa compounds.

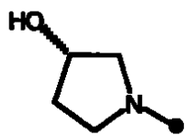
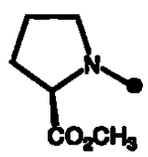
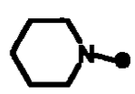
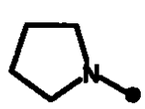
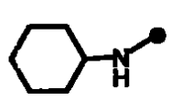
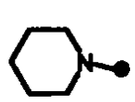
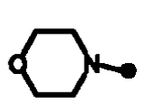
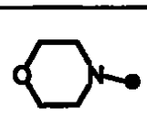
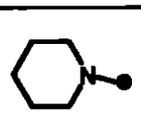
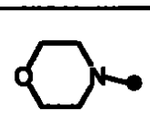
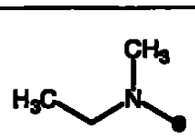
Table 4. Specific examples of formula III-A-a compounds

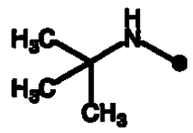
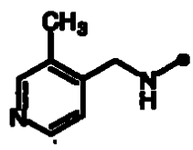
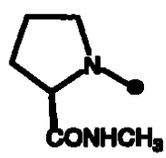
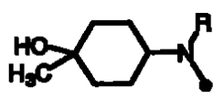
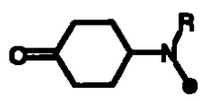
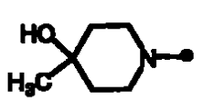
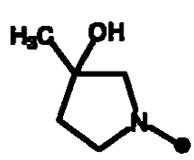
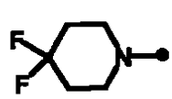
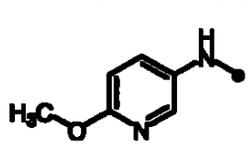


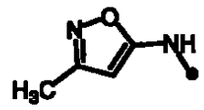
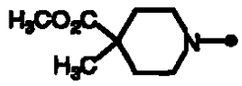
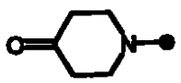
III-A-aa

No.	R^1	R^{6b}/R^{6b}	Q	G
64	H	CH_3/CH_3	CH_2	
65	H	CH_3/CH_3	CH_2	
66	H	CH_3/CH_3	CH_2	
67	H	CH_3/CH_3	CH_2	$HO-CH_2CH_2N(CH_3)-$
68	H	CH_3/CH_3	CH_2	
69	H	CH_3/CH_3	CH_2	
70	H	CH_3/CH_3	CH_2	
71	H	CH_3/CH_3	CH_2	

No.	R^1	R^{6b}/R^{6b}	Q	G
72	H	CH_3/CH_3	CH_2	Et_2N-
73	H	CH_3/CH_3	CH_2	
74	H	CH_3/CH_3	CH_2	
75	H	CH_3/CH_3	CH_2	
76	H	CH_3/CH_3	CH_2	
77	H	CH_3/CH_3	CH_2	
78	H	CH_3/CH_3	CH_2	
79	H	CH_3/CH_3	CH_2	
80	H	CH_3/CH_3	CH_2	
81	CH_3	CH_3/CH_3	CH_2	

No.	R^1	R^{6b}/R^{6b}	Q	G
82	H	CH_3/CH_3	CH_2	
83	H	CH_3/CH_3	CH_2	
84	CH_3	H/H	CH_2	
85	CH_3	H/H	CH_2	
86	H	CH_3/CH_3	CH_2	
87	CH_3	CH_3/CH_3	CH_2	
88	CH_3	CH_3/CH_3	CH_2	
89	F	CH_3/CH_3	CH_2	
90	F	CH_3/CH_3	CH_2	
91	H	CH_3/CH_3	*R*-CH(CH_3)	
92	H	CH_3/CH_3	CH_2	

No.	R^1	R^{6b}/R^{6b}	Q	G
93	H	CH_3/CH_3	CH_2	
94	H	CH_3/CH_3	CH_2	Me_2N-
95	H	CH_3/CH_3	CH_2	$(CH_3O)(CH_3)N$-
96	H	CH_3/CH_3	CH_2	
97	H	CH_3/CH_3	CH_2	
98	H	CH_3/CH_3	CH_2	
99	H	CH_3/CH_3	CH_2	
100	H	CH_3/CH_3	CH_2	
101	H	CH_3/CH_3	CH_2	
102	H	CH_3/CH_3	CH_2	
103	H	CH_3/CH_3	CH_2	

No.	R^1	R^{6b}/R^{6b}	Q	G
104	H	CH_3/CH_3	CH_2	
105	H	CH_3/CH_3	CH_2	
106	H	CH_3/CH_3	CH_2	

[0050] Based on their IκB kinase inhibitory properties and other pharmacological properties, compound example numbers 1-30, 39-62 and 64-106 are preferred. More preferred are compound example numbers 1, 2, 7, 10, 11, 13, 16, 17, 19-27, 39-62, 64-106.

[0051] The compounds of the present invention may be administered to humans or other mammals by a variety of routes, including oral dosage forms and injections (intravenous, intramuscular, intraperitoneal, subcutaneous, and the like). Numerous other dosage forms containing compounds of the invention can be readily formulated by one skilled in the art, utilizing the suitable pharmaceutical excipients (or carriers) as defined below.

[0052] Examples of pharmaceutically acceptable excipients (or carriers) and methods of manufacture for various compositions can be found in A. Gennaro (ed.), Remington: The Science and Practice of Pharmacy, 20th Edition, Lippincott Williams & Wilkins, Baltimore, MD (2000), which is incorporated in its entirety by reference herein. Pharmaceutically acceptable excipients (or carriers) include flavoring agents, pharmaceutical-grade

dyes or pigments, solvents, co-solvents, buffer systems, surfactants, preservatives, sweetener agents, viscosity agents, fillers, lubricants, glidants, disintegrants, binders and resins.

[0053] Conventional flavoring agents may be used, such as those described in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., pp. 1288-1300 (1990), which is incorporated in its entirety by reference herein. The pharmaceutical compositions of the invention generally contain from about 0 to 2% of flavoring agents.

[0054] Conventional dyes and/or pigments may also be used, such as those described in the Handbook of Pharmaceutical Excipients, by the American Pharmaceutical Association & the Pharmaceutical Society of Great Britain, pp. 81-90 (1986), which is incorporated in its entirety by reference herein. The pharmaceutical compositions of the invention generally contain from about 0 to 2% of dyes and/or pigments.

[0055] The pharmaceutical compositions of the invention generally contain from about 0.1 to 99.9% of solvent(s). A preferred solvent is water. Preferred co-solvents include ethanol, glycerin, propylene glycol, polyethylene glycol, and the like. The pharmaceutical compositions of the invention may include from about 0 to 50% of co-solvents.

[0056] Preferred buffer systems include acetic, boric, carbonic, phosphoric, succinic, maleic, tartaric, citric, acetic, benzoic, lactic, glyceric, gluconic, glutaric and glutamic acids and their sodium, potassium and ammonium salts. Particularly preferred buffers are phosphoric, tartaric, citric and acetic acids and salts thereof. The pharmaceutical compositions of the invention generally contain from about 0 to 5% of a buffer.

[0057] Preferred surfactants include polyoxyethylene sorbitan fatty acid esters, polyoxyethylene monoalkyl ethers, sucrose monoesters and lanolin esters and ethers, alkyl sulfate salts and sodium, potassium and ammonium salts of fatty acids. The pharmaceutical compositions of the invention generally contain from about 0 to 2% of surfactants.

[0058] Preferred preservatives include phenol, alkyl esters of parahydroxybenzoic acid, o-phenylphenol benzoic acid and salts thereof, boric acid and salts thereof, sorbic acid and salts thereof, chlorobutanol, benzyl alcohol, thimerosal, phenylmercuric acetate and nitrate, nitromersol, benzalkonium chloride, cetylpyridinium chloride, methyl paraben and propyl paraben. Particularly preferred preservatives are the salts of benzoic acid, cetylpyridinium chloride, methyl paraben and propyl paraben. The pharmaceutical compositions of the invention generally contain from about 0 to 2% of preservatives.

[0059] Preferred sweeteners include sucrose, glucose, saccharin, sorbitol, mannitol and aspartame. Particularly preferred sweeteners are sucrose and saccharin. Pharmaceutical compositions of the invention generally contain from about 0 to 5% of sweeteners.

[0060] Preferred viscosity agents include methylcellulose, sodium carboxymethylcellulose, hydroxypropyl-methylcellulose, hydroxypropylcellulose, sodium alginate, carbomer, povidone, acacia, guar gum, xanthan gum and tragacanth. Particularly preferred viscosity agents are methylcellulose, carbomer, xanthan gum, guar gum, povidone, sodium carboxymethylcellulose, and magn sium aluminum silicate. Pharmaceutical compositions

of the invention generally contain from about 0 to 5% of viscosity agents.

[0061] Preferred fillers include lactose, mannitol, sorbitol, tribasic calcium phosphate, dibasic calcium phosphate, compressible sugar, starch, calcium sulfate, dextro and microcrystalline cellulose. Pharmaceutical compositions of the invention generally contain from about 0 to 75% of fillers.

[0062] Preferred lubricants/glidants include magnesium stearate, stearic acid and talc. Pharmaceutical compositions of the invention generally contain from about 0 to 7%, preferably, about 1 to 5% of lubricants/glidants.

[0063] Preferred disintegrants include starch, sodium starch glycolate, crospovidone and croscarmelose sodium and microcrystalline cellulose. Pharmaceutical compositions of the invention generally contain from about 0 to 20%, preferably, about 4 to 15% of disintegrants.

[0064] Preferred binders include acacia, tragacanth, hydroxypropylcellulose, pregelatinized starch, gelatin, povidone, hydroxypropylcellulose, hydroxypropylmethylcellulose, methylcellulose, sugar solutions, such as sucrose and sorbitol, and ethylcellulose. Pharmaceutical compositions of the invention generally contain from about 0 to 12%, preferably, about 1 to 10% of binders.

[0065] Additional agents known to a skilled formulator may be combined with the compounds of the invention to create a single dosage form. Alternatively, additional agents may be separately administered to a mammal as part of a multiple dosage form.

[0066] For preparing pharmaceutical compositions containing the inventive compounds, inert, pharmaceutically

acceptable excipients or carriers can be either solid or liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets and suppositories. Generally, the powders and tablets may be comprised of from about 5 to 95 weight percent of active ingredient. Suitable solid carriers are known in the art, for example, magnesium carbonate, magnesium stearate, talc, sugar and lactose. Tablets, powders, cachets and capsules can be used as solid dosage forms suitable for oral administration. Examples of pharmaceutically acceptable carriers and methods of manufacture for various compositions may be found in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co. (1990).

[0067] Liquid form preparations include solutions, suspensions and emulsions. Common liquid form preparations include water and water-propylene glycol solutions for parenteral injection or addition of sweeteners and opacifiers for oral solutions, suspensions and emulsions. Liquid form preparations may also include solutions for intranasal administration.

[0068] Aerosol preparations suitable for inhalation include solutions and solids in powder form, which may be combined with a pharmaceutically acceptable carrier, such as an inert compressed gas (e.g., nitrogen).

[0069] Also included are solid form preparations that may be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions and emulsions.

[0070] The compounds of the invention may also be delivered transdermally. The transdermal compositions can take the form f creams, lotions, aerosols and emulsions

and may be included in a transdermal patch of a matrix or reservoir type as is conventional in the art for this purpose.

[0071] The preferred mode of administering the compounds of the invention is oral. Preferably, the pharmaceutical preparation is in a unit dosage form. In such a form, the preparation is subdivided into suitable sized unit doses containing appropriate quantities of the active component, for example, an effective amount to achieve the desired purpose.

[0072] The quantity of active ingredient (compound) in a unit dose of preparation may be varied or adjusted from about 0.01 to 4,000 mg, preferably, from about 0.01 to 1,000 mg, more preferably, from about 0.01 to 500 mg, and even more preferably, from about 0.01 to 250 mg, according to the particular application. A typical recommended daily dosage regimen for oral administration will usually range from about 0.02 to 2,000 mg/day, in one to four divided doses. For convenience, the total daily dosage may be divided and administered in portions during the day as required. Typically, pharmaceutical compositions of the invention will be administered from about 1 to 5 times per day, or alternatively, as a continuous infusion. Such administration can be used for chronic or acute therapy. The amount of active ingredient that may be combined with excipient or carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will usually contain from about 5 to 95% of active compound (w/w). Preferably, such preparations will contain from about 20 to 80 wt.% f active compound.

[0073] The pharmaceutically acceptable excipients or carriers employed in conjunction with the compounds of the invention are used at a concentration sufficient to provide a practical size to dosage relationship. The pharmaceutically acceptable excipients or carriers, in total, can comprise from about 0.1 to 99.9% by weight of the pharmaceutical compositions of the invention, preferably, from about 20 to 80% by weight.

[0074] Upon improvement of a patient's condition, a maintenance dose of a compound, composition or combination of the invention may be administered, if applicable. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

[0075] Specific dosage and treatment regimens for any particular patient may be varied and will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex and diet of the patient, the time of administration, the rate of excretion, the specific drug combination, the severity and course of the symptoms being treated, the patient's disposition to the condition being treated and the judgment of the treating physician. Determination of the proper dosage regimen for a particular situation is within the skill of the art. The amount and frequency of the administration of compounds of the invention or their pharmaceutically acceptable salts may be regulated according to the judgment of th attending

clinician, based on the factors recited above. As a skilled artisan will appreciate, lower or higher doses than those recited above may be required.

[0076] The inventive compounds are understood to provide efficacious treatment of a variety of diseases, symptoms and disorders, particularly, those which are inflammatory r immune-related in nature, including a reasonable time of onset upon administration, and a reasonable duration after administration. While food, diet, pre-existing conditions, alcohol and other systemic conditions could lengthen the time delay for an inventive drug to work after its administration, it is understood that optimum dosages will result in an efficacious drug treatment within and for a reasonable amount of time.

[0077] The inventive compounds can exist in unsolvated as well as solvated forms, including hydrated forms. In general, the solvated forms, with pharmaceutically acceptable solvents, such as water, ethanol and the like, are equivalent to the unsolvated forms for purposes of this invention.

[0078] The inventive compounds may form pharmaceutically acceptable salts with organic and inorganic acids. Examples of suitable acids for salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic and other mineral and carboxylic acids well known to those skilled in the art. The salts are prepared by contacting the free base forms with a sufficient amount of the desired acid to produce a salt in a conventional manner. The free base forms may be regenerated by treating the salt with a suitable dilute aqueous base solution, such as dilute aqueous sodium

hydroxide, potassium carbonate, ammonia or sodium bicarbonate. The free base forms may differ somewhat from their respective salt forms in certain physical properties, such as solubility in polar solvents, but the salts are otherwise equivalent to their respective free base forms for purposes of the invention.

[0079] On account of their pharmacological properties, the compounds according to the invention are suitable for the prophylaxis treatment and therapy of diseases, disorders and symptoms that involve increased activity of IkB kinase. These include, for example, joint inflammation (e.g., rheumatoid arthritis (RA), rheumatoid spondylitis, gouty arthritis, traumatic arthritis, rubella arthritis, psoriatic arthritis, osteoarthritis, and other arthritic conditions), acute synovitis, tuberculosis, atherosclerosis, muscle degeneration, cachexia, Reiter's syndrome, endotoxaemia, sepsis, septic shock, endotoxic shock, gram negative sepsis, gout, toxic shock syndrome, pulmonary inflammatory diseases (e.g., asthma, acute respiratory distress syndrome, chronic obstructive pulmonary disease, silicosis, pulmonary sarcoidosis, and the like), bone resorption diseases, reperfusion injuries, carcinoses, leukemia, sarcomas, lymph node tumors, skin carcinoses, lymphoma, apoptosis, graft versus host reaction, graft versus host disease (GVHD), allograft rejection and leprosy.

[0080] Furthermore, the inventive compounds may be used in the treatment of immune-related diseases, symptoms and disorders, for example, infections, such as viral infections (e.g., HIV, cytomegalovirus (CMV), influenza, adenovirus, the Herpes group of viruses, and the like), parasitic infections (e.g., malaria, such as cerebral

malaria), and yeast and fungal infections (e.g., fungal meningitis). In addition, the inventive compounds can be useful for treating fever and myalgias due to infection, acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), cachexia secondary to infection or malignancy, cachexia secondary to AIDS or cancer, keloid and scar tissue formation, pyresis, diabetes, and inflammatory bowel diseases (IBD) (e.g., Crohn's disease and ulcerative colitis). The compounds of the invention are also useful in the treatment of diseases or injuries to the brain in which over-expression of TNF-α has been implicated, such as multiple sclerosis (MS), ischemic brain injury, e.g. cerebral infarction (stroke) and head trauma. The compounds of the invention are also useful in the treatment of psoriasis, Alzheimer's disease, carcinomatous disorders (potentiation of cytotoxic therapies), cardiac infarct, chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome (ARDS).

[0081] In one embodiment the compounds of this invention are useful for treating cancer, especially for treating cancers where IKK activity is abnormally high. The cancer types that may be treated include lymphoma, such as diffuse large B-cell, primary mediastinal B-cell, and mantle cell; multiple myeloma; osteolytic bone metastasis; head and neck squamous cell cancer; prostate cancer; pancreatic cancer and non-small cell lung cancer. In one embodiment, the compounds are useful for ABC lymphoma. For the treatment f cancer, the compounds may be used as a single agent or in combination with other agents known to be useful for the treatment of cancer. Examples of such other agents include bortezomib; capecitibine; gemcitabine; irinotecan; fludarabine; 5-fluorouricil or 5-flu rouricil/ leucovorin;

taxanes, including, e.g., paclitaxel and docetaxel; platinum agents, including, e.g., cisplatin, carboplatin, and oxaliplatin; anthracyclins, including, e.g., doxorubicin and pegylated liposomal doxorubicin; mitoxantrone; dexamethasone; vincristine; etoposide; prednisone; thalidomide; Herceptin; temozolomide; and alkylating agents such as melphalan, chlorambucil, and cyclophosphamide.

[0082] The compounds of formula (I) are especially useful for treating inflammatory and immune-related diseases, disorders and symptoms, more especially, inflammatory ones such as RA, asthma, IBD, psoriasis, COPD and MS. It will be appreciated that the present compounds are useful for treating diseases, disorders or symptoms related to the activity of NF-κB, TNF-α, and other enzymes in pathways where IKK is known to modulate activity.

[0083] The compounds of this invention are also useful for treating a bone associated disease, symptom or disorder in which there is a deficit or deficiency of bone - either as a result of decreased new bone formation or an increase in bone resorption or a combination of both. Specific examples include osteoporosis, periodontal disease, osteomyelitis, rheumatoid arthritis, aseptic joint loosening and osteolytic lesions (typically cancer related). It is known that rheumatoid arthritis, which is characterized by inflammation of the joints, is also associated with destruction of cartilage and bone. Furthermore, it has been reported that an IKK inhibitor provided inhibition of cartilage and bone loss in a murine model of collagen-induced arthritis. See McIntyre et al., *Arthritis & Rheumatism* (2003), 48(9), 2652-2659.

[0084] Osteoporosis is a broad term applied to a number of distinct diseases in which there is decreased bone mass. These include primary osteoporosis (*e.g.*, post-menopausal, senile osteoporosis and juvenile osteoporosis) and secondary osteoporosis. Examples of secondary osteoporosis would be those associated with chronic diseases (*e.g.*, chronic renal disease, hepatic insufficiency, gastrointestinal malabsorption, chronic immobilization and chronic inflammatory diseases, including rheumatoid arthritis, osteoarthritis, periodontal disease and aseptic prosthetic joint loosening), endocrine dysfunction related diseases (*e.g.*, diabetes, hyperthyroidism, hyperparathyroidism, hypogonadism and hypopituitarism), drug and substance related symptoms (*e.g.*, corticosteroid, heparin, anticonvulsants, alcohol and immunosupressants), and hematological disorders (e.g., metastatic disease, myeloma, leukemia, gaucher's disease and anemia). Inhibition of ither IkB directly or the NF-kB pathway indirectly has been reported to be useful for the treatment of osteoporosis and osteoarthritis. See, for example, PCT applications WO 2003104219, WO 2003103658, WO 2003029242, WO 2003065972, and WO 9965495. Accordingly, this invention also provides a method of treating or preventing bone loss in a patient in need thereof, comprising administering to the patient a compound of this invention. Also provided is a method of generating bone formation in a patient comprising administering a compound of this invention.

[0085] Another embodiment of the invention provides a method of inhibiting activation of NF-κB dependent gene expression associated with the inhibition of IKK catalytic activity and/or IκB phosphorylation, comprising administering to a patient in need thereof an amount of the

compound according to claim 1 or the pharmaceutically acceptable salt or solvate thereof, or a pharmaceutical composition thereof, which is effective to inhibit IKK catalytic activity and/or IκB phosphorylation, thereby inhibiting activation of NF-κB dependent gene expression.

[0086] In one embodiment of the invention, there is provided a method of treating or preventing an inflammatory or immune-related physiological disorder, symptom or disease in a patient in need of such treatment, comprising administering to the patient an amount of at least one compound according to claim 1, or the pharmaceutically acceptable salt or solvate thereof, or a pharmaceutical composition thereof, which is effective to treat or prevent the inflammatory or immune-related physiological disorder, symptom or disease. Preferably, the inflammatory disease, disorder or symptom is rheumatoid arthritis, asthma, psoriasis, psoriatic arthritis, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease or multiple sclerosis.

[0087] The invention comprises a compound having the formula (I), a method for making an inventive compound, a method for making a pharmaceutical composition from at least one inventive compound and at least one pharmaceutically acceptable carrier or excipient, and a method of using one or more inventive compounds to treat a variety of disorders, symptoms and diseases, particularly ones that are inflammatory or immune-related in nature. The inventive compounds and their pharmaceutically acceptable salt and neutral compositions may be formulated together with a pharmaceutically acceptable excipient or carrier and the resulting composition may be administered *in vivo* to mammals, such as primates, e.g. chimpanzees and

humans (e.g. males and females) and animals (*e.g.*, dogs, cats, cows, horses, and the like), to treat a variety of disorders, symptoms and diseases. Furthermore, the inventive compounds can be used to prepare a medicament that is useful for treating a variety of disorders, symptoms and diseases.

[0088] While one or more of the inventive compounds may be used in an application of monotherapy to treat a disorder, disease or symptom, they also may be used in combination therapy, in which the use of an inventive compound or composition (therapeutic agent) is combined with the use of one or more other therapeutic agents for treating the same and/or other types of disorders, symptoms and diseases. Combination therapy includes administration of the therapeutic agents concurrently or sequentially. Alternatively, the therapeutic agents can be combined into one composition which is administered to the patient.

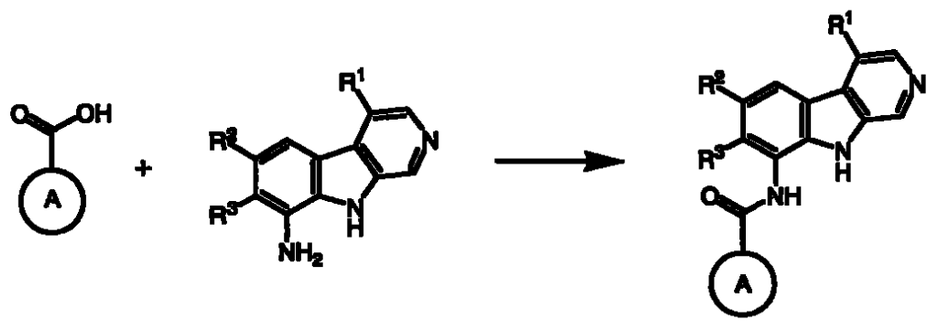
[0089] In one embodiment, the compounds of this invention are used in combination with other therapeutic agents, such as other inhibitors of IKK, other agents useful in treating NF-κB and TNF-α associated conditions, and agents useful for treating other disorders, symptoms and diseases. In particular, agents that induce apoptosis such as agents that disrupt cell cycle or mitochondrial function are useful in combination with the IKK inhibitors of this invention. Exemplary agents for combination with the IKK inhibitors include antiproliferative agents (e.g., methotrexate) and the agents disclosed in U.S. Pat. Application Publication No. US2003/0022898, p 14, para. [0173-0174], which is incorporated herein in its entirety. In some embodiments, a compound of the invention is administered in conjunction with a therapeutic agent

selected from the group consisting of cytotoxic agents, radiotherapy, and immunotherapy. Non-limiting examples of cytotoxic agents suitable for use in combination with the IKK inhibitors of the invention include capecitibine; gemcitabine; irinotecan; fludarabine; 5-fluorouracil or 5-fluorouracil/ leucovorin; taxanes, including, e.g., paclitaxel and docetaxel; platinum agents, including, e.g., cisplatin, carboplatin, and oxaliplatin; anthracyclins, including, e.g., doxorubicin and pegylated liposomal doxorubicin; mitoxantrone; dexamethasone; vincristine; toposide; prednisone; thalidomide; herceptin; temozolomide; and alkylating agents such as melphalan, chlorambucil, and cyclophosphamide. It is understood that other combinations may be undertaken while remaining within the scope of the invention.

[0090] Still another aspect of this invention is to provide a kit comprising separate containers in a single package, wherein the inventive pharmaceutical compounds, compositions and/or salts thereof are used in combination with pharmaceutically acceptable carriers to treat disorders, symptoms and diseases where IkB kinase plays a role.

[0091] The compounds of this invention may be prepared by methods known to those skilled in the art for analogous compounds, as illustrated by the general schemes below, and by reference to the preparative examples shown below.

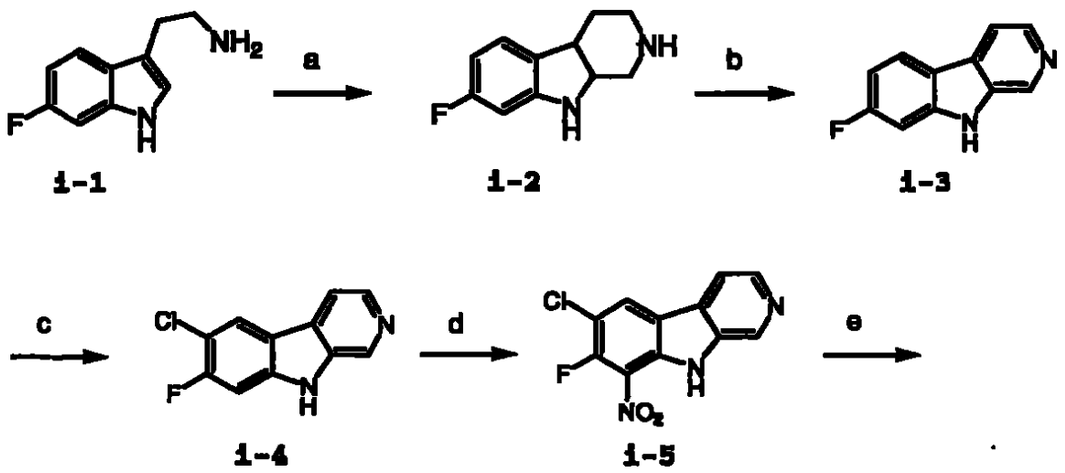
Scheme I



1a 2a I

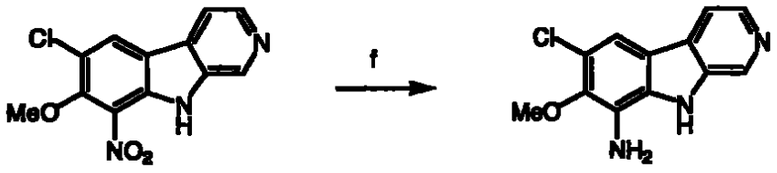
[0092] Scheme I above shows a general route for obtaining compounds of formula I. A Ring A carboxylic acid 1a may be coupled with the desired amino beta-carboline 2a to provide I. Many 1a intermediates that are useful for preparing compounds of this invention are readily available from known starting materials and chemical methods, especially in view of the synthetic examples detailed herein. Schemes II-IV describe routes for making the various β-carboline intermediates 2a.

Scheme II



i-1 i-2 i-3

i-4 i-5

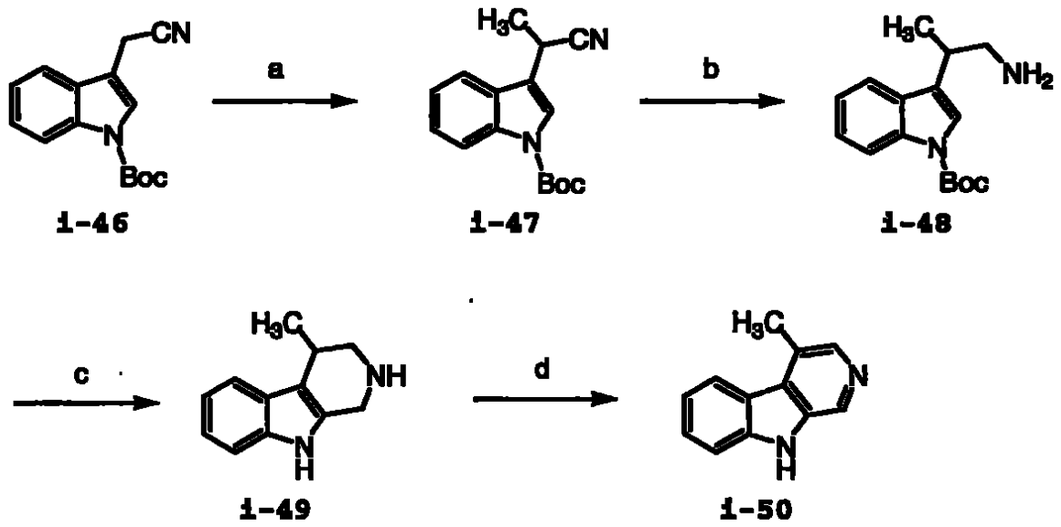


i-6 i-7

Steps: (a) (i) $HCOCO_2H$ (ii) HCl (b) Pd/C (c) NCS (d) $NaNO_2$ (e) NaOMe (f) Pt/ H_2

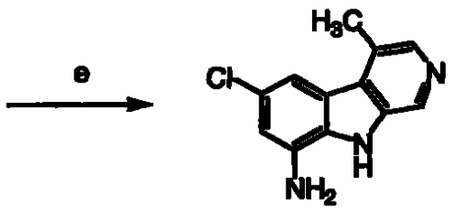
[0093] Scheme II above shows a route for making a beta-carboline moiety where R^1 is hydrogen, R^2 is chloro and R^3 is alkoxy. While the scheme is exemplified for R^3 being methoxy, it will be appreciated by one skilled in the art that beta-carbolines having other R^3 alkoxy groups may be obtained by replacing NaOMe in step (e) with other sodium or metal alkoxides.

Scheme III



i-46 i-47 i-48

i-49 i-50

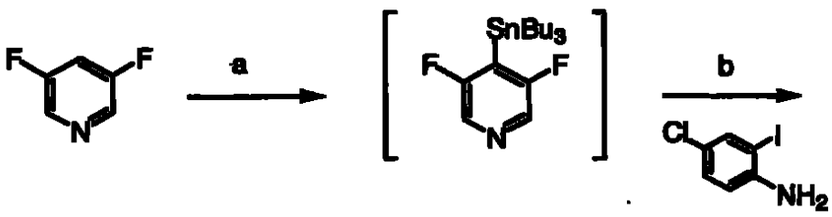


i-51

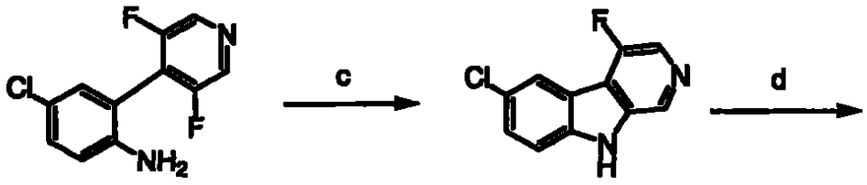
Steps: (a) NaHMDS, MeI (b) H_2, Raney Nickel, PtO (c) i. TFA ii. $CHOCO_2H$ iii. HCl (d) Pd/C, xylenes, 160 °C (e) i. NCS, 1N HCl ii. $NaNO_2$, TFA iii. H_2, Pt

[0094] Scheme III above shows a route for preparing a beta-carboline intermediate where R^1 is an alkyl such as methyl, R^2 is a halo such as chloro and R^3 is hydrogen. One skilled in the art will understand how the above scheme may be modified to obtain an R^1 alkyl group other than methyl r an R^2 halo group other than chloro.

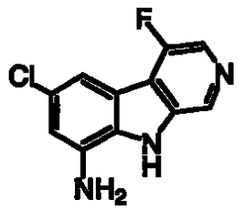
Scheme IV



i-63



i-64 i-65

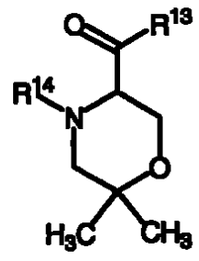


1-66

Steps: (a) i. LDA, THF, -78C, ii. $SnBu_3Cl$ (b) 5% $Pd(PPh_3)_2Cl_2$, 10% CuI, DMF, reflux overnight (c) 3eq NHMDS, THF, rt overnight (d) i. TFA/$NaNO_3$, ii. saturated $NaHCO_3$, iii. 5% Pt(S), NH_4O_2CH

[0095] Scheme IV above shows a route for making a beta-carboline intermediate where R^1 is fluoro, R^2 is chloro and R^3 is hydrogen. It will be appreciated that ready modification of this scheme will allow for the preparation of other intermediates. For example, another R^2 group may be introduced by replacing the 4-chloro-2-iodoaniline in step (b) with another 2-iodoaniline having a substituent other than chloro in the 4-position.

[0096] A particularly useful intermediate for making compounds of formula III-A-aa is intermediate 3a:

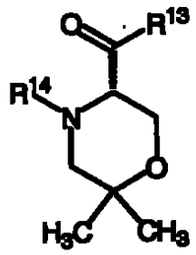


3a

where R^{13} is halo, OH, OR^{15}, or a carboxylic acid protecting group; R^{14} is an amino protecting group, hydrogen or -W-G as defined above; and R^{15} is an organic radical. Amino protecting groups are well-known in the art. Examples of suitable amino protecting groups include alkoxycarbonyl

groups such as t-butoxycarbonyl (t-BOC) and benzyl groups such as benzyl and para-methoxybenzyl. The carboxylic acid group at the 3-position of the morpholine ring may be protected as any stable ester group such as a simple alkyl or aryl ester such as a methyl, ethyl, benzyl, or pentafluorophenyl ester. In one embodiment, R^{14} is -W-G and R^{13} is -OH, halo, or a carboxylic acid protecting group. Various protecting groups are described in detail in Protecting Groups in Organic Synthesis, Theodora W. Greene and Peter G. M. Wuts, 3rd edition, 1999, published by John Wiley and Sons.

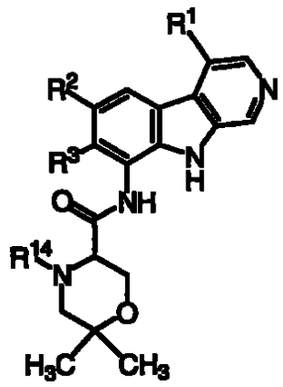
[0097] A preferred enantiomer of intermediate 3a is (*S*)-3a:



(*S*)-3a

where R^{13} and R^{14} are as described above.

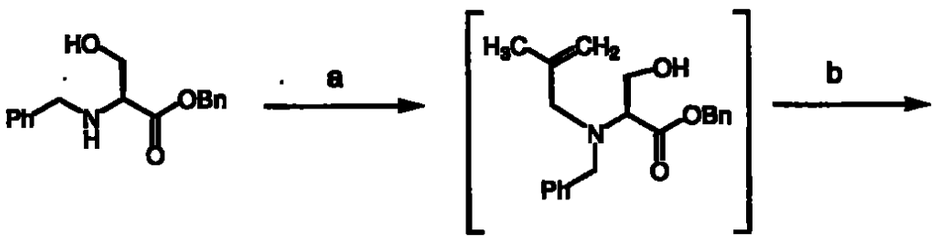
[0098] Intermediate 3a or (*S*)-3a, as the carboxylic acid or an activated form thereof (such as the acid chloride), may be coupled with an appropriate amino-beta-carboline as outlined in Scheme I above. When R^{14} is an amino protecting group, the amide coupling reaction provides further useful intermediates, shown below as compounds of formula IV:



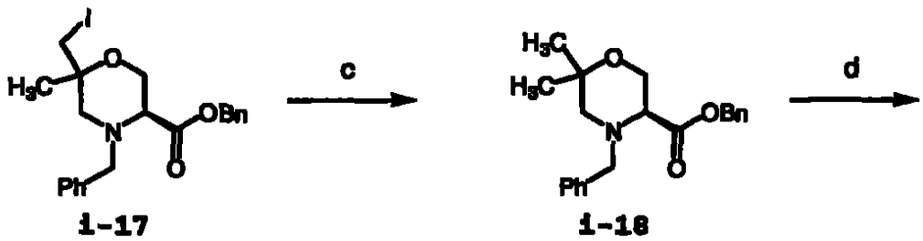
IV

where R^{14} is an amino protecting group and R^1, R^2 and R^3 are as described above. It will be appreciated by one of skill in the art that certain compounds of formula III-A-aa (where R^{6b} is each methyl) may be prepared from compounds of formula IV by removing the R^{14} protecting group and then attaching the -W-G portion using known methods. Alternatively, the compounds of formula III-A-aa may be prepared by first constructing intermediate 3a where R^{14} is -W-G and R^{13} is a carboxylic acid or derivative thereof. . The amide coupling reaction with an appropriate amino-beta-carboline then provides the compounds of formula III-A-aa directly.

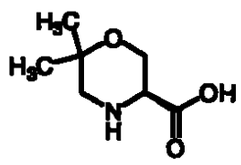
Scheme V



1-16



i-17 i-18

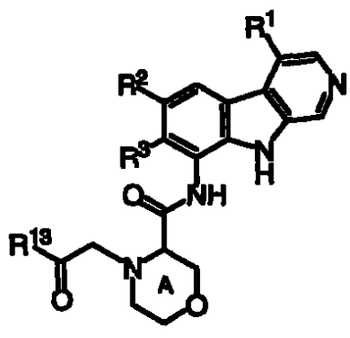


i-19

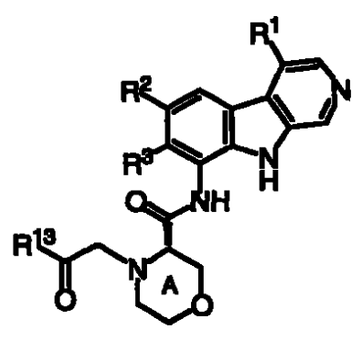
Steps: (a) K_2CO_3, KI, 3-Bromo-2-methyl-propene, MeCN (b) I_2, $NaHCO_3$, MeCN (c) Bu_3SnH, AIBN, toluene, 110°C (d) H_2, 20% $Pd(OH)_2/C$, 10% AcOH/MeOH

[0099] Scheme V above shows a route for making intermediates of formula 3a, including the unprotected i-19. The selective protection and deprotection of the amino and carboxylic acid groups in i-19 to provide various 3a intermediates will be within the knowledge of one skilled in the art.

[00100] Another useful intermediate for making compounds f formula III-A-aa is a compound of formula V, preferably (*S*)-V:



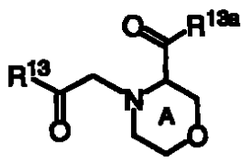
V



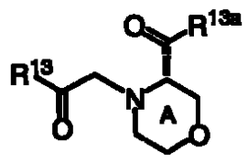
(*S*)-V

where R^{13} is halo or other leaving group, OH, OR^{15}, or a carboxylic acid protecting group, R^{15} is an organic radical such as a C_{1-6} aliphatic, aryl or benzyl, Ring A has 0-2 or 0-4 R^{6b}, and R^1, R^2 R^3, and R^{6b} are as defined above.

[00101] Another useful intermediate for making compounds of this invention is VI, preferably (*S*)-VI:



VI



(*S*)-VI

where one of R^{13} and R^{13a} is OH or a leaving group such as halo and the other is OR^{15} or a carboxylic acid protecting group, R^{15} is an organic radical such as a C_{1-6} aliphatic, aryl or benzyl, Ring A has 0-2, and R^{6b} is as defined above.

Synthesis Examples

[00102] The following abbreviations are used in the methods of preparation: RT or rt is room temperature; h, hr or hrs is hour or hours; min is minutes; TFA is trifluoroacetic acid; DMSO is dimethylsulfoxide; NCS is N-chlorosuccinimide; EDCI is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; EtOAc is ethyl acetate; DIEA is diisopropylethylamine; DCM is dichloromethane; DDQ is dichloro dicyano benzoquinone; mCPBA is meta-chloroperbenzoic acid; MeOH is methanol; EtOH is ethanol; MeCN is acetonitrile; TLC is thin layer chromatography; AIBN is azobisisobutyronitrile; NH_4OAc is ammonium acetate; NaOAc is sodium acetate; Et_2O is diethyl ether; AcOH is

acetic acid; and DMF is dimethylformamide. TBTU is N,N,N',N'-tetramethyl-o-(benzotriazol-1-yl)uranium tetrafluoroborate.

INTERMEDIATE 1: 7-fluoro-2,3,4,9-tetrahydro-1H-β-carboline-1-carboxylic acid

[00103] To 10 g (46.6 mmol) of commercially available 6-fluorotryptamine hydrochloride was added 50 ml of 1M acetate buffer (pH 4.4) to give a suspension that was stirred at room temperature (RT). A suspension of glyoxylic acid monohydrate (1.1 eq, 51.28 mmol, 4.72 g) in ethyl acetate was then added to the stirred suspension in one portion. The suspension was stirred overnight (16 h) at RT and the precipitated solid was collected by filtration and washed with both H_2O and ethyl acetate. The sample was then dried in vacuo to give a light yellow solid in quantitative yield.

^{1}H-NMR (300 MHz, acetic acid-d_4): δ 3.04 (m, 2H), 3.56 (m, 1H), 3.83 (m, 1H), 6.80 (m, 1H), 7.13 (dd, 1H), 7.34 (dd, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.17 min.

MS ($M+H^+$): 235.0.

INTERMEDIATE 2: 7-fluoro-2,3,4,9-tetrahydro-1H-β-carboline

[00104] 7-fluoro-2,3,4,9-tetrahydro-1H-β-carboline-1-carboxylic acid (5 g, 21.36 mmol) was suspended in 130 ml of 3N HCl in a 500 ml round-bottom flask and refluxed overnight (16 hr) with stirring. Upon cooling, a light brown solid precipitated out, which was collected by filtration and washed with H_2O. The salt obtained by filtration above was then dissolved in hot methanol (200

ml) and treated with 3M K_2CO_3 (5-10 ml) such that the pH is around 9. 100 ml of H_2O was added to this mixture, which was then allowed to stir at RT. The methanol was evaporated on a rotary evaporator to give a white aqueous suspension of the desired free base, which was collected by filtration (3.2 g, 79% yield).

^{1}H-NMR (300 MHz, methanol-d_4): δ 2.73 (t, 2H), 3.11 (t, 2H), 3.94 (s, 2H), 6.73 (m, 1H), 6.94 (m, 1H), 7.30 (dd, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.25 min.

MS ($M+H^+$): 191.1.

INTERMEDIATE 3: 7-fluoro-9H-β-carboline

[00105] 7-fluoro-2,3,4,9-tetrahydro-1H-β-carboline (3.5 g, 18.42 mmol) was suspended in xylenes (60 ml) in a 250 ml round-bottom flask equipped with a condenser that was open to the atmosphere, and heated. To this hot reaction mixture was added Pd/C (10 wt%, 0.2 eq, 700 mg) and the mixture refluxed in xylenes overnight (12-14 hours). The solution was then filtered through a pad of celite and the collected filtrate was then evaporated on a rotary evaporator to give the desired product as a brown/tan solid (3.0 g, 88% yield).

^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.10 (m, 1H), 7.37 (dd, 1H), 8.10 (d, 1H), 8.28 (dd, 1H), 8.35 (dd, 1H), 8.89 (s, 1H), 11.74 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.88 min.

MS ($M+H^+$): 187.1.

INTERMEDIATE 4: 6-chloro-7-fluoro-9H-β-carboline

[00106] 7-fluoro-9H-β-carboline (2.15 g, 11.58 mmol) was suspended in 100 ml of 1N HCl. To this mixture was added NCS (1.85 g, 13.89 mmol, 1.2 eq) and the resulting mixture was stirred at RT overnight. The reaction mixture was then filtered to give a light yellow solid (2.1 g, 83% yield).
^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.86 (d, 1H), 8.64 (d, 1H), 8.79 (d, 1H), 8.91 (d, 1H), 9.33 (s, 1H), 13.05 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 2.19 min.
MS ($M+H^+$): 221.1.

INTERMEDIATE 5: 6-chloro-7-fluoro-8-nitro-9H-β-carboline

[00107] 6-chloro-7-fluoro-9H-β-carboline (2.1 g, 9.54 mmol) was taken in a round-bottom flask (250 ml) and $NaNO_3$ (1.136 g, 13.36 mmol, 1.4 eq) was added. TFA (48 ml) was then added to the flask and the resulting mixture refluxed overnight. The TFA is then removed on a rotary evaporator. The resulting slurry is suspended in water (50 ml) and sonicated thoroughly. The resulting suspension is then filtered to give a yellow solid (2.0 g, 80% yield).
^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.21 (d, 1H), 8.46 (d, 1H), 9.04 (m, 2H), 12.55 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 2.24 min.
MS ($M+H^+$): 266.2.

INTERMEDIATE 6: 6-chloro-7-methoxy-8-nitro-9H-β-carboline

[00108] Methanol (0.462 ml, 11.4 mmol) was added to a stirring suspension of NaH (684 mg, 17.1 mmol) in DMF (10 ml) under an argon atmosphere. The resulting solution was allowed to stir at RT for 20 min. 6-chloro-7-fluoro-8-

nitro-9H-β-carboline (500 mg, 1.9 mmol) was added to the stirring solution and the resulting mixture was allowed to stir at RT. Upon addition of H_2O, a brown solid precipitated out which was filtered to give the desired 6-chloro-7-methoxy-8-nitro-9H-β-carboline (510 mg, 97% yield).

^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.02 (s, 3H), 8.52 (d, 1H), 8.60 (d, 1H), 9.05 (s, 1H), 9.12 (s, 1H), 12.78 (b, 1H).

Retention Time (LC, method: ammonium acetate standard): 2.28min.

MS ($M+H^+$): 278.

INTERMEDIATE 7: 6-chloro-7-methoxy-9H-β-carboline-8-ylamine

[00109] 6-chloro-7-methoxy-8-nitro-9H-β-carboline (510 mg, 1.84 mmol) was suspended in 50 ml of methanol and 100 mg of Pd/C (10%) was added. The flask was fitted with a balloon of hydrogen and the reaction mixture was stirred overnight at RT. Upon filtration through a pad of celite and evaporation of the methanol, a dark brown solid was obtained. This residue was suspended in methanol (15 ml) and added, with vigorous stirring, to a solution of saturated $NaHCO_3$ (100 ml). The light brown solid that precipitated out was collected by filtration and dried thoroughly in vacuo to give the desired product (512 mg, quantitative yield).

^{1}H-NMR (300 MHz, methanol-d_4): δ 3.90 (s, 3H), 7.63 (s, 1H), 8.11 (d, 1H), 8.27 (d, 1H), 8.84 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.12 min.

MS ($M+H^+$): 248.

INTERMEDIATE 8: 6-chloro-9H-β-carboline-8-ylamine

[00110] The target compound was prepared according to the procedures outlined for *Intermediate 1* to *Intermediate 7* where the starting material for *Intermediate 1* was unsubstituted tryptamine. An alternative synthesis for 6-chloro-9H-β-carboline-8-ylamine is described on page 34, example 15 of PCT Application Publication No. WO 01/68648 A1, which is incorporated herein in its entirety.

METHOD A: COUPLING PROCEDURE FOR 6,7,8-SUBSTITUTED-β-CARBOLINES

[00111] 6,7,8-substituted-9H-β-carboline (1 mmol), EDCI (1.6 mmol) and the appropriate carboxylic acid (1.2 mmol) were taken in a round-bottom flask and suspended in pyridine (5 ml). The resulting mixture was heated at 60°C overnight. The pyridine was then removed by rotary evaporation and 5% Na_2CO_3 solution was added. The resulting solid that precipitated out was collected by filtration. Chromatographic purification gave the desired product.

METHOD B: COUPLING PROCEDURE FOR 6,8-SUBSTITUTED-β-CARBOLINES

[00112] 6,8-substituted-9H-β-carboline (1.0 mmol), EDCI (1.6 mmol) and the carboxylic acid (1.2 mmol) to be coupled were taken in a round-bottom flask and suspended in pyridine (5 ml). The resulting mixture was stirred overnight. The pyridine was then removed by rotary evaporation and 5% Na_2CO_3 solution was added. The resulting solid that precipitated out was collected by

filtration. Chromatographic purification gave the desired product.

EXAMPLE 1: N-(6-chloro-7-methoxy-9H-β-carbolin-8-yl)-2-methyl-nicotinamide

[00113] 6-chloro-7-methoxy-9H-β-carbolin-8-ylamine (100 mg, 0.4 mmol), EDCI (125 mg, 0.64 mmol) and 2-methyl nicotinic acid (66 mg, 0.48 mmol) were taken in a round-bottom flask and suspended in pyridine (2 ml). The resulting mixture was heated at 80°C overnight. The pyridine was then removed by rotary evaporation and 5% Na_2CO_3 solution was added. The resulting solid that precipitated out was collected by filtration. Chromatographic purification gave the desired product in 50-70% yield.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.71 (s, 3H), 3.89 (s, 3H), 7.45 (dd, 1H), 8.15 (d, 1H), 8.21 (d, 1H), 8.38 (d, 1H), 8.45 (s, 1H), 8.61 (d, 1H), 8.92 (s, 1H), 10.33 (s, 1H), 11.57 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.77 min.

MS ($M+H^+$): 367.1.

EXAMPLE 2: 4-methyl-pyrimidine-5-carboxylic acid (6-chloro-7-methoxy-9H-beta-carbolin-8-yl)-amide

[00114] The desired compound was prepared according to *Method A* from 6-chloro-7-methoxy-9H-beta-carbolin-8-ylamine and 4-methyl-5-pyrimidine carboxylic acid in 80% yield.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.75 (s, 3H), 3.92 (s, 3H), 8.19 (d, 1H), 8.41 (d, 1H), 8.50 (s, 1H), 8.96 (s, 1H), 9.20 (s, 1H), 9.25 (s, 1H), 10.63 (s, 1H), 11.67 (s,1H).

Retention Time (LC, method: formic acid standard): 0.95 min.
MS ($M+H^+$): 368.

INTERMEDIATE 9: 6-chloro-7-ethoxy-8-nitro-9H-β-carboline

[00115] Sodium ethoxide (232 mg, 3.4 mmol) was added to a solution of 6-chloro-7-fluoro-8-nitro-9H-β-carboline (200 mg, 0.76 mmol) in DMSO (4 ml) and the reaction mixture allowed to stir overnight. The reaction mixture was diluted with water and the pH of the solution was adjusted to about 4 by adding 1N HCl. The aqueous solution was extracted (3X) with EtOAc. The combined EtOAc layers were dried and evaporated. The crude product was purified by flash chromatography to give the desired product in 40-60% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.44 (t, 3H), 4.24 (q, 2H), 8.21 (d, 1H), 8.46 (d, 1H), 8.91 (s, 1H), 9.02 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 2.37min.
MS ($M+H^+$): 291.9.

INTERMEDIATE 10: 6-chloro-7-ethoxy-9H-β-carbolin-8-ylamine

[00116] 6-chloro-7-ethoxy-8-nitro-9H-β-carboline (160 mg, 0.55 mmol) was suspended in 4 ml of methanol and 25 mg of Pd/C (10%) was added. The flask was fitted with a balloon f hydrogen and the reaction mixture was stirred overnight at RT. Upon filtration through a pad of celite and evaporation of the methanol, a dark brown solid was obtained and determined to be the desired 6-chloro-7-cyclopropylmethoxy-9H-β-carbolin-8-ylamine (80 mg, 55%).

^{1}H-NMR (300 MHz, Methanol-d_4/$CDCl_3$): δ 1.26 (t, 3H), 3.91 (q, 2H), 7.34 (s, 1H), 7.71 (d, 1H), 8.02 (s, 1H), 8.56 (s, 1H).

Retention Time (LC, method: formic acid standard): 1.16 min.

MS ($M+H^+$): 262.0.

EXAMPLE 3: N-(6-chloro-7-ethoxy-9H-β-carbolin-8-yl)-2-methyl-nicotinamide

[00117] The desired compound was prepared according to *Method A* from 6-chloro-7-ethoxy-9H-β-carbolin-8-ylamine and 2-methylnicotinic acid in 40% yield.

^{1}H-NMR (300 MHz, MeOH-d_4): δ 1.39 (t, 3H), 2.76 (s, 3H), 4.14 (q, 2H), 7.43 (dd, 1H), 8.05 (d, 1H), 8.26 (m, 3H), 8.55 (d, 1H), 8.79 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.98 min.

MS ($M+H^+$): 381.3.

INTERMEDIATE 11: 6-chloro-7-(N,N)-dimethylamino-8-nitro-9H-β-carboline

[00118] N,N-dimethylamine hydrochloride (278 mg, 3.4 mmol) was added to a stirring solution of 6-chloro-7-fluoro-8-nitro-9H-β-carboline (300 mg, 1.13 mmol) in DMSO (8 ml) under an argon atmosphere. This was followed by the addition of DIEA (0.83 ml, 4.65 mmol) and the reaction mixture was heated at 60°C overnight. After allowing the reaction mixture to cool to RT, water was added and a dark orange solid precipitated out. The solid was filtered, washed with water and dried to give the desired product (230 mg, 70% yield).

^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.06 (s, 6H), 7.23 (d, 1H), 7.53 (d, 1H), 7.66 (s, 1H), 8.05 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 2.41 min.
MS ($M+H^+$): 290.9.

INTERMEDIATE 12: 6-chloro-7-(N,N)-dimethylamino-9H-β-carboline-8-ylamine

[00119] 6-chloro-7-(N,N)-dimethylamino-8-nitro-9H-β-carboline (828 mg, 2.86 mmol) was suspended in 30 ml of methanol and 166 mg of Pd/C (10%) was added. The flask was fitted with a balloon of hydrogen and the reaction mixture was stirred overnight at ambient temperature. Upon filtration through a pad of celite and evaporation of the methanol, a dark brown solid was obtained and determined t be the desired 6-chloro-7-(N,N)-dimethylamino-9H-β-carboline-8-ylamine (500 mg, 67% yield).
^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 6H), 5.42 (s, 2H), 7.45 (s, 1H), 7.95 (d, 1H), 8.23 (d, 1H), 8.86 (d, 1H).
Retention Time (LC, method: ammonium acetate standard): 2.32 min.
MS ($M+H^+$): 261.1.

EXAMPLE 4: N-(6-chloro-7-7-(N,N)-dimethylamino-9H-β-carbolin-8-yl)-2-methyl-nicotinamide

[00120] The desired compound was prepared according to *Method A* from 6-chloro-7-(N,N)-dimethylamino-9H-β-carboline-8-ylamine and 2-methylnicotinic acid in 40-60% yield.
^{1}H-NMR (300 MHz, Methanol-d_4/$CDCl_3$): δ 2.82 (s, 3H), 2.94 (s, 6H), 7.33 (m, 1H), 8.02 (m, 3H), 8.34 (d, 1H), 8.61 (d, 1H), 8.95 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 2.29 min.

MS ($M+H^+$): 380.3.

INTERMEDIATE 13: 6-chloro-7-(4-methyl-piperazin-1-yl)-8-nitro-9H-β-carboline

[00121] To a DMSO solution (4 ml) of 200 mg (0.755 mmol) of 6-chloro-7-fluoro-8-nitro-9H-β-carboline was added 1-methylpiperazine (226 mg, 2.26 mmol) and DIEA (400 mg, 3.09 mmol) via a syringe. The reaction was allowed to stir at RT overnight. Upon addition of water, an orange solid precipitated out. The solid was filtered, washed and dried to give 236 mg (91% yield) of the desired 6-chloro-7-(4-methyl-piperazin-1-yl)-8-nitro-9H-β-carboline.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.24 (s, 3H), 2.48 (m, 4H), 3.13 (m, 4H), 8.13 (d, 1H), 8.40 (d, 1H), 8.73 (s, 1H), 8.94 (s, 1H), 12.05 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.72min.

MS ($M+H^+$): 346.

INTERMEDIATE 14: 6-chloro-7-(4-methyl-piperazin-1-yl)-8-amino-9H-β-carboline

[00122] Suspended 236 mg of 6-chloro-7-(4-methyl-piperazin-1-yl)-8-nitro-9H-β-carboline in 100ml of methanol and added 10% Pd/C (48 mg) under argon. The flask was flushed with hydrogen (3X) and the reaction mixture was stirred under a hydrogen atmosphere at RT overnight. The reaction mixture was filtered and Pd/C was removed using a pad of celite. The reaction mixture was evaporated to remove solvent and purified by flash chromatography to give

119 mg (55% yield) of the desired 6-chloro-7-(4-methyl-piperazin-1-yl)-8-amino-9H-β-carboline.

^{1}H-NMR (300 MHz, methanol-d_4): δ 2.39 (s, 3H), 2.46 (m, 2H), 2.87 (m, 4H), 3.83 (m, 2H), 7.50 (s, 1H), 7.97 (d, 1H), 8.24 (d, 1H), 8.78 (s, 1H).

Retention Time (LC, method: ammonium acetate polar): 1.32min.

MS ($M+H^+$): 316.

EXAMPLE 5: 2-chloro-N-[6-chloro-7-(4-methyl-piperazin-1-yl)-9H-β-carbolin-8-yl]-nicotinamide

[00123] The desired compound was prepared according to *Method A* from 6-chloro-7-(4-methyl-piperazin-1-yl)-8-amino-9H-β-carboline and 2-chloro-nicotinic acid in 25% yield.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.21 (s, 3H), 2.33 (m, 2H), 2.54(m, 2H), 3.24 (m, 4H), 7.73 (dd, 1H), 8.12 (d, 1H), 8.35 (d, 1H), 8.37 (s, 1H), 8.48 (dd, 1H), 8.61 (dd, 1H), 8.91 (s, 1H), 10.40 (s, 1H), 11.33 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.46min.

MS ($M+H^+$): 455.

INTERMEDIATE 15: Resolution of rac-terebic acid

[00124] Terebic acid was dissolved in a 10:1 mixture of EtOAc-MeOH [10 g of terebic acid (17.7 g of salt) in 550 ml] and heated to 50-55°C, followed by addition of (S)-(-)-α-methyl-benzylamine. The reaction mixture was stirred for 2 minutes and then left at RT for 15 minutes. The reaction mixture was then seeded with enriched salt (prepared on a smaller scale using 3 recrystallization cycles), sonicated for 10-15 seconds and left at RT overnight. The s lid was

filtered off, washed with EtOAc and dried under vacuum. Recrystallization was done in the same solvent mixture by re-dissolving the salt (24 mg/ml). This mixture was then heated to a gentle reflux for a short period of time (few crystals remained in suspension). The mixture was left at RT over night. The solid was processed as previously described.

[00125] Enantiomeric excess was determined in a crude fashion using proton NMR of the corresponding amide obtained from TBTU coupling.

[00126] *Regeneration of (R)-(+)-terebic acid*: The salt was dissolved in water (320 mg/ml), heated to 65°C and 1.2 equivalent of aqueous 6M HCl was added. The reaction mixture was then left at 4°C overnight. The solid was filtered off, washed with small portions of cold water and dried under a high vacuum (yield of 25-30%).

^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.30 (s, 3H), 1.52 (s, 3H), 2.74 (dd, 1H), 2.85 (dd, 1H), 3.25 (t, 1H).

Retention Time (LC, method: ammonium acetate standard): 0.33 min.

MS ($M+H^+$): 159.0.

EXAMPLE 6: 2,2-dimethyl-5-oxo-tetrahydro-furan-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00127] The desired compound was prepared according to *Method B* from 6-chloro-9H-β-carboline-8-ylamine and (R)-terebic acid (*Intermediate 15*) in 80-90% yield.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.44 (s, 3H), 1.59 (s, 3H), 2.91 (dd, 1H), 3.08 (dd, 1H), 3.38 (dd, 1H), 7.85 (m, 1H), 8.17 (d, 1H), 8.25 (d, 1H), 8.39 (d, 1H), 9.05 (s, 1H), 10.26 (s, 1H), 11.72 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.22 min.
MS ($M+H^+$): 358.3.

EXAMPLE 7: 1,2,2-trimethyl-5-oxo-pyrrolidine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00128] The desired compound was prepared according to *Method B* from 6-chloro-9H-β-carboline-8-ylamine and 1,2,2-trimethyl-5-oxo-pyrrolidine-3-carboxylic acid in 68% yield. ^{1}H-NMR (DMSO-d6, 300MHz) δ 1.23 (s, 3H), 1.45 (s, 3H), 2.59 (dd, 1H),2.66(s, 3H), 2.70 (dd, 1H), 3.17 (t, 1H), 7.91 (m, 1H), 8.18 (d, 1H), 8.24 (d, 1H), 8.40 (d, 1H), 9.07 (s, 1H), 10.16 (s, 1H), 11.32 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 1.17 min.
MS ($M+H^+$): 371.3.

INTERMEDIATE 16: N-benzyl-serine benzyl ester

[00129] To a mixture of L-serine-benzyl ester-HCl (2.3 g), benzaldehyde(1.05 eq.) and sodium acetate (1 eq.) in methanol was added sodium cyanoborohydride (1.0 eq.). The resulting mixture was stirred at ambient temperature for 15 hrs, then partitioned into ether and aqueous saturated sodium bicarbonate. The separated organic phase was extracted with 1M HCl (3X). Combined aqueous extracts were washed with ether, basified with aqueous 4.5M K_2CO_3 and extracted with ether. Combined organic extracts were washed with brine, dried over sodium sulfate and concentrated to dryness to give 2.32 g of the desired compound (waxy solid, 81% yield).

^{1}H-NMR (300MHz, $CDCl_3$): δ 3.48 (dd, 1H), 3.64 (dd, 1H), 3.74 (d, 1H), 3.80 (dd, 1H), 3.88 (d, 1H), 5.18 (s, 2H), 7.25-7.39 (m, 10H).

MS ($M+H^+$): 286.

INTERMEDIATE 17: 4-benzyl-6-iodomethyl-6-methyl-morpholine-3-carboxylic acid benzyl ester

[00130] To a solution of N-benzyl-serine benzyl ester (*Intermediate 16*, 6.35 g) in 90 ml of MeCN at ambient temperature was added 3-bromo-2-methyl-propene (5.6 ml), KI (740 mg) and K_2CO_3 (7.7 g). The reaction mixture was stirred at ambient temperature for 72 hrs. 1 ml of 3-bromo-2-methyl-propene was added and the reaction mixture was stirred for another 15 hrs. Only a small amount of starting material remained based on TLC (1:1; EtOAc-hexane). To the resulting mixture was added 11.2 g of iodine. After 4 hrs of stirring, TLC (10% EtOAc/hexane) showed complete conversion. The reaction mixture was partitioned into ether (300 ml) and 0.5 M $Na_2S_2O_3$ (100 ml). The separated organic phase was washed successively with water, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to dryness. The residue was purified on silica (5% EtOAc/Hexane) to give 6.65 g (yellowish oil, 64% yield, about 4:1 mixture) of compound 4-benzyl-6-iodomethyl-6-methyl-morpholine-3-carboxylic acid benzyl ester. Major Component: ^{1}H-NMR (300MHz, $CDCl_3$): δ 1.22 (s, 3H), 2.50 (d, 1H), 3.16-3.36 (m, 3H), 3.75-3.95 (m, 4H), 4.06 (dd, 1H), 5.16 (d, 1H), 5.21 (d, 1H), 7.28-7.36 (m, 10H).

MS ($M+H^+$): 466.

INTERMEDIATE 18: 4-benzyl-6,6-dimethyl-morpholine-3-carboxylic acid benzyl ester

[00131] To a solution of 4-benzyl-6-iodomethyl-6-methyl-morpholine-3-carboxylic acid benzyl ester (*Intermediate 17*, 1.23 g) and tributyltin hydride (1.8 ml, 2.5 eq.) in 11 ml of toluene under gentle reflux was added over 1.5 hr a solution of AIBN in toluene (25 mg/1ml). The mixture was allowed to cool down and was concentrated to dryness. The residue was partitioned into 15% 1M HCl in acetonitrile and hexane. The separated hexane phase was extracted two times with the acetonitrile solution. The combined acetonitrile solutions were washed with hexane two times and concentrated. The residue was partitioned into ether and 1M K_2CO_3. The separated ether phase was washed successively with 0.4M $Na_2S_2O_3$ and brine, dried over $MgSO_4$ and concentrated. The residue was purified on silica (7.5% EtOAc/hexane) to give 760 mg (oil, 85% yield) of compound 4-benzyl-6,6-dimethyl-morpholine-3-carboxylic acid benzyl ester.

^{1}H-NMR (300MHz, $CDCl_3$): δ 1.21 (s, 3H), 1.24 (s, 3H), 2.12 (d, 1H), 2.84 (d, 1H), 3.29 (t, 1H), 3.58 (d, 1H), 3.95-4.05 (m, 2H), 5.15 (d, 1H), 5.21 (d, 1H), 7.28-7.36 (m, 10H).

MS ($M+H^+$):340.

INTERMEDIATE 19: 6,6-dimethyl-morpholine-3-carboxylic acid

[00132] To a solution of 4-benzyl-6,6-dimethyl-morpholine-3-carboxylic acid benzyl ester (*Intermediate 18*, 1.25 g) in 40 ml of 10% AcOH/MeOH (under nitrogen) was added 250 mg of 20% $Pd(OH)_2$ on charcoal. The reaction mixture was purged with hydrogen (balloon) and was stirred at ambient temperature for 72 hrs. To the resulting gray

mixture was added 4 ml of water to help dissolution. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was co-evaporated with ethanol (2X) and then triturated with EtOAc. The generated white solid was filtered off and dried under high vacuum to give 559 mg of 6,6-dimethyl-morpholine-3-carboxylic acid (95% yield).

^{1}H-NMR (300MHz, D_2O): δ 1.35 (s, 3H), 1.38(s, 3H), 3.11 (d, 1H), 3.32 (d, 1H), 3.81-3.87 (m, 1H), 4.05 (bt, 1H), 4.17 (bd, 1H).

MS ($M+H^+$): 160.

INTERMEDIATE 20: 4,6,6-trimethyl-morpholine-3-carboxylic acid

[00133] To a suspension of 6,6-dimethyl-morpholine-3-carboxylic acid (*Intermediate 19*, 540 mg) in 17 ml of ethanol (under nitrogen) was added 100 mg of 10% Pd on charcoal and 830 ul (~3 eq.) of 37% aqueous formaldehyde. The mixture was purged with hydrogen (balloon) and stirred at ambient temperature for 5 hrs. To the resulting gray mixture were added 4 ml of water and 4 ml of methanol to help dissolution. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was triturated with EtOAc. The generated white solid was filtered off and dried under high vacuum to give 574 mg of 4,6,6-trimethyl-morpholine-3-carboxylic acid (97% yield).

^{1}H-NMR (300MHz, D_2O): δ 1.35 (s, 3H), 1.43(s, 3H), 2.94(s, 3H), 3.08 (d, 1H), 3.41 (d, 1H), 3.68 (dd, 1H), 3.96 (t, 1H), 4.14 (dd, 1H).

MS ($M+H^+$): 174.

EXAMPLE 8: 4,6,6-trimethyl-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00134] The desired compound was prepared according to *Method B* from 6-chloro-9H-β-carboline-8-ylamine and 4,6,6-trimethyl-morpholine-3-carboxylic acid in 61% yield.
^{1}H-NMR (DMSO-d6, 300MHz) δ 1.15 (s, 3H), 1.39 (s, 3H), 1.98 (d, 1H), 2.26 (s, 3H), 2.72 (d, 1H), 2.80 (dd, 1H), 3.79(m, 2H), 7.91 (s, 1H), 8.03-8.08 (m, 2H), 8.22 (d, 1H), 8.97 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 1.33 min.
MS ($M+H^+$): 373.2.

EXAMPLE 9: 2,2-Dimethyl-5-oxo-tetrahydro-furan-3-carboxylic acid (6-chloro-7-methoxy-9H- β-carbolin-8-yl)-amide

[00135] The desired compound was prepared according to *Method A* from 6-chloro-7-methoxy-9H-β-carboline-8-ylamine and (R)-terebic acid in a 60-80% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.48 (s, 3H), 1.61 (s, 3H), 3.03 (m, 2H), 3.51 (m, 1H), 3.86 (s, 3H), 8.16 (m, 1H), 8.37 (m, 1H), 8.43 (s, 1H), 8.94 (s, 1H), 10.10 (s, 1H), 11.33 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 1.94 min.
MS ($M+H^+$): 388.

INTERMEDIATE 21: 6-chloro-7-cyclopropylmethoxy-8-nitro-9H-β-carboline

[00136] Cyclopropylmethyl alcohol (0.921 ml, 11.4 mmol) was added to a stirring suspension of NaH (455 mg, 11.4 mmol) in DMF (20 ml) under an argon atmosphere. The resulting solution was allowed to stir at RT for 20 min.

6-chloro-7-fluoro-8-nitro-9H-β-carboline (500 mg, 1.9 mmol) was added to the stirring solution and the resulting mixture was allowed to stir at RT. Upon addition of H_2O, a brown solid precipitated out which was filtered to give the desired 6-chloro-7-cyclopropylmethyoxy-8-nitro-9H-β-carboline (510 mg, 85%).

^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.35 (m, 2H), 0.59 (m, 2H), 1.32 (m, 1H), 4.04 (d, 2H), 8.21 (d, 1H), 8.46 (d, 1H), 8.90 (s, 1H), 9.02 (s, 1H), 12.32 (b, 1H)

Retention Time (LC, method: ammonium acetate standard): 2.63min.

MS ($M+H^+$): 318.

INTERMEDIATE 22: 6-chloro-7-cyclopropylmethoxy-9H-β-carbolin-8-ylamine

[00137] 6-chloro-7-cyclopropylmethoxy-8-nitro-9H-β-carboline (510 mg, 1.61 mmol) was suspended in 12 ml of methanol and 100 mg of Pd/C (10%) was added. The flask was fitted with a balloon of hydrogen and the reaction mixture was stirred overnight at RT. Upon filtration through a pad of celite and evaporation of the methanol, a dark brown solid was obtained. This residue was suspended in methanol (10 ml) and added, with vigorous stirring, to a solution of saturated $NaHCO_3$ (100 ml). The light brown solid that precipitated out was collected by filtration and dried thoroughly in vacuo to give the desired 6-chloro-7-cyclopropylmethoxy-9H-β-carbolin-8-ylamine (371 mgs, 80% yield).

^{1}H-NMR (300 MHz, methanol-d_4): δ 0.36 (m, 2H), 0.61 (m, 2H), 1.37 (m, 1H), 3.88 (d, 2H), 7.58 (s, 1H), 7.96(d, 1H), 8.23 (d, 1H), 8.76 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 2.28min.

MS ($M+H^+$): 288.

EXAMPLE 10: N-(6-chloro-7-cyclopropylmethoxy-9H-β-carbolin-8-yl)-2-methyl-nicotinamide

[00138] The desired compound was prepared according to *Method A* from 6-chloro-7-cyclopropylmethoxy-9H-β-carbolin-8-ylamine and 2-methylnicotinic acid in a 40-60% yield.

^{1}H-NMR (300 MHz, MeOH-d_4): δ 0.29 (m, 2H), 0.55 (m, 2H), 1.29 (m, 1H), 2.78 (s, 3H), 3.95 (m, 2H), 7.46 (dd, 1H), 8.08 (m, 1H), 8.30 (m, 3H), 8.59 (m, 1H), 8.81 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 2.18 min.

MS ($M+H^+$): 405.

INTERMEDIATE 23: 6-chloro-7-(N,N)-dimethylaminoethoxy-8-nitro-9H-β-carboline

[00139] N,N-dimethylaminoethyl alcohol (6.0 eq) was added to a stirring suspension of NaH (6.0 eq) in DMF under an argon atmosphere. The resulting solution was allowed to stir at RT for 20 min. 6-chloro-7-fluoro-8-nitro-9H-β-carboline (1.0 eq) was added to the stirring solution and the resulting mixture was allowed to stir at RT. Upon addition of H_2O, a brown solid precipitated out which was filtered to give the desired 6-chloro-7-(N,N)-dimethylaminoethoxy-8-nitro-9H-□-carboline (quantitative yield).

^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.23 (s, 6H), 2.74 (t, 2H), 4.28 (t, 2H), 8.21(d, 1H), 8.46 (d, 1H), 8.90 (s, 1H), 9.02 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.26 min.

MS ($M+H^+$): 335.

INTERMEDIATE 24: 6-chloro-7-(N,N)-dimethylaminoethoxy-9H-β-carbolin-8-ylamine

[00140] 6-chloro-7-(N,N)-dimethylaminoethoxy-8-nitro-9H-β-carboline (500 mg, 1.5 mmol) was suspended in 12 ml of methanol and 100 mg of Pd/C (10%) was added. The flask was fitted with a balloon of hydrogen and the reaction mixture was stirred overnight at RT. Upon filtration through a pad of celite and evaporation of the methanol, a dark brown solid was obtained. The residue was suspended in methanol (10 ml) and added, with vigorous stirring, to a solution of saturated $NaHCO_3$ (100 ml). The solid that precipitated out was collected by filtration and dried thoroughly in vacuo to give the desired 6-chloro-7-(N,N)-dimethylaminoethoxy-9H-β-carboline-8-ylamine (380 mgs, 83%).

^1H-NMR (300 MHz, methanol-d_4): δ 2.43 (s, 6H), 2.84 (t, 2H), 4.11 (t, 2H), 7.47 (s, 1H), 7.88 (d, 1H), 8.20 (d, 1H), 8.72 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.34min.

MS ($M+H^+$): 305.

EXAMPLE 11: N-[6-cloro-7-(2-dimethylamino-ethoxy)-9H-β-carbolin-8-yl]-2-methyl-nicotinamide

[00141] The desired compound was prepared according to *Method A* from 6-chloro-7-(N,N)-dimethylaminoethoxy-9H-β-carboline-8-ylamine and 2-methylnicotinic acid in a 40-60% yi 1d.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.92 (s, 6H), 2.49 (m, 2H), 2.68 (s, 3H), 4.25 (m, 2H), 7.50 (dd, 1H), 8.16 (m, 2H), 8.38 (d, 1H), 8.42 (s, 1H), 8.66 (m, 1H), 9.00 (s, 1H), 11.27 (s, 1H), 11.78 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 1.52 min.
MS ($M+H^+$): 424.

EXAMPLE 12: 2-amino-cyclopentanecarboxylic acid (6-chloro-7-methoxy-9H-β-carbolin-8-yl)-amide

[00142] 6-chloro-7-methoxy-9H-β-carboline-8-ylamine and 2-tert-butoxycarbonylamino-cyclopentanecarboxylic acid were reacted using *Method A*. To this product was added ~5ml of 4N HCl/dioxane and the resulting mixture was allowed to stir at RT. The reaction was followed by LC-MS until completion. Evaporation was allowed to remove all the solvent which gave a crude HCl salt. The desired product was then purified by preparative HPLC.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.68 (m, 1H), 1.84 (m, 2H), 2.06 (m, 2H), 2.20 (m, 1H), 3.46 (m, 1H), 3.67 (m, 1H), 3.89 (s, 3H), 8.24 (d, 2H), 8.61 (d, 1H), 8.72 (s, 1H), 8.78 (d, 1H), 9.19 (s, 1H), 10.58 (s, 1H), 13.20 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 1.54 min.
MS ($M+H^+$): 359.

INTERMEDIATE 25: 1-(2-dimethylamino-ethyl)-5-oxo-pyrrolidine-3-carboxylic acid

[00143] A mixture of commercially available itaconic acid and N,N-dimethylethylenediamine was heated up to 160 °C for about 20-25 minutes. The mixture was allowed to cool to 100 °C and then diluted with MeOH to prevent

solidification. The product was obtained in a 56% yield after crystallization from MeOH/EtOAc.

^{1}H-NMR (300MHz, D_2O): δ 2.63 (dd, 1H), 2.80 (dd, 1H), 2.95 (s, 6H), 3.15-3.25 (m, 1H), 3.32-3.44 (m, 1H), 3.44-3.76 (m, 4H), 3.82-3.94 (m, 1H).

Retention Time (LC, method: ammonium acetate standard): 0.13 min.

MS ($M+H^+$): 201.0.

EXAMPLE 13: 1-(2-Dimethylamino-ethyl)-5-oxo-pyrrolidine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00144] Prepared according to *Method B* from 6-chloro-9H-β-carboline-8-ylamine and 1-(2-dimethylamino-ethyl)-5-oxo-pyrrolidine-3-carboxylic acid in 70 - 80% yield as the di-HCl salt.

^{1}H-NMR (300MHz, D_2O): δ 2.97 (dd, 1H), 3.03 (s, 6H), 3.08 (dd, 1H), 3.51 (t, 2H), 3.76-3.89 (m, 3H), 3.95 (dd, 1H), 4.01 (dd, 1H), 7.75 (d, 1H), 8.24 (d, 1H), 8.45 (dd, 1H), 8.52 (dd, 1H), 9.10 (bs, 1H).

Retention Time (LC, method: ammonium acetate standard): 0.99 min.

MS ($M+H^+$): 400.

EXAMPLE 14: 1-(2-dimethylamino-ethyl)-5-oxo-pyrrolidine-3-carboxylic acid (6-chloro-7-methoxy-9H-β-carbolin-8-yl)-amide

[00145] Prepared according to *Method A* from 6-chloro-7-methoxy-9H-β-carboline-8-ylamine and 1-(2-dimethylamino-ethyl)-5-oxo-pyrrolidine-3-carboxylic acid in 60% yield following purification using a semi-preparative Chiralcel OD column with 85/7.5/7.5 hexane/EtOH/MeOH as the eluant.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.44 (s, 6H), 2.75 (t, 2H), 2.88 (d, 2H), 3.56 (t, 2H), 3.66 (m, 1H), 3.85 (m, 2H), 3.90 (s, 3H), 8.03 (d, 1H), 8.21 (s, 1H), 8.28 (d,1H), 8.79 (s,1H).

Retention Time (LC, method: ammonium acetate standard): 1.42 min.

MS ($M+H^+$): 430.

INTERMEDIATE 26: 6-chloro-7-fluoro-9H-β-carbolin-8-ylamine

[00146] A slurry of 6-chloro-7-fluoro-8-nitro-9H-β-carboline (500 mg, 1.88 mmol) in MeOH (25 ml) was degassed with argon. Palladium on charcoal (20% w/w on C, 50 mg) was added and the reaction vessel was flushed with hydrogen. The slurry was stirred under a balloon of hydrogen for 6 hr, then filtered through celite and concentrated under reduced pressure to yield 6-chloro-7-fluoro-9H-β-carbolin-8-ylamine (400 mg) as a brown solid.

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.48 (br s, 1); 8.99 - 8.98 (m, 1); 8.37 - 8.35 (m, 1); 8.11 - 8.09 (m, 1); 7.74 - 7.72 (m, 1); 5.65 (br s, 2).

HCOOH standard conditions.

DAD R_t = 1.00 min.

MS ($M+H^+$): 236.

EXAMPLE 15: N-(6-chloro-7-fluoro-9H-β-carbolin-8-yl)-2-methyl-nicotinamide

[00147] A solution of 6-chloro-7-fluoro-9H-β-carbolin-8-ylamine (100 mg, 0.424 mmol) in pyridine (2.5 ml) was stirred at RT. 2-methyl nicotinic acid (70 mg, 0.509 mmol) was added, followed by EDCI (130 mg, 0.678 mmol). The suspension was stirred at 100 °C for a day. The pyridine was removed under reduced pressure and the resulting dark

oil was triturated with saturated aqueous $NaHCO_3$. The precipitate which formed was filtered and washed with MeOH. The material was treated with 2M HCl in Et_2O to yield a gray solid, the di-HCl salt N-(6-chloro-7-fluoro-9H-β-carbolin-8-yl)-2-methyl-nicotinamide (110 mg).

^{1}H-NMR (300 MHz, DMSO-d6): δ 13.15 (s, 1); 11.03 (s, 1); 9.33 (s, 1); 8.99 - 8.97 (m, 1); 8.92 - 8.89 (m, 1); 8.79 - 8.73 (m, 2); 8.50 (m, 1); 7.70 (m, 1); 2.80 (s, 3).

HCOOH standard conditions.

DAD R_t = 0.91 min.

MS ($M+H^+$): 355.

INTERMEDIATE 27: 6-chloro-7-methylsulfanyl-8-nitro-9H-β-carboline

[00148] A 250 ml round-bottom flask with magnetic stirrer was charged with 6-chloro-7-fluoro-8-nitro-β-carboline (*Intermediate 5*, 3.959 g, 14.9 mmol) and 100 ml anhydrous DMF. The resulting orange mixture was cooled to 0 °C (ice and water bath) and sodium thiomethoxide (1.809 g, 25.8 mmol) in powder form was added slowly thereto. The reaction mixture was stirred for 1 hr at 0 °C, warmed to RT, and added slowly to a stirring mixture of 4:1 H_2O / saturated aqueous sodium bicarbonate (500 ml). The precipitated solid was collected via suction filtration and air-dried to afford 4.017 g of 6-chloro-7-methylsulfanyl-8-nitro-9H-β-carboline as an orange powder. The crude material was used directly in subsequent steps.

^{1}H-NMR (300 MHz, Methanol-d_4): δ 8.91 (1H, d) 8.63 (1H, s) 8.42 (1H, d) 8.17 (1H, dd) 2.54 (3H, s).

LCMS (formic acid standard method) retention time= 1.43 min.

MS ($M+H^+$): 294.

INTERMEDIATE 28: 6-chloro-7-methylsulfanyl-9H-β-carbolin-8-ylamine

[00149] A 500 ml round-bottom flask with magnetic stirrer was charged with 6-chloro-7-methylsulfanyl-8-nitro-9H-β-carboline (4.011 g, 13.6 mmol) and 200 ml anhydrous ethanol. To the resulting mixture was added aqueous ammonium chloride (75 ml of 0.33 M solution, 24.7 mmol), aqueous hydrochloric acid (10 ml of 1 M solution, 10 mmol), and iron powder (7.734 g, 138 mmol). The resulting mixture was heated to 60 °C (oil bath) and stirred vigorously for 3.5 hr. The reaction was cooled to RT, diluted with EtOAc (75 ml) and activated charcoal (ca. 2.5 g) was added. The resulting mixture was stirred at RT for an additional 1.5 hr, filtered through a pad of celite, and the resulting filtrate concentrated (rotavap, then vacuum pump) to aff rd 5.153 g of a yellowish-orange colored solid. The solid was redissolved in MeOH (50-100 ml) and slowly added to saturated aqueous sodium bicarbonate (500 ml) with stirring. The mixture was stirred at RT for 45 min and the resulting solid collected via suction filtration and air-dried to afford 3.476 g of 6-chloro-7-methylsulfanyl-9H-β-carbolin-8-ylamine as a tan solid which was used without further purification in subsequent steps.

^{1}H-NMR (300 MHz, Methanol-d_4): δ 8.87 (1H, s) 8.35-8.24 (1H, m) 8.16-8.06 (1H, m) 7.67 (1H, s) 2.32 (3H, s).

LCMS (ammonium acetate standard method) retention time= 2.12 min.

MS ($M+H^+$): 264.

EXAMPLE 16: N-(6-chloro-7-methylsulfanyl-9H-β-carbolin-8-yl)-2-methyl-nicotinamide

[00150] A 250 ml round-bottom flask with magnetic stirrer was charged with 6-chloro-7-methylsulfanyl-9H-β-carbolin-8-ylamine (*Intermediate 28*, 2.336 g, 8.86 mmol) and 2-methylnicotinic acid (3.219 g, 23.4 mmol) in 80 ml anhydrous pyridine. To the resulting reaction mixture at RT was added solid 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (7.080 g, 36.9 mmol) and the reaction mixture was heated to 100 °C (oil bath) for 2 days. The resulting mixture was cooled to RT and concentrated (rotavap) to afford a brown residue. The residue was redissolved in MeOH (50 ml), slowly added to a stirring mixture of 5:1 H_2O / saturated aqueous sodium bicarbonate (600 mlL) and stirred at RT for ~18 hr. The precipitated solid was collected via suction filtration, washed with Et_2O (2 x 150 ml), and air-dried to afford 3.036 g of crude N-(6-chloro-7-methylsulfanyl-9H-β-carbolin-8-yl)-2-methyl-nicotinamide as a brown solid. The crude material was purified via HPLC (yields = ~40-60%)
^{1}H-NMR (300 MHz, DMSO-d6): δ 11.72 (1H, s) 10.50 (1H, s) 8.97 (1H, s) 8.62 (1H, dd) 8.57 (1H, s) 8.41 (1H, d) 8.33-8.27 (1H, m) 8.21 (1H, d) 2.73 (3H, s) 2.41 (3H, s).
LCMS (ammonium acetate standard method) retention time= 1.89 min.
MS ($M+H^+$): 383.

INTERMEDIATE 29: 6-chloro-7-ethylsulfanyl-8-nitro-9H-β-carboline

[00151] A 25 ml round-bottom flask with a magnetic stirrer was charged with 6-chloro-7-fluoro-8-nitro-9H-β-carboline (102 mg, 0.38 mmol) in 5 ml of anhydrous DMF. To

the resulting orange mixture at RT was slowly added sodium thioethoxide (80% pure, 69.7 mg, 0.66 mmol) in powder form. The reaction mixture was stirred at RT for 45 minutes, then added drop-wise to a 5:1 mixture of H_2O / saturated sodium bicarbonate (~30 ml). The resulting precipitated solid was collected by suction filtration, washed with 1:1 hexanes / diethyl ether (2 x 20 ml), and air-dried to afford 95.0 mg of 6-chloro-7-ethylsulfanyl-8-nitro-9H-β-carboline as an range solid (79%).

^{1}H-NMR (300 MHz, CD_3OD, ppm) δ 8.91 (1H, s) 8.63 (1H, s) 8.42 (1H, d) 8.18 (1H, d) 3.04 (2H, q) 1.20 (3H, t).

Retention Time (LC, formic acid standard method): 1.71 min.

MS ($M+H^+$): 308.

INTERMEDIATE 30: 6-chloro-7-ethylsulfanyl-9H-β-carbolin-8-ylamine

[00152] A 50 ml round-bottom flask with magnetic stirr r was charged with 6-chloro-7-ethylsulfanyl-8-nitro-9H-β-carboline (85.0 mg, 0.28 mmol) in 10 ml anhydrous ethanol. To the resulting orange mixture at RT was added 0.33 M aqueous ammonium chloride (2.0 ml, 0.66 mmol) and iron powder (680 mg, 12.2 mmol). The reaction mixture was heated to 60 °C and stirred vigorously for 20 hr. Next, the mixture was cooled to RT, diluted with ethyl acetate (15 ml), and activated charcoal (~180 mg) was added. The resulting mixture was filtered through a pad of celite and the filtrate was concentrated to afford 77.8 mg of 6-chloro-7-ethylsulfanyl-9H-β-carbolin-8-ylamine as a yellow solid (> 99%).

^{1}H-NMR (300 MHz, CD_3OD, ppm) δ 8.94 (1H, s) 8.33-8.29 (1H, m) 8.21-8.18 (1H, m) 7.73 (1H, s) 2.85 (2H, q) 1.21 (3H, t).

LCMS (ammonium acetate standard method) retention time= 2.13 min.
(M^+ = 278; M^- = 276).

EXAMPLE 17: N-(6-chloro-7-ethylsulfanyl-9H-β-carbolin-8-yl)-2-methyl-nicotinamide

[00153] A 25 ml round-bottom flask with magnetic stirrer was charged with 6-chloro-7-ethylsulfanyl-9H-β-carbolin-8-ylamine (37.2 mg, 0.13 mmol) and 2-methylnicotinic acid (36.2 mg, 0.26 mmol) in 3 ml anhydrous pyridine. To the resulting light-orange mixture at RT was added solid 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (73.2 mg, 0.38 mmol) and the resulting reaction mixture was heated to 80 °C for 5 days. Next, the reaction mixture was cooled to RT and concentrated to afford a brown, viscous syrup. The syrup was dissolved in a minimal amount of MeOH (~2 ml), slowly added to a 5:1 mixture of H_2O / saturated sodium bicarbonate (~20 ml), and stirred at RT for 2.5 hr. The resulting precipitated solid was collected via suction filtration, washed with 1:1 hexanes / diethyl ether (2 x 20 ml), and air-dried to afford 21.0 mg of N-(6-chloro-7-ethylsulfanyl-9H-β-carbolin-8-yl)-2-methyl-nicotinamide as a tan solid (~38%). LCMS (ammonium acetate standard method) retention time = 2.33 min. (M^+ = 397; M^- = 395). ^{1}H-NMR (300 MHz, CD_3OD, ppm) δ 8.89 (1H, s) 8.63-8.58 (1H, m) 8.44-8.38 (2H, m) 8.36 (1H, d) 8.15 (1H, d) 7.52-7.44 (1H, m) 2.98 (2H, q) 2.84 (3H, s) 1.20 (3H, t).

INTERMEDIATE 31: [2-(6-chloro-8-nitro-9H-β-carbolin-7-ylsulfanyl)-ethyl]-dimethyl-amine

[00154] A 25 ml round-bottom flask with magn tic stirrer was charged with 6-chloro-7-fluoro-8-nitro-9H-β-carboline

(98 mg, 0.37 mmol) in 2 ml of anhydrous DMF. A second 10 ml round-bottom flask with magnetic stirrer was charged with 2-dimethylamino-ethanethiol hydrochloride (100 mg, 0.70 mmol) in 2 ml anhydrous DMF. To the resulting suspension was added n-butyllithium (0.43 ml of 1.6 M solution in hexanes, 0.69 mmol) via syringe and the mixture was stirred for 5 min at RT. Next, the thioanion solution was added via syringe to 6-chloro-7-fluoro-8-nitro-9H-β-carboline, and the resulting red solution was stirred at RT for 30 min. The reaction mixture was slowly added to a 5:1 mixture of H_2O/saturated aqueous sodium bicarbonate (30 ml) and allowed to sit at RT for several hours. The resulting precipitated solid was collected via suction filtration and air-dried to afford 109 mg of [2-(6-chloro-8-nitro-9H-β-carbolin-7-ylsulfanyl)-ethyl]-dimethyl-amine as an orange solid (83%).

LCMS (ammonium acetate standard method) retention time= 1.35 min.

(M^+ = 351; M^- = 349).

^{1}H-NMR (300 MHz, CD_3OD, ppm) δ 8.92 (1H, d, J = 1.0 Hz) 8.66 (1H, s) 8.43 (1H, d) 8.19 (1H, dd) 3.18-3.13 (2H, m) 2.57-2.52 (2H, m) 2.21 (6H, s).

INTERMEDIATE 32: 6-chloro-7-(2-dimethylamino-ethylsulfanyl)-9H-β-carbolin-8-ylamine

[00155] A 50 ml round-bottom flask with a magnetic stirrer was charged with [2-(6-chloro-8-nitro-9H-β-carbolin-7-ylsulfanyl)-ethyl]-dimethyl-amine (106 mg, 0.30 mmol) in 8 ml of anhydrous ethanol. To the resulting orange mixture at RT was added 0.33 M aqueous ammonium chl ride (1.95 ml, 0.64 mmol) and iron powder (540 mg, 9.67 mmol). The reaction mixture was heated to 60 °C and

stirred vigorously for 20 hr. Next, the mixture was cooled to RT, diluted with ethyl acetate (20 ml) and activated charcoal (ca. 150 mg) was added. The resulting mixture was filtered through a pad of celite and the resulting filtrate concentrated to afford 103 mg of 6-chloro-7-(2-dimethylamino-ethylsulfanyl)-9H-β-carbolin-8-ylamine as a yellow solid. The crude product was used directly in the coupling step.

LCMS (ammonium acetate standard method) retention time= 1.34 min.

(M^+ = 321; M^- = 319).

^{1}H-NMR (300 MHz, CD_3OD, ppm) δ 8.87 (1H, s) 8.30 (1H, d) 8.06 (1H, d) 7.75 (1H, s) 3.23-3.13 (4H, m) 2.84 (6H, s).

EXAMPLE 18: N-[6-chloro-7-(2-dimethylamino-ethylsulfanyl)-9H-β-carbolin-8-yl]-2-methyl-nicotinamide

[00156] A 25 ml round-bottom flask with magnetic stirrer was charged with 6-chloro-7-(2-dimethylamino-ethylsulfanyl)-9H-β-carbolin-8-ylamine (45.2 mg, 0.14 mmol) and 2-methylnicotinic acid (39.0 mg, 0.28 mmol) in 4.5 ml of anhydrous pyridine. To the resulting light-orange mixture at RT was added solid 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (95.0 mg, 0.49 mmol). The resulting reaction mixture was heated to 80 °C for 3 days. Next, the reaction was cooled to RT and concentrated to afford a brown, viscous syrup. The syrup was dissolved in a minimal amount of MeOH (~2 ml), slowly added to a 5:1 mixture of H_2O / saturated sodium bicarbonate (~25 ml), and extracted with ethyl acetate (3 x 30 ml). The combined organic layers were washed with brine (1 x 30 ml), dried over Na_2SO_4, filtered and concentrated to afford a brown residue (79.6 mg). The residue was redissolved in MeOH (~5

ml), and filtered through a cotton plug. To the resulting filtrate was added HCl in 1,4-dioxane (1.0 ml, 4.0 mmol), the resulting solution stirred at RT for 3 hr, and added drop-wise to diethyl ether (30 ml) with stirring. The resulting precipitated product was collected via suction filtration, washed with ether and air-dried to afford 36.3 mg of N-[6-chloro-7-(2-dimethylamino-ethylsulfanyl)-9H-β-carbolin-8-yl]-2-methyl-nicotinamide tris-hydrochloride as a yellow powder.

^{1}H-NMR (300 MHz, CD_3OD, ppm) δ 8.95 (1H, s) 8.62 (1H, dd) 8.43 (1H, s) 8.38-8.34 (2H, m) 8.16 (1H, d) 7.50 (1H, dd) 3.09 (2H, t) 2.85 (3H, s) 2.30 (2H, t) 1.99 (6H, s).

LCMS (ammonium acetate standard method) retention time= 1.56 min.

(M^+ = 440; M^- = 438).

INTERMEDIATE 33: morpholine-3(S),4-dicarboxylic acid 4-tert-butyl ester

[00157] A suspension of morpholine-3(S)-carboxylic acid (2.00 g, 15.3 mmol) in DMF (75 ml) was stirred at RT. Triethylamine (7.47 ml, 53.6 mmol) and di-tert-butyl dicarbonate (BOC_2O, 4.02 g, 18.4 mmol) were added. The suspension was stirred at RT for one hour, during which time the reaction formed a clear yellow solution. The solution was concentrated to a reduced volume (~25 ml) and diluted with water (15 ml) and 1N HCl (15 ml). The mixture was poured into a separatory funnel, diluted further with water (100 ml) and brine (100 ml), and extracted with Et_2O (3 x 100 ml). The organic layer was washed with brine, dried, filtered and concentrated to yield a white solid. The solid, which contained excess BOC_2O, was dissolved in Et_2O (500 ml) and extracted with 1N NaOH (3 x 100 ml). The

aqueous layer was acidified with 6N HCl to approximately a pH of 2, then extracted quickly with Et_2O (3 x 100 ml). The Et_2O layer was dried, filtered and concentrated to yield white solid (3.07 g).

^{1}H-NMR (300 MHz, DMSO-d6): δ 12.95 (br s, 1); 4.34 - 4.30 (m, 1); 4.18 - 4.10 (m, 1); 3.83 - 3.74 (m, 1); 3.59 - 3.51 (m, 2); 3.39 - 3.32 (m, 1); 3.21 - 2.95 (m, 1); 1.41 - 1.36 (m, 9).

NH_4OAc standard conditions.

ELSD R_t = 1.08 min.

M-H = 230.

EXAMPLE 19: 4-methyl-morpholine-3(S)-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00158] A slurry of morpholine-3(S)-carboxylic acid (3.00 g, 22.9 mmol) in EtOH (115 ml) was stirred at RT. A solution of aqueous CH_2O (3.42 ml, 45.8 mmol, 37% w/w in H_2O) was added, followed by $Pd(OH)_2$ (600 mg, 20% w/w on charcoal). The flask was charged with hydrogen (1 atm) and the grey slurry was stirred for 24 hr at RT under a balloon of hydrogen. The flask was purged with nitrogen and the black slurry was diluted with MeOH, filtered through filter paper and concentrated to a reduced volume. The pale grey solution was filtered through a 0.45 μm syringe filter to remove residual $Pd(OH)_2$ and concentrated to yield a clear colorless oil. The oil was placed under high vacuum for 24 hr and a white, solid foam was isolated. The foam was dissolved in pyridine (200 ml) and 6-chloro-9H-β-carbolin-8-ylamine (3.74 g, 17.2 mmol) was added, followed by EDCI (5.87 g, 30.6 mmol). The clear pale orange solution was stirred at RT for 24 hr. The solution was diluted with H_2O (300 ml) and poured into a separatory funnel containing

EtOAc (300 ml). The mixture was shaken and the layers were separated. The aqueous layer was extracted with EtOAc (3 x 150 ml) and the combined organic layers were washed with H_2O and brine. The organic layer was dried, filtered and concentrated to yield a brown oil which was placed under high vacuum. The resulting brown foam was triturated with MeOH and the precipitate which formed was filtered and washed with MeOH. The resulting pale yellow solid was purified via chiral HPLC to yield a white solid (3.23 g). ^{1}H-NMR (300 MHz, DMSO-d6): δ 11.36 (s, 1); 10.02 (s, 1); 9.04 (s, 1); 8.38 (d, 1); 8.22 - 8.21 (m, 1); 8.15 (d, 1); 7.91 - 7.90 (m, 1); 4.00 (dd, 1); 3.85 - 3.81 (m, 1); 3.69 - 3.58 (m, 2); 2.99 - 2.95 (dd, 1); 2.89 - 2.85 (m, 1); 2.32 (s, 3); 2.32 - 2.24 (m, 1).
NH_4OAc standard conditions.
DAD R_t = 1.89 min.
M+H = 345.
Chiral preparative HPLC: 10% v/v EtOH/Hexanes.
Chiralcel OD column.
R_t = 11.5 - 14 min.
Enantiopurity of product ≥ 99% ee.

METHOD C: PROCEDURE FOR 4-MORPHOLINE SUBSTITUTED ANALOGS

[00159] As outlined for *Intermediate 34*, *Intermediate 35* and Example 20:

INTERMEDIATE 34: 3(S)-(6-chloro-9H-β-carbolin-8-ylcarbamoyl)-morpholine-4-carboxylic acid tert-butyl ester

[00160] A solution of morpholine-3(S),4-dicarboxylic acid 4-tert-butyl ester (2.83 g, 12.7 mmol) in pyridine (106 ml) was stirred at RT. 6-chloro-9H-β-carbolin-8-ylamine (2.30 g, 10.6 mmol) was added, followed by EDCI (4.06 g,

21.2 mmol). The clear orange-to-brown solution was stirred at RT for 14 hr. The solution was diluted with H_2O (120 ml) and poured into a separatory funnel containing EtOAc (200 ml), H_2O (100 ml) and brine (100 ml). The mixture was shaken and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 50 ml) and the combined organic layers were washed with brine. The organic layer was dried, filtered and concentrated to a reduced volume, then added drop-wise to a stirring solution of 1:1 Et_2O/hexanes (500 ml). The precipitate which formed was filtered and washed with 1:1 Et_2O/Hexanes. The filtrate was concentrated to a reduced volume and a second crop of precipitate was collected. The solid product was placed under high vacuum for 2 hr to yield 3(S)-(6-chloro-9H-β-carbolin-8-ylcarbamoyl)-morpholine-4-carboxylic acid tert-butyl ester as a pale yellow to pale brown solid (4.36 g).

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.30 (s, 1); 10.13 (s, 1); 9.06 (s, 1); 8.40 - 8.38 (m, 1); 8.19 - 8.16 (m, 2); 7.98 (s, 1); 4.67 - 4.47 (m, 2); 3.96 - 3.60 (m, 2); 3.64 - 3.39 (m, 3); 1.42 (s, 9).

NH_4OAc standard conditions.

DAD R_t = 2.31 min.

M+H = 431.

INTERMEDIATE 35: 2(R)-[3(S)-(6-chloro-9H-β-carbolin-8-ylcarbamoyl)-morpholin-4-ylmethyl]-pyrrolidine-1-carboxylic acid tert-butyl ester

[00161] A solution of 3(S)-(6-chloro-9H-β-carbolin-8-ylcarbamoyl)-morpholine-4-carboxylic acid tert-butyl ester (1.00 g, 2.32 mmol) in CH_2Cl_2 (6 ml) was stirred at RT. Trifluoroacetic acid (6 ml) was added and the solution was

stirred at RT for 45 min, then concentrated to a residue. The residue was concentrated once more from CH_2Cl_2 to yield a yellow-brown solid which was dissolved in THF (13 ml) under argon. Gentle warming was sometimes needed to ensure complete dissolution. A solution of N-(tert-butoxycarbonyl)-D-prolinal (693 mg, 3.48 mmol) in THF (2 ml) was added, followed by sodium triacetoxyborohydrid (738 mg, 3.48 mmol). The solution was stirred at RT for 30 min, then quenched via addition of 1N aqueous NaOH (30 ml). The mixture was poured into a separatory funnel containing EtOAc (100 ml), H_2O (100 ml), and brine (100 ml). The mixture was shaken and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 50 ml) and the combined organic layers were washed with brine. The organic layer was dried, filtered and concentrated to yield a light brown solid. Column chromatography (2%-4% MeOH/CH_2Cl_2) yielded 2(R)-[3(S)-(6-Chloro-9H-β-carbolin-8-ylcarbamoyl)-morpholin-4-ylmethyl]-pyrrolidine-1-carboxylic acid tert-butyl ester as a white solid (915 mg).

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.30 (s, 1); 9.88 (s, 1); 9.04 (s, 1); 8.39 - 8.37 (m, 1); 8.20 - 8.15 (m, 2); 7.95 (s, 1); 3.99 - 3.82 (m, 3); 3.69 - 3.63 (m, 2); 3.44 - 3.32 (m, 1); 3.27 - 3.11 (m, 3); 2.92 - 2.80 (m, 1); 2.44 - 2.32 (m, 1); 1.99 - 1.67 (m, 5); 1.33 (s, 9).

HCOOH standard conditions.

DAD R_t = 1.39 min.

M+H = 514.

Chiral HPLC.

[00162] The enantiopurity of the sample was checked. The samples were ≥ 97% ee.

Chiralpak AD column.

15% v/v EtOH/Hexanes containing 0.1% Et_2NH.

EXAMPLE 20: 4-pyrrolidin-2(R)-ylmethyl-morpholine-3(S)-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide, HCl salt)

[00163] To a solution of 2(R)-[3(S)-(6-chloro-9H-β-carbolin-8-ylcarbamoyl)-morpholine-4-ylmethyl]-pyrrolidine-1-carboxylic acid tert-butyl ester (850 mg, 1.65 mmol) in MeOH (16 ml) was added concentrated aqueous HCl (13 ml). The solution was stirred at RT for 30-45 min, during which time a fine yellow precipitate formed. The reaction mixture was concentrated to yield 4-pyrrolidin-2(R)-ylmethyl-morpholine-3(S)-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide, HCl salt) as a pale yellow solid (755 mg).

^{1}H-NMR (300 MHz, DMSO-d6): δ. 13.30 (s, 1); 11.56 (br s, 1); 9.63 (br s, 1); 9.46 (s, 1); 8.87 - 8.85 (m, 1); 8.66 - 8.57 (m, 2); 8.25 (s, 1); 4.38 - 4.26 (m, 1); 4.24 - 4.08 (m, 1); 4.04 - 3.86 (m, 2); 3.86 - 3.68 (m, 2); 3.57 - 3.39 (m, 1); 3.39 - 3.02 (m, 4); 2.99 - 2.76 (m, 1); 2.10 - 1.84 (m, 3); 1.75 - 1.56 (m, 1).

HCOOH standard conditions.

DAD R_t = 0.81 min.

M+H = 414.

INTERMEDIATE 36: cis-2-(tert-butoxycarbonylamino)-cyclopentanecarboxylic acid (6-chloro-9H-carbolin-8-yl) amide

[00164] A solution of cis-2-(tert-butoxycarbonylamino)-cyclopentane carboxylic acid (550 mg, 2.4 mmol) in pyridine (10 ml) was stirred at RT. 6-chloro-9H-β-carbolin-8-ylamine (436 mg, 2.0 mmol) was added, followed by EDCI

(615 mg, 3.2 mmol), and the orange solution was stirred at RT for 1.5 hr. The solution was diluted with H_2O (20 ml) and poured into a separatory funnel containing H_2O (50 ml) and EtOAc (100 ml). The mixture was shaken and the layers were separated. The aqueous layer was extracted with EtOAc (100 ml). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to orange oily solids which were subsequently triturated with 5% MeOH in Et_2O (20 ml) and captured by filtration to yield cis-2-(tert-butoxycarbonylamino)-cyclopentanecarboxylic acid (6-chloro-9H-carbolin-8-yl) amide as a light yellow solid (740 mg).

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.17 (s, 1); 9.89 (s, 1); 9.04 (s, 1); 8.36 (d, 1); 8.18 - 8.08 (m, 2); 7.95 (s, 1); 6.92 (d, 1); 4.32 - 4.22 (m, 1); 3.16 - 3.09 (m, 1); 2.13 - 2.01 (m, 1); 1.96 - 1.75 (m, 3); 1.74 - 1.59 (m, 1); 1.58 - 1.42 (m, 1); 1.07 (s, 9).

NH_4OAc standard conditions.

DAD R_t = 2.52 min.

M+H = 429.

EXAMPLE 21: cis-2-amino-cyclopentanecarboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00165] A solution of 2-(tert-butoxycarbonylamino)-cyclopentanecarboxylic acid (6-chloro-9H-β-carbolin-8-yl) amide (736 mg, 1.72 mmol) in trifluoroacetic acid (5 ml) was stirred at RT for 20 min, then concentrated to an orange oil. The oil was dissolved in MeOH (5 ml) and neutralized with a saturated aqueous sodium bicarbonate solution (25 ml). The resulting mixture was further diluted with H_2O (25 ml) and EtOAc (100 ml). The aqueous layer was removed and extracted with EtOAc (100 ml). The

rganic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated to yellow solids (507 mg). These solids were dissolved in MeOH (5 ml) and a solution of HCl in dioxane (4 M, 1.5 ml) was added. The bright yellow solution was stirred 30 min, then concentrated to yield cis-2-amino-cyclopentanecarboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide as a yellow powder (600 mg).
^{1}H-NMR (300 MHz, DMSO-d6): δ 9.29 (s, 1); 8.75 (d, 1); 8.53 (d, 1); 8.37 (s, 1); 8.02 (s, 1); 4.05 - 3.95 (m, 1); 3.42 - 3.34 (m, 1); 2.46 - 1.80 (m, 6).
NH_4OAc standard conditions.
DAD R_f = 1.65 min.
M+H = 329.

EXAMPLE 22: 4-(2-amino-ethyl)-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide, HCl salt

[00166] *Method C* was followed using racemic morpholine-3-carboxylic acid reductively alkylated with 2-aminoacetaldehyde.
^{1}H-NMR (300 MHz, MeOH-d4): δ 9.37 (s, 1); 8.76 (d, 1); 8.55 (d, 1); 8.44 (d, 1); 8.06 (d, 1); 4.68 - 4.55 (m, 2); 4.17 - 3.99 (m, 3); 3.84 - 3.73 (m, 2); 3.57 - 3.39 (m, 4).
NH_4OAc standard conditions.
DAD R_f = 1.69 min.
M+H = 374.

EXAMPLE 23: 4-(2(S)-amino-propyl)-morpholine-3(S)-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide, HCl salt (first eluting diastereomer)

[00167] *Method C* was followed using racemic morpholine-3-carboxylic acid reductively alkylated with the appropriate

alanine aldehyde. The diastereomers were separated via column chromatography prior to the deprotection step.
^{1}H-NMR (300 MHz, DMSO-d6): δ 9.32 (s, 1); 8.76 (d); 8.55 (d, 1); 8.41 (s, 1); 8.08 (s, 1); 4.38 (d, 1); 4.32 - 4.21 (m, 1); 4.16 - 4.09 (m, 1); 4.04 - 3.95 (m, 2); 3.79 - 3.57 (m, 2); 3.47 - 3.40 (m, 1); 3.22 - 3.05 (m, 2); 1.44 (d, 3).
NH_4OAc standard conditions.
DAD R_t = 1.38 min.
M+H = 388.

EXAMPLE 24: 4-(2(S)-amino-propyl)-morpholine-3(R)-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide, HCl salt (second eluting diastereomer)

[00168] *Method C* was followed using racemic morpholine-3-carboxylic acid reductively alkylated with the appropriate alanine aldehyde. The diastereomers were separated via column chromatography prior to the deprotection step.
^{1}H-NMR (300 MHz, DMSO-d6): δ 9.34 (s, 1); 8.77 (d, 1); 8.55 (d, 1); 8.42 (s, 1); 8.06 (s, 1); 4.42 (d, 1); 4.30 - 4.12 (m, 1); 4.07 - 3.92 (m, 3); 3.89 - 3.74 (m, 1); 3.65 - 3.49 (m, 1); 3.25 - 2.90 (m, 3); 1.36 (d, 3).
NH_4OAc standard conditions.
DAD R_t = 1.57 min.
M+H = 388.

EXAMPLE 25: 2-amino-cyclohexanecarboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00169] A solution of cis-2-(tert-butoxycarbonylamino)-cyclohexane carboxylic acid (255 mg, 1.05 mmol) in pyridine (10 ml) was stirred at RT. 6-chloro-9H-β-carbolin-8-ylamine (218 mg, 1.00 mmol) was added, followed by EDCI

(315 mg, 1.64 mmol) and the slightly turbid pale orange solution was stirred at RT for 16 hr. The solution was diluted with H_2O (20 ml) and poured into a separatory funnel containing H_2O (50 ml) and EtOAc (50 ml). The mixture was shaken and the layers were separated. The aqueous layer was extracted with EtOAc (50 ml) and the combined organic layers were washed with brine. The organic layer was dried, filtered and concentrated to yield a yellow oil which was placed under high vacuum for 4 hr. The resulting yellow-brown glass was slurried in CH_2Cl_2 (10 ml) at RT. Trifluoroacetic acid (5 ml) was added and the slurry instantly dissolved to form a clear orange solution. The solution was stirred at RT for 45 min, then concentrated to a brown residue. The residue was azeotroped from toluene (3 x 10 ml) to yield a yellow solid. A solution of dilute aqueous Na_2CO_3 was prepared by adding a small volume of 10% aqueous Na_2CO_3 to H_2O (50 ml) until the aqueous solution reached a pH of 10. The yellow solid was dissolved in minimal MeOH and was added drop-wise to the aqueous solution with stirring. The precipitate which formed was filtered, washed with H_2O and placed under high vacuum to yield 2-amino-cyclohexanecarboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide as pale yellow solid (147 mg).

^{1}H-NMR (300 MHz, DMSO-d6): δ 9.00 (s, 1); 8.37 - 8.34 (m, 1); 8.16 - 8.13 (m, 2); 7.83 (m, 1); 5.66 - 5.00 (br s, 2); 3.42 - 3.40 (m, 1); 2.70 - 2.62 (m, 1); 2.02 - 1.90 (m, 1); 1.70 - 1.54 (m, 5); 1.42 - 1.29 (m, 2).

NH_4OAc standard conditions.

DAD R_t = 1.46 min.

M+H = 343.

EXAMPLE 26: 4-(2(R)-amino-propyl)-morpholine-3(S)-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide, HCl salt

[00170] *Method C* was followed using racemic morpholine-3-carboxylic acid reductively alkylated with the appropriate alanine aldehyde. The diastereomers were separated via column chromatography prior to the deprotection step.
^{1}H-NMR (300 MHz, DMSO-d6): δ 9.35 (s, 1); 8.77 (m, 1); 8.55 (m, 1); 8.43 (s, 1); 8.01 (s, 1); 4.45 (d, 1); 4.26 (m, 1); 4.09-3.91 (m, 3); 3.79 (m, 1); 3.63 (m, 1); 3.28-2.99 (m, 3); 1.37 (d, 3).
NH_4OAc standard conditions.
DAD R_f = 1.39 min.
M+H = 388.

EXAMPLE 27: 4-(2(R)-amino-3-phenyl-propyl)-morpholine-3(S)-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide, HCl salt

[00171] *Method C* was followed using racemic morpholine-3-carboxylic acid reductively alkylated with the appropriate alanine aldehyde. The diastereomers were separated via column chromatography prior to the deprotection step.
^{1}H-NMR (300 MHz, DMSO-d6): δ 9.34 (s, 1); 8.77 (d, 1); 8.55 (d, 1); 8.42 (s, 1); 8.04 (s, 1); 7.44 - 7.23 (m, 5); 4.39 (d, 1); 4.65 - 4.07 (m, 1); 4.40 - 3.82 (m, 4); 3.50 - 3.25 (m, 1); 3.30 - 3.14 (m, 1); 3.11 - 2.88 (m, 3); 2.85 - 2.69 (m, 1).
NH_4OAc standard conditions.
DAD R_f = 1.90 min.
M+H = 464.

METHOD D: CHROMATOGRAPHY CONDITIONS

LCMS

Column type: Phenomenex Luna C18(2) columns, 5 um, size 50 x 4.6 mm

Run time: 5.00 minute run

NH_4OAc Conditions:

Solvent A:

10 mM NH_4OAc

99% H_2O

1% MeCN

Solvent B:

10 mM NH_4OAc

5% H2O

95% MeCN

Standard gradient:

Initial conditions - 95% A, 5% B

3.5 minute gradient from 5%-100% B

3.5 - 4.3 minutes hold at 100% B

4.3 - 5 minutes initial conditions

Polar gradient:

Initial conditions - 70% A, 30% B

3.5 minute gradient from 70%-100% B

3.5 - 4.3 minutes hold at 100% B

4.3 - 5 minutes initial conditions

Nonpolar gradient:

Initial conditions - 100% A

3.5 minute gradient from 0%-50% B

3.5 - 4.3 minutes hold at 100% B

4.3 - 5 minutes initial conditions

HCOOH Conditions:

Solvent C:

0.1% HCOOH

99% H_2O

1% MeCN

Solvent D:

0.1% HCOOH

5% H_2O

95% MeCN

Standard gradient:

Initial conditions - 95% C, 5% D

3.5 minute gradient from 5%-100% D

3.5 - 4.3 minutes hold at 100% D

4.3 - 5 minutes initial conditions

Polar gradient:

Initial conditions - 70% C, 30% D

3.5 minute gradient from 70%-100% D

3.5 - 4.3 minutes hold at 100% D

4.3 - 5 minutes initial conditions

Nonpolar gradient:

Initial conditions - 100% C

3.5 minute gradient from 0%-50% D

3.5 - 4.3 minutes hold at 100% D

4.3 - 5 minutes initial conditions

INTERMEDIATE 37: 6-chloro-2,3,4,9-tetrahydro-1H-β-carboline, HCl salt

[00172] 5-chlorotryptamine hydrochloride (5 g, 20 mmol, 1 equiv.) was dissolved in 40 ml 3 M NaOAc buffer (pH = 4.8) and 40 ml water. Glyoxalic acid (1.84 g, 20 mmol, 1 equiv.) was added in one portion and the solution was stirred at RT overnight. The resulting thick slurry was filtered and the light green solids were suspended in 100 ml 6N HCl and heated at 125 °C under a reflux condenser for

1 hour with intermittent additions of conc HCl (2 ml every 15 min). After cooling to RT, 4.38 g (90%) of 6-chloro-2,3,4,9-tetrahydro-1H-β-carboline, HCl salt as blue-grey solid was isolated by filtration.

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.33 (br, 2H), 9.62 (br, 2H), 7.53 (d, 1), 7.39 (d, 1), 7.09 (dd, 1), 4.33 (br, 2H), 2.92 (t, 2)

Formic acid standard conditions.

DAD RT = 1.56 min.

M+H = 207.

INTERMEDIATE 38: (6-chloro-1,3,4,9-tetrahydro-β-carbolin-2-yl)-phenyl-methanone

[00173] 6-chloro-2,3,4,9-tetrahydro-1H-β-carboline, HCl salt (10.2 g, 42 mmol, 1 equiv.) was suspended in 100 ml of dry pyridine under N_2 and cooled to 0 °C in an ice water bath. Benzoyl chloride (7.3 ml, 63 mmol, 1.5 equiv.) was added drop-wise to the cold solution after which the reaction was removed from the ice bath and allowed to stir overnight at room temperature. The reaction was quenched by the addition of water until choked with solids. These solids were captured by filtration, washed with a saturated aqueous sodium bicarbonate solution, re-suspended in water, sonicated, and refiltered to give 1.27 g (94%) of (6-chloro-1,3,4,9-tetrahydro-β-carbolin-2-yl)-phenyl-methanone as crystalline orange solids.

^{1}H-NMR (300 MHz, DMSO-d6): δ 10.65 - 10.59 (br, 1H), 7.0 - 7.5 (m, 9H), 4.60 - 4.83 (br, 2H), 3.62 - 3.99 (br, 2H), 2.75 (br., 2H).

Formic acid standard conditions.

DAD RT = 2.68 min.

M+H = 311.

INTERMEDIATE 39: 2-benzoyl-6-chloro-1,2,3,9-tetrahydro-β-carbolin-4-one

[00174] (6-chloro-1,3,4,9-tetrahydro-β-carbolin-2-yl)-phenyl-methanone (1.76 g, 5.66 mmol, 1 equiv.) and DDQ (2.31 g, 10.2 mmol, 1.8 equiv.) were mixed as solids and cooled to -78 °C. 15 ml of a 9:1 THF/H_2O solution was cooled to -78 °C and the resulting slurry was added to the cooled solids followed by an additional 15 ml of THF (also cooled to -78 °C). The deep blue solution was stirred at -78 °C for 2 hours and then gradually warmed to room temperature and stirred an additional two hours. The reaction was quenched by the addition of 1 N NaOH, and extracted with 3 x 150 ml EtOAc. The combined organic layers were washed with 2 x 100 ml 1 N HCl, 1 x 100 ml brine, dried over $MgSO_4$, filtered and concentrated to yield 1.38 g (75%) of 2-benzoyl-6-chloro-1,2,3,9-tetrahydro-β-carbolin-4-one as oily orange solids.
^{1}H-NMR (300 MHz, DMSO-d6): δ 12.11 - 12.48 (br, 1H), 7.29 - 7.88 (m, 8H), 4.93 - 5.18 (br, 2H), 4.60 - 4.46 (br, 2H).
Exact Mass: 324.07.
Formic acid standard conditions
DAD RT = 2.15 min.
M+H = 325.

INTERMEDIATE 40: 4-amino-6-chloro β-carboline

[00175] Crude 2-benzoyl-6-chloro-1,2,3,9-tetrahydro-β-carbolin-4-one (4 g) was dissolved in 30 ml of anhydrous hydrazine and stirred at reflux (130 °C il bath) under N_2 for 6 hours, after which the reaction mixture was allowed

to cool to room temperature and sit overnight. The precipitated yellow solids were removed by filtration and washed with water, 2 x 5 ml, to yield 785 mg (30%) of 4-amino-6-chloro β-carboline as an off white solid. Additional water was added to the combined filtrates until no further precipitation occurred. These solids were also removed by filtration to give 1.056 g (39%) of 4-amino-6-chloro β-carboline as yellow solids, (69% total yield). ^{1}H-NMR (300 MHz, DMSO-d6): δ 11.48 (s, 1H), 8.44 (s, 1H), 8.13 (s, 1H), 7.77 (s, 1H), 7.42 - 7.52 (m, 2H), 5.86 (s, 2H).
Formic acid standard conditions.
DAD RT = 1.68 min.
M+H = 218.

INTERMEDIATE 41: N-(6-chloro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide

[00176] 4-amino-6-chloro β-carboline (1.05 g, 4.82 mmol, 1 equiv.) was dissolved in 4 ml of anhydrous pyridine and 20 ml of THF and cooled to 0 °C under N_2. Trifluoroacetic anhydride (3.4 ml, 24 mmol, 5 equiv.) was added drop-wise to the cooled solution. Upon complete addition, the reaction was removed from the ice bath and stirred at room temperature for ~1.5 hours. The reaction was quenched by the slow addition of water (10 ml) and extracted 2 x 150 ml EtOAc, washed 2 x 100 ml saturated aqueous sodium bicarbonate, 1 x 100 ml brine, dried over $MgSO_4$, filtered and concentrated to orange solids. These solids were titurated by in 10 - 15 ml Et_2O and captured by filtration to give 1.23 g (81%) of N-(6-chloro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide as a yellow solid.

^{1}H-NMR (300 MHz; DMSO-d6): δ 12.11 (s, 1H), 11.89 (s, 1H), 8.92 (s, 1H), 8.33 (s, 1H), 7.82 (s, 1H), 7.60-7.70 (m, 2H).
Formic acid standard conditions.
DAD RT = 2.12 min.
M+H = 314.

INTERMEDIATE 42: N-(6-chloro-8-nitro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide

[00177] N-(6-chloro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide (125 mg, 0.4 mmol, 1 equiv.) was dissolved in 2 ml TFA and $NaNO_2$ (541 mg, 7.84 mmol, 2 equiv.) was added in one portion. The solution was stirred at room temperature for 4 hr. Volatiles were removed by rotovap, and the resulting oily orange solids were suspended in water, neutralized with a saturated aqueous sodium bicarbonate solution and filtered to give 132 mg (92%) of N-(6-chloro-8-nitro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide.
^{1}H-NMR (300 MHz, DMSO-d6): δ 12.87 (s, 1H), 12.03 (s, 1H), 9.11 (s, 1H), 8.56 (s, 1H), 8.53 (s, 1H), 8.26 (s, 1H).
Formic acid standard conditions.
DAD RT = 2.27 min.
M+H = 359.

INTERMEDIATE 43: N-(8-amino-6-chloro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide

[00178] The crude N-(6-chloro-8-nitro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide (130 mg, 0.36 mmol) was dissolved in 7 ml of MeOH and the reaction vessel was vacuum purged 3 X with N_2. Platinum (20 mg, 20% wt. on activated carbon) was added quickly, and the reaction

vessel was again vacuum purged 3 X with N_2, followed by 3 additional vacuum purge cycles with H_2. The reaction was allowed to stir under H_2 at 1 atm overnight. Upon completion, the reaction vessel was purged of H_2 and filtered over celite. The celite was washed several times with methanol until the filtrates were clear. The combined filtrates were concentrated to give N-(8-amino-6-chloro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide as a yellow solid (112 mg, 95%).

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.83 (s, 1H), 8.98 (s, 1H), 8.30 (s, 1H), 7.10 (s, 1H), 6.82 (s, 1H), 5.87 (br, 2H).

Formic acid standard conditions.

DAD RT = 1.95 min.

M+H = 329.

INTERMEDIATE 44: N-[6-chloro-4-(2,2,2-trifluoro-acetylamino)-9H-β-carbolin-8-yl]-2-methyl-nicotinamide

[00179] N-(8-amino-6-chloro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide (90 mg, 0.274 mmol, 1 equiv.) and 2-methylnicotinic acid (45 mg, 0.329 mmol, 1.2 equiv.) were dissolved in 1.5 ml of anhydrous pyridine under N_2. EDCI (84 mg, 0.438 mmol, 1.6 equiv.) was added in one portion and the reaction mixture was stirred at room temperature for 2 hours. The reaction was quenched with water and the resulting dark solids captured by filtration. These solids were titurated in a 3:1 methanol - DMSO solution to give N-[6-chloro-4-(2,2,2-trifluoro-acetylamino)-9H-β-carbolin-8-yl]-2-methyl-nicotinamide as light yellow solids (41 mg, 3%).

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.94 (br, 1H), 11.78 (s, 1H), 10.61 (s, 1), 9.00 (s, 1), 8.64 (d, 1), 8.38 (s, 1H), 8.13

(d, 1), 8.01 (s, 1H), 7.73 (s, 1H), 7.45 (m, 1H), 2.68 (s, 3H).

Formic acid standard conditions.

DAD RT = 1.98 min.

M+H = 448.

INTERMEDIATE 45: N-[6-chloro-4-(2,2,2-trifluoro-acetylamino)-9H-β-carbolin-8-yl]-nicotinamide

[00180] N-(8-amino-6-chloro-9H-β-carbolin-4-yl)-2,2,2-trifluoro-acetamide (90 mg, 0.274 mmol, 1 equiv.) and 2-methylnicotinic acid (40 mg, 0.329 mmol, 1.2 equiv.) were dissolved in 1.5 ml of anhydrous pyridine under N_2. EDCI (84 mg, 0.438 mmol, 1.6 equiv.) was added in one portion and the reaction mixture was stirred at room temperature 2 hours. The reaction was quenched and treated following the preceding protocol to obtain 38 mg (32%) of N-[6-chloro-4-(2,2,2-trifluoro-acetylamino)-9H-β-carbolin-8-yl]-nicotinamide.

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.89 (br, 1H), 10.73 (s, 1), 9.28 (s, 1), 8.97 (s, 1), 8.84 (d, 1), 8.43 (d, 1), 8.37 (s, 1), 7.85 (s, 1), 7.76 (s, 1), 7.67 (m, 1).

Formic acid standard conditions.

DAD RT = 1.94 min.

M+H = 434.

EXAMPLE 28: N-(4-amino-6-chloro-9H-β-carbolin-8-yl)-2-methyl-nicotinamide

[00181] N-[6-chloro-4-(2,2,2-trifluoro-acetylamino)-9H-β-carbolin-8-yl]-nicotinamide (41 mg, 0.092 mmol, 1 equiv.) was suspended in 5 ml of MeOH and a 2 ml aqueous solution f K_2CO_3 (127 mg, 0.92 mmol, 10 equiv.) was added thereto. The resulting clear solution was heated at 60 °C for 16 hr

and then allowed to cool to RT. Additional water was added producing fine solids that were captured by filtration, washed once with 10 ml of 5% MeOH in Et_2O, and dried under high vacuum to give 18 mg of N-(4-amino-6-chloro-9H-β-carbolin-8-yl)-2-methyl-nicotinamide as powdery yellow solids (56% yield).

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.18 (s, 1H), 10.41 (s, 1H), 8.62 (d, J = 3.6, 1H),), 8.32 (s, 1H), 8.20 (s, 1H), 8.11 (d, J = 7.5, 1H), 7.91 (s, 1H), 7.79 (s, 1H), 7.43 (m, 1H), 5.91 (br, 2H), 2.66 (s, 3H).

Formic acid standard conditions.

DAD RT = 1.63 min.

M+H = 352.

EXAMPLE 29: N-(4-amino-6-chloro-9H-β-carbolin-8-yl)-nicotinamide

[00182] N-[6-chloro-4-(2,2,2-trifluoro-acetylamino)-9H-β-carbolin-8-yl]-nicotinamide (38 mg, 0.088 mmol, 1 equiv.) was suspended in 5 ml of MeOH and a 2 ml aqueous solution of K_2CO_3 (121 mg, 0.92 mmol, 10 equiv.) was added thereto. The resulting clear solution was heated at 60 °C for 11 hr. After cooling to RT, fine solids precipitated that were captured by filtration and washed with 10 ml of water to give 2.78 mg (10%) of N-(4-amino-6-chloro-9H-β-carbolin-8-yl)-nicotinamide.

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.32 (s, 1H), 10.56 (s, 1H), 9.26 (s, 1H), 8.82 (d, 1), 8.42 (d, 1), 8.36 (s, 1H), 8.18 (s, 1H), 7.79 (s, 1H), 7.70 (s, 1H), 7.64 (m, 1H), 5.91 (br, 2H).

Formic acid standard conditions.

DAD RT = 1.55 min.

M+H = 338.

INTERMEDIATE 46: 3-cyanomethyl-indole-1-carboxylic acid tert-butyl ester

[00183] A solution of 3-indoleacetonitrile (10 g, 64 mmol) in DMF (160 ml) was stirred at RT. K_2CO_3 (13.3 g, 96 mmol) and di-tert-butyl dicarbonate (15.35 g, 70 mmol) were added thereto and the reaction mixture was stirred at RT for 12 hr. H_2O (100 ml) was added to the reaction mixtur and the resulting precipitate was captured by filtration. The solids were dissolved in hot methanol (20 ml) and the solution was allowed to cool slowly, producing light orang solids that were isolated by filtration to give 3-cyanomethyl-indole-1-carboxylic acid tert-butyl ester (9.2 g).

^{1}H-NMR (300 MHz, DMSO-d6): δ 8.08 (d, 1); 7.70 - 7.66 (m, 2); 7.42 - 7.29 (m, 2); 4.12 (s, 2); 1.63 (3, 9).

NH_4OAc standard conditions.

DAD R_t = 3.31 min.

M+H = 257.

INTERMEDIATE 47: 3-(cyano-methyl-methyl)-indole-1-carboxylic acid tert-butyl ester

[00184] A stirred solution of 3-cyanomethyl-indole-1-carboxylic acid tert-butyl ester (2.15 g, 8.39 mmol) in THF (40 ml) was cooled to -78 °C under an argon atmosphere. Sodium bis(trimethylsilyl)amide (1 M in THF, 10 ml, 10 mmol) was added thereto and the cold solution was stirred for 30 minutes. Iodomethane (627 uL, 10 mmol) was added thereto and the reaction mixture was stirred 1.5 hr while gradually warming to 0 °C. H_2O (100 ml) was added thereto and the solution was brought to RT and diluted with EtOAc

(250 ml). The aqueous layer was removed and extracted with EtOAc (250 ml). The combined organic layers were washed with aqueous HCl (1N, 3 x 50 ml), followed by brine, then dried over $MgSO_4$, filtered, and concentrated to an orange oil. Purification via column chromatography (hexanes:ethyl acetate) gave 3-(cyano-methyl-methyl)-indole-1-carboxylic acid tert-butyl ester (1.8 g).

^{1}H-NMR (300 MHz, DMSO-d6): δ 8.09 (d, 1); 7.73 (d, 1); 7.69 (s, 1); 7.74 - 7.29 (m, 2); 4.56 (q, 1); 1.66 (d, J = 7.2, 3); 1.63 (s, 9).

INTERMEDIATE 48: 3-(2-amino-1-methyl-methyl)-indole-1-carboxylic acid tert-butyl ester

[00185] A solution of 3-(cyano-methyl-methyl)-indole-1-carboxylic acid tert-butyl ester (850 mg, 3.14 mol) in methanol (15 ml) was stirred at RT. A Raney-Nickel catalyst (50% g /wt suspension in H_2O, 1 ml) was added thereto and the reaction vessel was capped and vacuum purged 3 times with argon, followed similarly by hydrogen. The mixture was stirred 22 hr under 1 atm. of hydrogen, vacuum purged with argon and filtered through celite. The filtrate was concentrated to an oil (670 mg) and determined by LCMS to be composed mainly of the desired compound 3-(2-amino-1-methyl-methyl)-indole-1-carboxylic acid tert-butyl ester.

NH_4OAc standard conditions.

DAD R_t = 1.78 min.

M+H = 275.

INTERMEDIATE 49: 4-methyl-2,3,4,9-tetrahydro-1H-β-carboline-1-carboxylic acid

[00186] Crude 3-(2-amino-1-methyl-methyl)-indole-1-carboxylic acid tert-butyl ester (670 mg, approx. 2.44 mmol) was dissolved in trifluoroacetic acid (2 ml) and stirred 30 min at RT, then concentrated to an oily solid under reduced pressure. The resulting oil was dissolved in a 3 M of NaOAc:AcOH buffer solution (pH = 4.8, 12 ml) and H_2O (6 ml) at RT. Glyoxalic acid (225 mg, 2.44 mmol) was added thereto and the reaction mixture was stirred for 4 hr at RT, then concentrated to dryness. The resulting solids were determined to be composed mainly of the desired compound 4-methyl-2,3,4,9-tetrahydro-1H-β-carboline-1-carboxylic acid by LCMS and used subsequently thereafter without purification.

NH_4OAc standard conditions.

DAD R_t = 1.24 min.

M+H = 231.

INTERMEDIATE 50: 4-methyl-2,3,4,9-tetrahydro-1H-β-carboline

[00187] Crude 4-methyl-2,3,4,9-tetrahydro-1H-β-carboline-1-carboxylic acid (approx. 2.44 mmol) was suspended in H_2O (5 ml) and HCl (12 N, 5 ml) and the suspension was heated at 120 °C for 1 hr, then allowed to cool to RT. Dark orange-brown solids were removed by filtration and then dissolved in methanol (5 ml). A saturated sodium bicarbonate solution (20 ml) was added thereto, producing a thick yellow slurry. This reaction mixture was filtered to yield yellow solids composed mainly of 4-methyl-2,3,4,9-tetrahydro-1H-β-carboline (386 mg) as determined by LCMS.

NH_4OAc standard conditions.

DAD R_t = 1.23 min.

M+H = 187.

INTERMEDIATE 51: 4-Methyl-9H-β-carboline

[00188] 4-methyl-2,3,4,9-tetrahydro-1H-β-carboline (214 mg, 1.17 mmol) was suspended in xylenes (10 ml). Pd (10% wt. on carbon, 21 mg) catalyst was added thereto and the reaction mixture was stirred at 160 °C for 24 hours, then cooled to RT and filtered through celite. The filtrate was concentrated to dryness to give 4-methyl-9H-β-carboline (210 mg).

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.73 (br s, 1); 8.78 (br s, 1); 8.19 (d, 1); 8.13 (s, 1); 7.62 (br s, 1); 7.53 (t, 1); 7.25 (t, 1); 2.78 (s, 3).

NH_4OAc standard conditions.

DAD R_t = 2.24 min.

M+H = 183.

INTERMEDIATE 52: 6-Chloro-4-methyl-9H-β-carboline

[00189] 4-methyl-9H-β-carboline (97 mg, 0.532 mmol) was dissolved in HCl (1N, 4 ml) and stirred at RT. NCS (85 mg, 0.637 mmol) was added thereto and the reaction mixture was stirred for 5 hr. Saturated sodium bicarbonate solution (20 ml) was added thereto and the reaction mixture was extracted twice with EtOAc (100 ml). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give 6-chloro-4-methyl-9H-β-carbolin (108 mg) as an oil.

^{1}H-NMR (300 MHz, DMSO-d6): δ 8.67 (s, 1); 8.19 (s, 1); 8.11 (s, 1); 7.57 - 7.54 (m, 2); 2.82 (s, 3).

NH_4OAc standard conditions.

DAD R_t = 2.48 min.

M+H = 217.

INTERMEDIATE 53: 6-chloro-4-methyl-8-nitro-9H-β-carboline

[00190] A solution of 6-chloro-4-methyl-9H-β-carboline (100 mg, 0.462 mmol) in trifluoroacetic acid (10 ml) was stirred at RT. $NaNO_3$ (106 mg, 1.25 mmol) was added thereto and the reaction mixture was stirred 30 min, then concentrated to give an orange residue. The residue was dissolved in MeOH (5 ml) and neutralized by an addition of a saturated sodium bicarbonate solution (20 ml) causing the formation of yellow solids that were captured by filtration, washed with H_2O (10 ml) and Et_2O (2 x 10 ml) to give 6-chloro-4-methyl-8-nitro-9H-β-carboline (82 mg).
^{1}H-NMR (300 MHz, DMSO-d6): δ 8.90 (s, 1); 8.65 (s, 1); 8.55 (s, 1); 8.29 (s, 1); 2.89 (s, 3).
NH_4OAc standard conditions.
DAD R_t = 3.00 min.
M+H = 262.

INTERMEDIATE 54: 6-chloro-4-methyl-9H-β-carbolin-8-ylamine

[00191] A solution of 6-chloro-4-methyl-8-nitro-9H-β-carboline (80 mg, 0.31 mmol) in MeOH (10 ml) was stirred at RT. Platinum (10% wt. on carbon, 24 mg) catalyst was added thereto and the reaction vessel was capped and vacuum purged 3 times with argon, followed similarly by hydrogen. The reaction mixture was stirred 1.5 hr under 1 atm hydrogen, then vacuum purged with argon, diluted with DCM (10 ml) and filtered through a 0.2 uM syringe filter. The filtrate was concentrated to yield 6-chloro-4-methyl-9H-β-carbolin-8-ylamine (67 mg) as a light brown oil.
^{1}H-NMR (300 MHz, DMSO-d6): δ 9.04 (s, 1); 8.29 (s, 1); 7.74 (d, 1); 7.10 (d, 1); 2.98 (s, 3).
NH_4OAc standard conditions.

DAD R_f = 1.89 min.

M+H = 231.

EXAMPLE 30: N-(6-chloro-4-methyl-9H-β-carbolin-8-yl)-nicotinamide

[00192] A solution of 6-chloro-4-methyl-9H-β-carbolin-8-ylamine (43 mg, 0.19 mmol) in pyridine (4 ml) was stirred at RT under an argon atmosphere. Nicotinoyl chloride hydrochloride (40 mg, 0.22 mmol) was added thereto and the reaction mixture was stirred for 12 hr. The solution was diluted with H_2O (5 ml) and poured into a separatory funnel containing H_2O (5 ml) and EtOAc (25 ml). The reaction mixture was shaken and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 25 ml). The combined organic layers were washed with a saturated sodium bicarbonate solution (15 ml), followed by brine, then dried over $MgSO_4$, filtered, and concentrated to yield N-(6-chloro-4-methyl-9H-β-carbolin-8-yl)-nicotinamide (5.2 mg) as an orange viscous oil.

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.67 (s, 1); 10.70 (s, 1); 9.27 (s, 1); 8.83 (s, 2); 8.45 (s, 1); 8.20 - 8.12 (m, 2); 7.83 (s, 1); 7.66 (s, 1); 2.80 (s, 3).

NH_4OAc standard conditions.

DAD R_f = 1.92 min.

M+H = 337.

INTERMEDIATE 55: 1,1-Dioxo-1λ^6-thiomorpholine-3,4-dicarboxylic acid 4-tert-butyl ester

[00193] Thiomorpholine-3,4-dicarboxylic acid 4-tert-butyl ester (120 mg, 0.485 mmol) was dissolved in Et_2O (8 ml). To the solution was added mCPBA (172 mg, 0.994 mmol), followed later by a second portion of mCPBA (84 mg,

0.485 mmol). The precipitate which formed was filtered, washed with Et_2O and dried to yield a white solid of 1,1-dioxo-1λ^6-thiomorpholine-3,4-dicarboxylic acid 4-tert-butyl ester (74 mg).

EXAMPLE 31: 1,1-Dioxo-1λ^6-thiomorpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00194] A slurry of 6-chloro-7-fluoro-9H-β-carbolin-8-ylamine (43 mg, 0.198 mmol), 1,1-dioxo-1λ^6-thiomorpholine-3,4-dicarboxylic acid 4-tert-butyl ester (72 mg, 0.257 mmol), and EDCI (76 mg, 0.396 mmol) in pyridine (2 ml) was heated to 70 °C. After 1 hr, the solvent was removed under reduced pressure and the resulting dark oil was dissolved in MeOH (1 ml). The MeOH solution was added drop-wise to a stirring solution of aqueous $NaHCO_3$ and a yellow precipitate was formed. The solid was filtered, dried, and dissolved in 2 M HCl in Et_2O. After stirring overnight the resulting yellow solid was filtered and dried to yield a yellow solid, the di-HCl salt of 1,1-Dioxo-1λ^6-thiomorpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide (78 mg).

^{1}H-NMR (300 MHz, MeOH-d4): δ 9.26 (s, 1); 8.74 (d, 1); 8.54 (d, 1); 8.37 (d, 1); 7.94 (d, 1); 5.01 (dd, 1); 4.15 - 3.75 (m, 4); 3.64 - 3.58 (m, 2).

NH_4OAc standard conditions.

DAD R_f = 1.57 min.

M+H = 379.

INTERMEDIATE 56: 6,6-Dimethyl-morpholine-3,4-dicarboxylic acid 4-tert-butyl ester

[00195] To a suspension of 6,6-dimethyl-morph line-3-carboxylic acid (5.56 g, 34.9 mmol) in dioxane (58 mL) was

added aqueous potassium carbonate (1M, 58 mL). To the resulting clear colorless solution was added di-tert-butyl dicarbonate (9.14 g, 41.9 mmol). The solution was stirred at room temperature overnight. The reaction mixture was diluted with water (200 mL) and the pH of the solution was confirmed to be approximately 7. The reaction mixture was poured into a separatory funnel and extracted with Et_2O (2 x 100 mL) to remove excess di-tert-butyl dicarbonate. The aqueous layer was acidified by addition of 6N aqueous HCl with stirring until a pH of 3 was reached. The mixture was quickly extracted with Et_2O (2 x 200 mL) and the organic layers were combined, dried over magnesium sulfate, filtered, and concentrated to yield a clear colorless oil. The oil was dissolved in Et_2O (50 mL), triturated with hexanes (150 mL), and concentrated to yield a white solid. The solid product was placed on the high-vacuum pump for several hours, after which 8.85 g of 6,6-dimethyl-morpholine-3,4-dicarboxylic acid 4-tert-butyl ester was obtained (97% yield).

^{1}H-NMR (300 MHz, DMSO-d6): δ 12.95 (s, 1); 4.35 ("dd", 1); 3.98 - 3.83 (m, 2); 3.48 ("dd", 1); 2.81 ("dd", 1); 1.39 ("dd", 9); 1.15 (s, 3); 1.08 ("dd", 3).

NH_4OAc standard conditions.

DAD R_t = 0.98 min.

M-H = 258.

INTERMEDIATE 57: (S)-5-(6-Chloro-9H-β-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholine-4-carboxylic acid tert-butyl ester

[00196] The desired compound was prepared according to *Method C* from 6-chloro-9H-β-carboline-8-ylamine and 6,6-

dimethyl-morpholine-3,4-dicarboxylic acid 4-tert-butyl ester in 87% yield.

^{1}H-NMR (DMSO-d6, 300MHz) δ 11.33 (s, 1); 10.14 (s, 1); 9.05 (s, 1); 8.38 (d, 1); 8.21 (d, 1); 8.16 (d, 1); 7.94 (s, 1); 4.70 - 4.56 (m, 1); 4.25 - 4.14 (m, 1); 4.07 (dd, 1); 3.64 - 3.56 (m, 1); 3.30 - 3.14 (m, 1); 1.41 ("dd", 9); 1.21 (s, 3); 1.15 (s, 3).

NH_4OAc standard conditions.

DAD R_t = 1.84 min.

M+H = 459.

Chiral HPLC: ≥ 95% ee.

Chiralpak AD column.

15% v/v EtOH/Hexanes containing 0.1% Et_2NH.

INTERMEDIATE 58: [(S)-4-((S)-2-Amino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide trifluoroacetate salt

[00197] The desired compound was prepared using the same procedure as for Intermediate 35 starting from (S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholine-4-carboxylic acid tert-butyl ester and using Boc-alaninal in the reductive alkylation step in 60% yield.

^{1}H-NMR (300 MHz, D_2O): δ 1.16 (d, 3H), 1.25 (s, 3H), 1.28 (s, 3H), 2.41 (d, 1H), 2.59 (dd, 1H), 2.89 (dd, 1H), 2.95 (d, 1H), 3.35-3.50 (m, 2H), 3.95-4.15 (m, 2H), 7.59 (d, 1H), 7.97 (d, 1H), 8.11 (d, 1H), 8.30 (d, 1H), 8.40 (d, 1H), 8.94 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.57 min.

MS ($M+H^+$): 416.2.

[00198] METHOD E: Coupling procedure using [(S)-4-((S)-2-Amino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide trifluoroacetate salt [(S)-4-((S)-2-Amino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide trifluoroacetate salt (1.0 mmole), TBTU (1.2 mmoles), the acid (1.25 mmoles) to be coupled and Et_3N (4-6 mmoles, basic pH) were taken into acetonitrile(10 ml). The resulting mixture was stirred at ambient temperature for 4-15 hrs. The reaction mixture was then partitioned into EtOAc and 10% aqueous Na_2CO_3 solution. The separated aqueous phase was further extracted with EtOAc. The combined extracts were successively washed with 10% aqueous Na_2CO_3 solution and brine, dried over Na_2SO_4 and concentrated completely. The residue was purified on silica (2-7%MeOH/CH_2Cl_2) to give the corresponding product.

EXAMPLE 39: 6,6-Dimethyl-4-[2-(2,2,2-trifluoro-acetylamino)-propyl]-morpholine-3-carboxylic acid (6-chloro-9*H*-β-carbolin-8-yl)-amide:

[00199] 4-(2-Amino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9*H*-β-carbolin-8yl)-amide (3 CF_3COOH salt) (1.5g) was suspended in dichloromethane (80 mL) along with 5 equivalents of triethylamine. Trifluoroacetic anhydride (56 µL, 2 equivalents) was added and the mixture stirred at room temperature for an hour. The solvent was removed by rotary evaporation. The product was purified by silica gel flash chromatography (5% methanol / dichloromethane, product Rf 0.3) to afford 1g. ^{1}H-NMR (300 MHz, relative to $CDCl_3$ peak at 7.3 ppm) δ 10 (s, 1H), 9.7 (d, 1H) , 8.7 (s, 1H), 8.6 (s, 1H), 8.2 (d,

1H), 7.6 (s, 2H), 6.6 (s, 1H), 4.3 (m, 1H), 3.9 (m, 1H), 3.8 (t, 1H), 3.2 (m, 1H), 2.7-2.9 (m, 2H), 2.5 (m, 1H), 2.2 (d, 1 H), 1.4 (d, 3H), 1.3 (s, 3H), 1.2 (s, 3H).

LCMS (ammonium acetate standard method) retention time = 1.84 min.

(M^+ = 512; M^- = 510).

EXAMPLE 40: 4-((S)-2-Acetylamino-propyl)-6,6-dimethyl-morpholine-3-(S)-carboxylic acid (6-chloro-9H-b-carbolin-8-yl)-amide

[00200] The desired compound was prepared according to the previous example from 4-(2-Amino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9*H*-β-carbolin-8yl)-amide (3 CF_3COOH salt) and acetic anhydride.

^{1}H-NMR (300 MHz, methyl-d_3 alcohol-d): δ 1.15 (d, 3H), 1.23 (s, 3H), 1.39 (s, 3H), 1.98 (s, 3H), 2.24 (d, 1H), 2.38 (m, 1H), 2.68 (m, 1H), 2.92 (d, 1H), 3.24 (m, 1H), 3.98 (m, 2H), 4.22 (m, H), 7.78 (d, 1H), 7.98 (m, 2H), 8.27 (d, 1H), 8.84 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.54 min.

MS ($M+H^+$): 458.

EXAMPLE 41: 4-((S)-2-Methanesulfonylamino-propyl)-6,6-dimethyl-morpholine-3-(S)-carboxylic acid (6-chloro-9H-b-carbolin-8-yl)-amide

[00201] The desired compound was prepared according to Method E.

^{1}H-NMR (300 MHz, methyl-d_3 alcohol-d): δ 1.28 (s, 3H), 1.29 (d, 3H), 1.43 (s, 3H), 2.28 (d, 1H), 2.57 (m, 1H), 2.66 (m, 1H), 2.98 (s, 3H), 3.03 (d, 1H), 3.34 (m, 1H), 3.66 (m,

1H), 4.05 (m, 2H), 7.67 (d, 1H), 8.10 (m, 2H), 8.32 (d, 1H), 8.89 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.54 min.

MS ($M+H^+$): 494.

EXAMPLE 42: 4-{2-[(-4,6-Dimethyl-pyrimidine-5-carbonyl)-amino]-propyl}-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00202] The desired compound was prepared using [4-(2-Amino-propyl)-6,6-dimethyl-morpholin-3-ylmethyl]-(6-chloro-9*H*-beta-carbolin-8-yl)-amine and 4,6-dimethyl pyrimidine-5-carboxylic acid following Method E in 51% yield.

^{1}H-NMR (300MHz, DMSO): δ 11.27 (1H, s), 10.02 (1H, s), 9.0 (1H, s), 8.86 (1H, s), 8.5 (1H, d), 8.3 (1H, d), 8.22 (2H, m), 7.88 (1H, s), 4.1 (1H, m), 3.9 (2H, m), 2.99 (2H, m), 2.36 (6H, s), 2.1 (2H, m), 1.3 (3H, s), 1.24 (6H, m).

Retention time (LC, method: ammonium acetate standard): 1.50 min.

MS (M+H+): 551

EXAMPLE 43: (S)-6,6-Dimethyl-4-{(S)-2-[(2-methyl-pyridine-3carbonyl)amino]propyl}-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide.

[00203] The desired compound was prepared according to Method E from (S)-4-((S)-2-Amino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide trifluoroacetate salt and 2-methyl-nicotinic acid in 75% yield.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.21 (s,3H), 1.22 (d,3H), 1.36 (s,3H), 2.10 (d,1H), 2.42 (m,1H), 2.60 (m,1H), 2.99 (d,1H), 3.20 (m,1H), 3.92 (m,2H), 4.22 (m,1H), 7.22 (dd,1H), 7.65

(d,1H), 7.90 (s,1H), 8.16 (d,1H), 8.23 (s,1H), 8.31 (d,1H),8.38 (d,1H), 8.45 (d,1H), 9.02 (s,1H),10.04 (s,1H), 11.26 (s,1H).

Retention Time (LC, method: ammonium acetate standard): 2.16min.

MS ($M+H^+$): 535.5.

EXAMPLE 44: 6,6-Dimethyl-4-{2-[(tetrahydro-pyran-4-carbonyl)-amino]-propyl}-morpholine-3-carboxylic acid (6-chloro-9H-b-carbolin-8-yl)-amide:

[00204] 4-(2-Amino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-b-carbolin-8-yl)-amide (3 CF_3COOH salt) (300 mg) was suspended in methylene chloride (12 mL) along with 3 equivalents of triethylamine. Morpholine-4-carbonyl chloride (70 mg, 1.3 equivalents) was added and the mixture stirred at room temperature overnight. The solvent was removed by rotary evaporation. The product was separated by preparative TLC on silica plates (10/90 methanol/ethyl acetate as eluent, product Rf 0.4) Yield: 83 mg.

^{1}H-NMR (300 MHz, relative to CD3OD peak at 3.3 ppm) δ 8.8 (s, 1H), 8.27 (d, 1H), 7.9-7.99 (m, 3H), 3.95-4.15 (m, 2H), 3.85-3.95 (m, 2H), 3.5-3.6 (m, 5H), 3.3-3.45 (m, 2H), 3.15-3.3 (m, 2H), 2.85-2.95 (d, 1H), 2.6-2.72 m, 1H), 2.3-2.43 (m, 1H), 2.2-2.28 (d, 1H), 2.0 (s, 2H), 1.35-1.45 (d, 3H), 1.05-1.25 (m, 6H).

LCMS (ammonium acetate standard method) retention time= 2.39 min.(M+ = 529; M- = 527).

EXAMPLE 45: 4-{(S)-2-[(1-Acetyl-pyrrolidine-2-(S)-carbonyl)-amino]-propyl}-6,6-dimethyl-morpholine-3-(S)-carboxylic acid (6-chloro-9H-b-carbolin-8-yl)-amide

[00205] The desired compound was prepared according to Method E.

^{1}H-NMR (300 MHz, methyl-d_3 alcohol-d): δ 1.21 (d, 3H), 1.28 (s, 3H), 1.39 (s, 3H), 1.95 (m, 3H), 2.05 (s, 3H), 2.17 (m, 1H), 2.28 (d, 1H), 2.51 (m, 1H), 2.74 (m, 1H), 3.05 (d, 1H), 3.30 (m, 1H), 3.55 (m, 1H), 3.61 (m, 1H), 4.05 (m, 2H), 4.18 (m, 1H), 4.34 (m 1H), 7.78 (d, 1H), 8.10 (m, 2H), 8.33 (d, 1H), 8.90 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 2.07 min.

MS ($M+H^+$): 555.

EXAMPLE 46: 6,6-Dimethyl-4-{-2-[(5-methyl-isoxazole-3-carbonyl)-amino]-propyl}-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00206] The desired compound was prepared using [4-(2-Amino-propyl)-6,6-dimethyl-morpholin-3-ylmethyl]-(6-chloro-9*H*-beta-carbolin-8-yl)-amine and 5-methylisoxazole carbonyl chloride following Method E in 61% yield.

^{1}H-NMR (300MHz, DMSO): δ 11.2 (1H, s), 9.98 (1H, s), 9.0 (1H, s), 8.7 (1H, d), 8.6 (1H, d), 8.2 (2H, m), 7.9 (1H, s). 6.47 (1H, s), 3.87 (2H, m), 3.17 (2H,m), 2.9 (1H, d), 2.7 (1H, m), 2.3 (4H), 2.1 (1H, d), 1.29 (3H, s), 1.15 (6H, m).

Retention time (LC, method: ammonium acetate standard): 1.81 min.

MS (M+H+): 526

EXAMPLE 47: 6,6-Dimethyl-4-[2-(3-methyl-ureido)-proplyl]-morpholine-3-carboxylic acid (6-chloro-9*H*-β-carbolin-8-yl)-amide:

[00207] 4-(2-Amino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9*H*-□-carbolin-8yl)-amide (3 HCl salt) (300mg) was suspended in dichloromethane (10 mL) triethyl amine (4 equivalents) and methyl isocyanate (2 equivalents) were added simultaneously. After one hour at room temperature solvent was removed by rotary evaporation. The product was purified by silica gel flash chromatography (5% methanol / dichloromethane, product Rf = 0.3) to afford 200mg.

^{1}H-NMR (300 MHz, relative to $CDCl_3$ peak at 7.3 ppm) δ 12.3 (s, 1H), 10.2 (s, 1H), 8.9 (s, 1H), 8.6 (s, 1H), 8.4 (d, 1H), 7.9 (d, 1H), 7.8 (s, 1H), 5.4 (s, 1H), 5.2 (d, 1H), 4.2 (s, 1H), 3.8 (m, 2H), 3.2 (m, 1H), 2.6-3 (m, 7H), 2.3 (d, 1H), 2.2 (t, 1H), 1.4 (s, 3H), 1.2 (s, 3H), 1.1 (d, 3H) LCMS (ammonium acetate standard method) retention time = 1.62 min. (M^+ = 473; M^- = 471).

EXAMPLE 49: {(S)-2-[(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-1-methyl-ethyl}-carbamic acid methyl ester.

[00208] To a solution of (S)-4-((S)-2-Amino-propyl)-6,6-dimethyl-morpholine-3 carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide hydrochloride salt (3.45g, 6.59 mmole) in 68ml of dry pyridine, was added in three portions over 1.5 hr, a 3M DCM solution of methyl chloroformate (9.2 ml, 27.6 mmole, 4.2 eq). After 2 h, 10 ml of water were added and the mixture was concentrated to dryness. The residue was partitioned int 150 ml 0f EtOAc and 100 ml of

an aqueous 0.5M solution of K_2CO_3. The separated aqueous phase was extracted with 50 ml of EtOAc. The combined organic extracts were successively washed with water (2X50 ml) and brine (50 ml), dried over Na_2SO_4 and concentrated to dryness. The residue was purified on silica (5% MeOH/CH_2Cl_2) to give 2.48 g(thick oil, 77% yield) of the desired product.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.15 (d, 3H), 1.28 (s, 3H), 1.42 (s, 3H), 2.33 (dd, 1H), 2.42 (d, 1H), 2.78 (dd, 1H), 2.86 (d, 1H), 3.32 (dd, 1H), 3.86 (s, 3H), 3.92 (t, 1H), 4.01 (dd, 1H), 4.18 (m, 1H), 4.78 (d, 1H), 7.95 (d, 1H), 7.97 (s, 1H), 8.29 (s, 1H), 8.50 (d, 1H), 8.98 (s, 1H), 9.88 (s, 1H), 10.94 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.70 min.

MS ($M+H^+$): 474.1

EXAMPLE 50: 4-{2-[(2,4-Dimethyl-pyridine-3-carbonyl)-amino]-propyl}-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9*H*-beta-carbolin-8-yl)-amide

[00209] The desired compound was prepared from [4-(2-Amino-propyl)-6,6-dimethyl-morpholin-3-ylmethyl]-(6-chloro-9*H*-beta-carbolin-8-yl)-amine nad 2,4-dimethyl nicotinic acid following Method E in 50% yield.

^{1}H-NMR (300MHz, DMSO): δ 11.27 (1H, s), 10 (1H, s), 8.9 (1H, s), 8.37 (2H, d), 8.24 (3H, m), 7.8 (1H, s), 7.04 (1H, d), 3.91 (2H, m), 3.1 (2H, m), 2.36 (4H, m), 2.1 (3H, m), 2.05 (1H, d), 1.3 (3H, s), 1.2 (6H, m).

Retention time (LC, method: ammonium acetate standard) : 1.53 min.

MS (M+H+): 550

EXAMPLE 51: 6,6-dimethyl-4-{2-[(pyrazine-2-carbonyl)-amino]-propyl}-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00210] The desired compound was prepared from [4-(2-Amino-propyl)-6,6-dimethyl-morpholin-3-ylmethyl]-(6-chloro-9H-beta-carbolin-8-yl)-amine and 2-pyrazine carboxylic acid following Method E in 62% yield.

^{1}H-NMR (300MHz, DMSO): δ 12.9 (1H, s), 10.89 (1H, s), 9.42 (1H, s), 9.0 (1H, s), 8.8 (1H, d), 8.6 (1H, d), 8.4 (1H, s), 8.2 (1H, m), 4.5 (1H, m), 4.1 (2H, m), 3.1 (1H, m), 2.1 (4H, m), 1.23 (9H, m).

Retention time (LC, method: ammonium acetate standard): 1.69 min.

MS (M+): 522.2

EXAMPLE 52: Pyridine-3,4-dicarboxylic acid 4-({2-[5-(6-chloro-9H-β-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl}-1-methyl-ethyl}-amide}3-methylamide

[00211] To a solution of [4-(2-Amino-propyl)-6,6-dimethyl-morpholin-3-ylmethyl]-(6-chloro-9H-beta-carbolin-8-yl)-amine (100 mg, 0.132 mmole) in 0.6 ml of dry acetonitrile, was added 3, 4-pyridinedicarboxylic anhydride(21mg, 0.15mmole) and triethylamine (102 ml, 0.8mmole). The reaction mixture was stirred at ambient temperature for 1h. The solvent was then removed under reduced pressure and the residue was taken up into pyridine (0.6 ml). To the resulting mixture was added 2M methylamine solution in THF (0.2 ml, 0.4 mmole) and EDCI (40 mg, 0.21 mmole).The reaction mixture was stirred for 4 hrs, the solvent was removed under reduce pressure and the residue was partitioned into EtOAc and 1M aqueous K_2CO_3.

The separated aqueous phase was extracted twice with EtOAc. The combined organic phases were successively washed with water and brine, dried over $MgSO_4$, and concentrated completely. The residue was purified on silica gel (10% MeOH-CH_2Cl_2) to give the title compound as a white solid in 36% yield.

^{1}H-NMR (300MHz, DMSO): δ 9.24 (1H, s), 8.17 (1H,s), 7.81 (2H, m), 7.5 (3H, m), 7.3 (1H, s), 7.0 (1H, s) 6.5 (1H, d), 3.08 (2H, d), 3.35 (1H, m), 2.4 (3H, m), 1.86 (4H, m), 0.5 (3H, s), 0.37 (6H, m).

Retention time (LC, method: ammonium acetate standard): 1.46 min.

MS (M+H+): 579

EXAMPLE 53: 6.6-Dimethyl-4-{(4-methyl-pyrimidine-5-carbonyl)-amino)-propyl}-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00212] The desired compound was prepared according to Method E from [4-(2-Amino-propyl)-6,6-dimethyl-morpholin-3-ylmethyl]-(6-chloro-9H-beta-carbolin-8-yl)-amine and 4-methyl-pyrimidine-5-carboxylic acid in 55% yield.

^{1}H-NMR (300MHz, DMSO): δ 11.26 (1H, s), 10.04 (1H, s) 9.04 (2H, m), 8.66 (1H, d) 8.3 (2H, m) 8.22-8.17 (2H, m), 7.9 (1H, s), 4.2 (1H, m), 3.93 (2H, m), 3.2 (1H, m), 2.98 (2H, m), 2.6 (3H, m), 2.1 (2H, m), 1.36 (3H, s), 1.23 (6H, m).

Retention time (LC, method: ammonium acetate standard): 1.55 min.

MS (M+H+): 537

EXAMPLE 54: (S)-6,6-Dimethyl-4-{(S)-2-[(4-methyl-pyridine-3-carbonyl)-amino]-propyl}-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide.

[00213] The desired compound was prepared according to Method E from (S)-4-((S)-2-Amino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide trifluoroacetate salt and 4-methyl-nicotinic acid in 79% yield.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.21 (s,3H), 1.22 (d,3H), 1.36 (s,3H), 2.10 (d,1H), 2.40 (m,1H), 2.62 (m,1H), 2.99 (d,1H), 3.22 (m,1H), 3.94 (m,2H), 4.23 (m,1H), 7.26 (d,1H), 7.90 (s,1H), 8.16 (d,1H), 8.23 (s,1H), 8.34-8.46 (m,3H), 9.02 (s,1H),10.04 (s,1H), 11.27 (s,1H).

Retention Time (LC, method: ammonium acetate standard): 2.22min.

MS ($M+H^+$): 535.5.

EXAMPLE 47: 4-[2-(2-Amino-2-methyl-propionyl)]-6,6-dimethyl-morpholine-3-carboxylic acid(6-chloro-9H-β-carbolin-8yl)-amide

[00214] A solution of {2-[5-(6-Chloro-9H-β-carbolin-8-ylcarbamoyl) 2,2-dimethyl-morpholin-4-yl]-1-methyl-ethyl}-carbamic acid tert-butyl ester (70.2 mg, 0.14 mmol) in TFA (2 mL) was stirred at room temperature. After 15 min, the reaction was concentrated and the crude product was azeotroped with CH_2Cl_2 (2 x 5 mL). A mixture of the crude intermediate, TBTU (54.0 mg, 0.17 mmol), triethylamine (0.2 mL, 1.43 mmol) and 2-tert-butoxycarbonylamino-2-methyl-propionic acid (45.0 mg, 0.22 mmol) in MeCN (1 mL) was stirred at room temperature for 18 h. The solution was diluted with H_2O (20 mL) and poured into a separatory funnel containing EtOAc (50 mL), and brine (50 mL). The

mixture was shaken and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organic layers were dried, filtered and concentrated. The crude product was purified by flash chromatography to yield a yellow solid (51.0 mg, 62%) which was shown by NMR and LCMS to be 4-[2-(2-amino-2-methyl-propionyl]-6,6-dimethyl-morpholine-3-carboxylic acid(6-chloro-9H-β-carbolin-8yl)-amide.

^{1}H-NMR (300 MHz, DMSO-d6): δ 12.94 (br s, 1); 11.36 (br s, 1); 10.16 (s, 1); 9.06 (s, 1); 8.39 (d, 1); 8.22 (d, 1); 8.19 (d, 1); 7.95 (s, 1); 4.77 - 4.52 (m, 1); 4.28 - 4.13 (m, 1); 4.13 - 4.00 (m, 1); 3.68 - 3.52 (m, 1); 3.22 - 3.12 (m, 1); 1.44 (s, 3); 1.41 - 1.38 (m, 6); 1.28 - 1.24 (m, 2); 1.22 (s, 3); 1.25 (s, 3); 1.11 - 1.07 (m, 1).

NH_4OAc standard conditions.

DAD R_t = 1.31 min.

M+H = 501.

EXAMPLE 55: 6,6 Dimethyl-4-(1,2,3,4-tetrahydroisoquinolin-3-ylmethyl)-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)amide

[00215] The desired compound was made following the procedure outlined in Method C using 6,6-dimethyl morpholine-3-carboxylic acid and (s)-tetrahydroisoquinoline aldehyde.

^{1}H-NMR (300MHz, D_2O): δ 9.1 (1H, s), 8.68 (1H, d), 8.52 (1H, d), 8.41 (1H, d), 7.68 (1H, d), 7.27 (1H, d), 7.06 (1H, m), 6.97 (1H, d), 6.84 (1H, m), 4.31 (2H, m), 4.09 (2H, m), 3.68 (1H, m), 3.56 (1H, t), 3.2 (2H, m), 3.06 (2H, m), 2.7 (2H, m) 1,48 (3H, s), 1.32 (3H, s)

Retention time (LC, method: ammonium acetate standard) : 2.43 min.

MS (M+H+): 505

INTERMEDIATE 59: 6,6-Dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide, HCl salt

[00216] To a clear brown solution of 5-(6-chloro-9H-β-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholine-4-carboxylic acid tert-butyl ester (10.4 g, 22.7 mmol) in methanol (41 mL) was added HCl in dioxane (4M, 91 mL). The reaction was stirred for 30 minutes at room temperature, during which time a pale brown precipitate began to form. The mixture was poured into a 250-mL volume of vigorously stirring Et_2O. The resulting slurry was stirred at room temperature for 15 minutes, then filtered to yield a pale orange solid. The solid was placed on the high-vacuum pump overnight, after which 9.71 g of 6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide was obtained (99% yield).

^{1}H-NMR (DMSO-d6, 300MHz) δ 13.47 (s, 1); 11.77 (s, 1); 9.42 (s, 1); 8.86 (d, 1); 8.66 (d, 1); 8.58 (d, 1); 8.25 (d, 1); 4.41 - 4.37 (m, 2); 4.05 (dd, 1); 3.32 - 3.28 (m, 1); 3.04 - 3.00 (m, 1); 1.34 (s, 3); 1.30 (s, 3).

NH_4OAc standard conditions.

DAD R_t = 1.48 min.

M+H = 359.

EXAMPLE 56: 4-(2-Amino-butyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide (*also INTERMEDIATE 60*)

[00217] *Method C* was followed, using 5-(6-chloro-9H-β-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholine-4-carboxylic acid tert-butyl ester and the appropriate aldehyde, (1-formyl-propyl)-carbamic acid tert-butyl ester.

^{1}H-NMR (DMSO-d6, 300MHz) δ 9.06 (s, 1); 8.37 (d, 1); 8.19 (d, 1); 8.15 (d, 1); 7.85 (d, 1); 6.75 (br s, 2); 3.96 - 3.85 (m, 2); 3.17 - 3.13 (m, 1); 2.89 (d, 1); 2.78 - 2.74 (m, 1); 2.67 - 2.59 (m, 1); 2.26 - 2.20 (m, 1); 2.14 (d, 1); 1.58 - 1.50 (m, 1); 1.32 (s, 3); 1.32 - 1.23 (m, 1); 1.18 (s, 3); 0.87 (t, 3).

NH_4OAc standard conditions.

DAD R_t = 1.27 min.

M+H = 430.

EXAMPLE 57: 6,6-Dimethyl-4-{2-[(2-methyl-pyridine-3-carbonyl)-amino]-butyl}-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide, HCl salt

[00218] To a solution of 4-(2-amino-butyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide (100 mg, 0.233 mmol) in pyridine (4 mL) was added 2-methyl-nicotinic acid (38.4 mg, 0.280 mmol) and EDCI (71.5 mg, 0.373 mmol). The solution was stirred overnight at room temperature, then diluted with water (5 mL). The mixture was poured into a separatory funnel and diluted further with water (20 mL). The mixture was extracted with EtOAc (2 x 20 mL), then the combined organic layers were washed with brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated to yield a yellow-brown oil which was purified via column chromatography. The resulting yellow solid was dissolved in methanol (2 mL) and HCl in Et_2O (2M, 2 mL) was added. The mixture was stirred for 5 minutes, then concentrated to yield 103 mg of 6,6-dimethyl-4-{2-[(2-methyl-pyridine-3-carbonyl)-amino]-butyl}-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide (71% yield).

^{1}H-NMR (DMSO-d6, 300MHz) δ 13.31 (br s, 1); 11.41 (br s, 1); 11.14 (br s, 1); 9.44 (s, 1); 8.85 (d, 1); 8.73 - 8.64 (m, 3); 8.52 (s, 1); 8.32 (s, 1); 7.72 (s, 1); 4.64 - 3.55 (m, 6); 3.24 - 3.06 (m, 1); 2.95 - 2.81 (m, 1); 2.71 (s, 3); 1.89 - 1.74 (m, 1); 1.55 - 1.41 (m, 1); 1.32 (s, 3); 1.23 (s, 3); 0.93 (t, 3).

NH_4OAc standard conditions.

DAD R_t = 1.66 min.

M+H = 549.

INTERMEDIATE 61: 4-(2-Amino-3-methyl-butyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00219] *Method C* was followed, using 5-(6-chloro-9H-β-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholine-4-carboxylic acid tert-butyl ester and the appropriate aldehyde, (1-formyl-2-methyl-propyl)-carbamic acid tert-butyl ester.

^{1}H-NMR (DMSO-d6, 300MHz) δ 9.05 (s, 1); 8.37 (d, 1); 8.21 (d, 1); 8.16 (dd, 1); 7.90 (d, 1); 6.71 (br s, 2); 3.93 - 3.86 (m, 2); 3.18 - 3.14 (m, 1); 2.91 (d, 1); 2.70 - 2.65 (m, 2); 2.21 (dd, 1); 2.15 (d, 1); 1.74 - 1.66 (m, 1); 1.31 (s, 3); 1.19 (s, 3); 0.85 (d, 3); 0.80 (d, 3).

NH_4OAc standard conditions.

DAD R_t = 1.27 min.

M+H = 444.

EXAMPLE 58: 6,6-Dimethyl-4-{3-methyl-2-[(2-methyl-pyridine-3-carbonyl)-amino]-butyl}-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide

[00220] To a solution of 4-(2-amino-3-methyl-butyl)-6,6-dimethyl-morph line-3-carboxylic acid (6-chloro-9H-β-

carbolin-8-yl)-amide (1.47 g; 3.31 mmol) in pyridine (35 mL) was added 2-methyl-nicotinic acid (544 mg, 3.97 mmol) and EDCI (1.02 g, 5.30 mmol). The solution was stirred 6.5 hours at room temperature, then diluted with water (100 mL). The mixture was poured into a separatory funnel and diluted further with water (50 mL) and EtOAc (150 mL). The layers were shaken and separated. The aqueous layer was extracted with EtOAc (3 x 50 mL), then the combined organic layers were washed with brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated to yield an orange semi-solid residue which was purified via column chromatography. The resulting yellow solid was placed on the high-vacuum pump overnight, after which 1.43 g of 6,6-dimethyl-4-{3-methyl-2-[(2-methyl-pyridine-3-carbonyl)-amino]-butyl}-morpholine-3-carboxylic acid (6-chloro-9H-β-carbolin-8-yl)-amide was obtained (77% yield).

^{1}H-NMR (DMSO-d6, 300MHz) δ 11.32 (s, 1); 10.08 (s, 1); 9.02 (s, 1); 8.46 (dd, 1); 8.38 (d, 1); 8.21 - 8.14 (m, 3); 7.97 (d, 1); 7.64 (dd, 1); 7.23 (dd, 1); 4.23 - 4.14 (m, 1); 3.99 - 3.87 (m, 2); 3.22 - 3.19 (m, 1): 3.02 (d, 1); 2.85 (dd, 1); 2.52 (s, 3); 2.30 (dd, 1); 2.11 (d, 1); 2.05 - 1.95 (m, 1); 1.32 (s, 3); 1.21 (s, 3); 0.93 (d, 3); 0.86 (d, 3)..

NH_4OAc standard conditions.

DAD R_t = 1.67 min.

M+H = 563.

EXAMPLE 59: 6,6-Dimethyl-4-{3-methyl-2-(S)-[(tetrahydro-furan-3-carbonyl)-amino]-butyl}-morpholine-3-(S)-carboxylic acid (6-chloro-9H-b-carbolin-8-yl)-amide

[00221] The desired compound was prepared following Method E from Intermediate 61 and the appropriate acid.

^{1}H-NMR (300 MHz, methyl-d_3 alcohol-d): δ 0.87 (m, 6H), 1.25 (d, 3H), 1.37 (d, 3H), 1.81 (m, 1H), 2.10-2.47 (m, 4H), 2.93 (m, 2H), 3.10 (m, 1H), 3.26 (m, 1H), 3.80 (m, 1H), 3.86-4.07 (m, 6H), 7.86 (d, 1H), 8.10 (m, 2H), 8.32 (d, 1H), 8.89 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 1.73 min. MS ($M+H^+$): 542.

INTERMEDIATE 62: {(S)-2-[(S)-5-(6-Chloro-4-methyl-9H-β-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-1-methyl-ethyl}-carbamic acid tert-butyl ester

[00222] A solution of (S)-4-((S)-2-tert-butoxycarbonylamino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (3.316 g, 10.5 mmol) (prepared by reductively alkylating (S)-6,6-dimethyl-morpholine-3-carboxylic acid with N-(tert-butoxycarbonyl)-L-alanal) in anhydrous pyridine (75 mL) was stirred at room temperature. 6-chloro-4-methyl-9H-β-carbolin-8-ylamine (1.869 g, 8.09 mmol) was added, followed by EDCI (2.894 g, 15.1 mmol). The reaction was stirred at room temperature for 14-18 hours under argon. The reaction was partially concentrated, diluted with H_2O (20 mL) and transferred to a separatory funnel. The mixture was diluted with brine (50 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried, filtered and concentrated to afford a dark residue. Column chromatography (0-8% MeOH / CH_2Cl_2) yielded {(S)-2-[(S)-5-(6-Chloro-4-methyl-9H-β-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-1-methyl-ethyl}-carbamic acid tert-butyl ester as a light tan solid (2.688 g).

^{1}H-NMR (300 MHz, DMSO-d6): δ 11.25 (s, 1) 9.94 (s, 1) 8.88 (s, 1) 8.18 (s, 1) 8.02 (s, 1) 7.92 (s, 1) 6.73 (d, 1) 3.95 - 3.85 (m, 2) 3.66 (br s, 1) 3.16 - 3.08 (m, 1) 2.88 (d, 1) 2.76 (s, 3) 2.51 - 2.40 (m, 1) 2.23 (dd, 1) 1.99 (d, 1) 1.34 (br s, 12) 1.17 (s, 3) 1.08 (d, 3).

NH_4OAc standard conditions.

ELSD R_t = 2.07 min.

M+H = 530.

EXAMPLE 60: (S)-6,6-Dimethyl-4-{(S)-2-[(2-methyl-pyridine-3-carbonyl)-amino]-propyl}-morpholine-3-carboxylic acid (6-chloro-4-methyl-9H-β-carbolin-8-yl)-amide

[00223] A solution of {(S)-2-[(S)-5-(6-Chloro-4-methyl-9H-β-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-1-methyl-ethyl}-carbamic acid tert-butyl ester (2.688 g, 5.08 mmol) in EtOH (60 mL) was stirred at room temperature. Concentrated HCl (10 mL) was added and the reaction stirred for 14 hours at room temperature under argon. The reaction was concentrated to afford a yellow solid (2.84 g). The solid was dissolved in anhydrous pyridine (40 mL) and stirred at room temperature under argon. Triethylamine (2.20 mL, 15.7 mmol) and EDCI (1.39 g, 7.28 mmol) were added. The reaction mixture was stirred at room temperature for 10 minutes and 2-methyl-nicotinic acid (0.868 g, 6.33 mmol) was added. The reaction was stirred at room temperature for 14-18 hours and diluted with H_2O (40 mL). The mixture was poured into a separatory funnel containing H_2O (40 mL), brine (40 mL), and EtOAc (40 mL). The mixture was shaken and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 40 mL) and the combined organic layers washed with brine. The organic

layer was dried, filtered, and concentrated. The resulting residue was dissolved in EtOAc (10-20 mL) and added dropwise to a stirring solution of 4:1 hexanes / Et_2O (300 mL). The precipitate which formed was collected via filtration and air dried to yield (S)-6,6-Dimethyl-4-{(S)-2-[(2-methyl-pyridine-3-carbonyl)-amino]-propyl}-morpholine-3-carboxylic acid (6-chloro-4-methyl-9H-β-carbolin-8-yl)-amide as a tan solid (3.151 g).
^{1}H-NMR (300 MHz, DMSO-d6): δ 11.28 (s, 1) 9.98 (s, 1) 8.81 (s, 1) 8.45 - 8.39 (m, 1) 8.26 (d, 1) 8.13 (s, 1) 7.97 (s, 1) 7.89 (s, 1) 7.64 - 7.56 (m, 1) 7.24 - 7.12 (m, 1) 4.25 - 4.10 (m, 1) 3.91 - 3.82 (m, 2) 3.20 - 3.10 (m, 1) 2.94 (d, 1) 2.71 (s, 3) 2.61 - 2.49 (m, 1) 2.37 (s, 3) 2.06 - 2.02 (m, 2) 1.30 (s, 3) 1.16 (d, 3) 1.15 (s, 3).
NH_4OAc standard conditions.
ELSD R_f = 1.57 min. M+H = 549.

INTERMEDIATE 63: 3,5-Difluoro-4-tributylstannanyl-pyridine

[00224] *n*-Butyl lithium (1.0 eq, 76 mmol, 47.6 mL, 1.6 M in hexanes) was added *via* dropping funnel to a solution of diisopropylamine (1.05 eq, 80 mmol, 11.2 mL) in THF (300 mL) at -78 °C under nitrogen (N_2). The solution was stirred for 30 min at -78 °C, then a solution of 3,5-difluoropyridine (1.05 eq, 80 mmol, 9.2 g) in THF (20 mL) was added dropwise *via* syringe. A beige precipitate was observed to form. The reaction stirred at -78 °C for 90 min then tributyltin chloride (1.0 eq, 76 mmol, 20.7 mL) was added dropwise *via* syringe and the resulting solution allowed to warm to RT over 2 h. Water (5 mL) was added, then roughly 250 mL of THF was removed on a rotary evaporat r. The resulting material was diluted with diethyl

ether (350 mL) and washed successively with water (2x200 mL), saturated sodium chloride solution (1x150 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford the 3,5-Difluoro-4-tributylstannanyl-pyridine as a colourless oil (27.5 g, 88%). This material was used crude without further purification.
Retention Time (LC, method: ammonium acetate standard): 3.35 min. MS ($M+H^+$): 406.

INTERMEDIATE 64: 4-Chloro-2-(3,5-difluoro-pyridin-4-yl)-phenylamine

[00225] Stille coupling: A dimethyl formamide (256 mL) solution of crude Intermediate 63 (1.1 eq, 70 mmol, 27.5 g) and 2-iodo-4-chloro-phenylamine (1.0 eq, 64 mmol, 16.2 g) was degassed with N_2 for 15 min. Dichlorobis(triphenylphosphine)palladium (II) (0.05 eq, 3.2 mmol, 2.2 g) and copper (I) iodide (0.1 eq, 6.4 mmol, 1.2 g) were added and the suspension heated at reflux for 15 h under N_2. The mixture was cooled to RT, filtered through a short plug of celite® and the dimethyl formamide removed on a rotary evaporator. The crude material was dissolved in acetonitrile (300 mL), washed with hexanes (2x 200 mL) then concentrated *in vacuo*. The material was then dissolved in ethyl acetate (400 mL) and washed successively with water (2x200 mL), saturated sodium bicarbonate solution (1x200 mL), saturated sodium chloride solution (200 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting solid was triturated with diethyl ether (50 mL) to remove the dark colour, then dissolved in the minimum volume of methanol, filtered to remove an insoluble impurity and concentrated in vacuo to afford the 4-Chloro-2-(3,5-difluoro-pyridin-4-yl)-phenylamine as a tan solid

(12.3 g, ~80%,) which was used in the subsequent step without further purification.
^{1}H-NMR (300 MHz, dmso-d_6): δ 5.28 (s, 2H), 6.77 (d, 1H), 7.08 (d, 1H), 7.19 (dd, 1H) and 8.58 (s, 2H).
Retention Time (LC, method: ammonium acetate standard): 1.70 min. MS ($M+H^+$): not observed.

INTERMEDIATE 65 : 6-Chloro-4-fluoro-9H-β-carboline

[00226] Sodium bis(trimethylsilyl)amide (3.0 eq, 130 mmol, 130 mL, 1.0M in THF) was added *via* dropping funnel to a solution of crude Intermediate 64 (1.0 eq, 43 mmol, 10.4 g) in THF at RT under N_2. After stirring for 15 h the excess base was quenched by the cautious addition of saturated ammonium chloride solution (100 mL) and the majority of the THF removed on a rotary evaporator. The resulting slurry was extracted with ethyl acetate (400 mL then 2x200 mL), then the combined organics were washed successively with saturated sodium bicarbonate solution (300 mL), saturated sodium chloride solution (300 mL), dried over sodium sulfate and filtered. Silica gel was added and the slurry concentrated on a rotary evaporator. The material was purified using a Biotage Flash 75 purification system (short column) eluting with 96:4 dichloromethane/methanol to afford the 6-Chloro-4-fluoro-9H-β-carboline as an off-white solid (7.8 g, 82%).
^{1}H-NMR (300 MHz, dmso-d_6): δ 7.71-7.61 (m, 3H), 8.11 (d, 1H) and 12.16 (s, 1H).
Retention Time (LC, method: ammonium acetate standard): 1.70 min. MS ($M+H^+$): 221.

INTERMEDIATE 66 : 6-chloro-4-fluoro-9H-β-carbolin-8-ylamine

[00227] Sodium nitrate (1.5 eq, 53 mmol, 4.5 g) was added portionwise to a solution of Intermediate 65 (1.0 eq, 35 mmol, 7.8 g) in trifluoroacetic acid (200 mL) and the resulting mixture heated at 70 °C for 3 h. After cooling to RT the trifluoroacetic acid was removed on a rotary evaporator to afford a crude solid which was suspended in a small volume of methanol and added dropwise to a vigorously stirred mixture of saturated sodium bicarbonate solution (500 mL). The resulting slurry was stirred for 15 min then the precipitated solids were collected by suction filtration, washed with water (300 mL) and then dried *in vacuo* to afford 6-chloro-4-fluoro-8-nitro-9H-β-carboline (about 9.5 g) which was used in the subsequent step without further purification.

Retention Time (LC, method: ammonium acetate standard): 1.79 min. MS ($M+H^+$): 266

[00228] Sulfated platinum (~0.1 eq, 1 g) was added to a suspension of 6-chloro-4-fluoro-8-nitro-9H-β-carboline (1.0 eq, 35 mmol, 9.3 g) and ammonium formate (3.0 eq, 105 mmol, 6.6 g) in ethanol (175 mL) and the resulting mixture heated at 75 °C for 4 h. After cooling to RT the mixture was filtered through a short plug of Celite® washing with copious amounts of methanol, and then the filtrate concentrated *in vacuo* to afford a beige solid. The solid was suspended in the minimum volume of methanol and added dropwise to a vigorously stirred mixture of saturated sodium bicarbonate solution and saturated sodium chloride solution. After stirring for 15 min the precipitated solids were collected by suction filtration, washed with water (200 mL) and dried *in vacuo* to afford 6-chloro-4-fluoro-9H-

β-carbolin-8-ylamine (5.8 g, 70% 2 steps) as a beige powder.

^{1}H-NMR (300 MHz, dmso-d_6): δ 5.76 (s, 2H), 6.81 (d, 1H), 7.29 (d, 1H), 8.24 (d, 1H), 8.82 (d, 1H), and 11.71 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.59 min. MS ($M+H^+$): 236.

EXAMPLE 61: 4-(2-Acetylamino-propyl)-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-4-fluoro-9H-b-carbolin-8-yl)-amide

[00229] The desired compound was prepared from Intermediate 66 and acetic anhydride.

^{1}H-NMR (300 MHz, dmso-d_6): δ 11.70 (s, 1H), 10.17 (s, 1H), 8.92 (s, 1H), 8.34 (d, 1H), 7.95 (s, 2H), 7.81 (d, 1H), 4.05-3.95 (m, 1H), 3.92-3.83 (m, 2H), 3.18-3.12 (m, 1H), 2.87 (d, 1H), 2.55-2.47 (m, 1H), 2.40-2.31 (m, 1H), 2.03 (d, 1H), 1.78 (s, 3H), 1.32 (s, 3H), 1.63 (s, 3H) and 1.08 (d, 3H).

Retention Time (LC, method: ammonium acetate standard): 1.73 min. MS ($M+H^+$): 474.

EXAMPLE 62: 6,6-Dimethyl-4-{2-[(2-methyl-pyridine-3-carbonyl)-amino]-propyl}-morpholine-3-carboxylic acid (6-chloro-4-fluoro-9H-β-carbolin-8-yl)-amide

[00230] The desired compound was prepared according to Method E from Intermediate 66 and methylnicotinic acid.

^{1}H-NMR (300 MHz, dmso-d_6): δ 11.63 (s, 1H), δ 10.10 (s, 1H), δ 8.90 (s, 1H), δ 8.44 (d, 1H), δ 8.34 (s, 1H), δ 8.29 (d, 1H), 7.94 (s, 2H), 7.65 (d, 1H), 7.21 (dd, 1H), 4.25-4.15 (m, 1H), 3.97-3.88 (m, 2H), 3.24-3.15 (m, 1H),

2.99 (d, 1H), 2.59 (t, 1H), 2.49 (s, 3H), 2.40 (dd, 1H), 2.09 (d, 1H), 1.35 (s, 3H), 1.21 (d, 3H) and 1.20 (s, 3H). Retention Time (LC, method: ammonium acetate standard): 1.67 min. MS ($M+H^+$): 553.

Example 63: [(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid

[00231] To a suspension of (S)-6,6-Dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide (2.6 g, 6.0 mmoles) in 60 ml of methanol was added 1.7 ml of triethylamine (2.0 eq.), sodium cyanoborohydride (575 mg, 9.1 mmoles) and glyoxylic acid (780 mg, 8.5 mmoles). The reaction mixture was stirred at ambient temperature for 1.5 hrs. Water was added (5 ml) and the mixture was concentrated to a thick yellow slurry. More water was then added (30 ml) and the resulting slurry was stirred at ambient temperature for 10 min. and was filtered. The collected yellow solid was washed with water and dried under high vacuum to give 1.80 g (71%) of the desired product.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.17 (s,3H), 1.30 (s,3H), 2.81 (d,1H), 3.34 (d,1H), 3.46 (d,1H), 3.56 (dd,1H), 3.84-3.90 (m,2H), 7.92 (s,1H), 8.15 (d,1H), 8.21 (s,1H), 8.36 (d,1H), 9.01 (s,1H), 10.28 (s,1H),11.40 (s,1H) .

Retention Time (LC, method: ammonium acetate standard): 1.26 min.

MS ($M+H^+$): 417.1.

METHOD F: Coupling procedure for reverse amides from *[(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid*

[00232] [(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid (1.0 mmol), EDCI (1.6 mmol) and the amine (1.2 mmol) to be coupled were taken in a round-bottom flask and suspended in pyridine (5 ml). The resulting mixture was stirred overnight. The pyridine was then removed under reduced pressure and the residue was partitioned in EtOAc and 5% aqueous Na_2CO_3 solution. The separated aqueous phase was further extracted with EtOAc. The combined extracts were successively washed with water and brine, dried over Na_2SO_4 and concentrated completely. The residue was purified on silica to give the desired product.

EXAMPLE 64: (S)-6,6-Dimethyl-4-(2-oxo-2-pyrrolidin-1-yl-ethyl)-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00233] The desired compound was prepared according to Method F from [(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid and pyrrolidine in 82% yield.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.21 (s,3H), 1.29 (s,3H), 1.75-1.92 (m,4H), 2.46 (d,1H), 2.77 (d,1H), 3.35-3.68 (m,7H), 3.94 (m,2H), 8.08 (s,1H), 8.19 (d,1H), 8.23 (s,1H), 8.41 (d,1H), 9.05 (s,1H), 10.71 (s,1H),11.51 (s,1H).

Retention Time (LC, method: ammonium acetate standard): 1.75 min.

MS ($M+H^+$): 470.3.

EXAMPLE 65: (S)-6,6-Dimethyl-4-(2-oxo-2-piperidin-1-yl-ethyl)morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00234] The desired compound was prepared according to Method F from [(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid and piperidine in 90% yield.

^{1}H-NMR (300 MHz, DMSO-d6): δ 1.17 (s,3H), 1.29 (s,3H), 1.35-1.60 (m,6H), 2.25 (d,1H), 2.71 (d,1H), 3.15 (d,1H), 3.26 (dd,1H), 3.37 (dd,1H), 3.45-3.65 (m, 2H), 3.65 (d, 1H), 3.70 (m, 1H), 3.89 (m, 2H) 7.95 (d,1H), 8.15 (d,1H), 8.20 (d,1H), 8.37 (d,1H), 9.01 (s,1H), 10.43 (s,1H),11.32 (s,1H).

Retention Time (LC, method: ammonium acetate standard): 1.86 min.

MS (M+H$^+$): 484.3.

EXAMPLE 66: (S)-6,6-Dimethyl-4-(2-morpholin-4-yl-2-oxo-ethyl)-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00235] The desired compound was prepared according to Method F from [(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid and morpholine in 86% yield.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.17 (s,3H), 1.30 (s,3H), 2.19 (d,1H), 2.71 (d,1H), 3.03 (d,1H), 3.16 (d,1H), 3.22 (dd,1H), 3.45-3.72 (m,7H), 3.80-3.98 (m, 3H), 7.94 (d,1H), 8.15 (d,1H), 8.21 (d,1H), 8.37 (d,1H), 9.03 (s,1H), 10.35 (s,1H),11.28 (s,1H) .

Retention Time (LC, method: ammonium acetate standard): 1.56 min.

MS ($M+H^+$): 486.3.

EXAMPLE 67: (S)-4-{[(2-Hydroxy-ethyl)-methyl-carbamoyl]-methyl}-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00236] The desired compound was prepared according to Method F from [(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid and 2-methylamino-ethanol in 55% yield.

^{1}HNMR (300 MHz, DMSO-d_6): δ 1.21 (s, 3H), 1.30 (s, 3H), 2.39 (m,1H), 2.75 (bd,1H), 2.96 (s,1.5H), 3.17 (s,1.5H), 3.50-3.65 (m,2.5H), 3.70-3.85 (m,1.5H), 3.93 (m, 2H), 4.69 (m, 0.5H), 4.94(m, 0.5H), 8.05 (d,1H), 8.18-8.25 (m, 2H), 8.42 (d,1H), 9.06 (s,1H), 10.68 (s,1H),11.38 (s,1H) .

Retention Time (LC, method: ammonium acetate standard): 1.47 min.

MS ($M+H^+$): 474.

EXAMPLE 68: (S)-6,6-Dimethyl-4-(pyridin-3-ylcarbamoylmethyl)-morpholine-3-carboxylic acid (6-chlor -9H-beta-carbolin-8-yl)-amide

[00237] The desired compound was prepared according to Method F from [(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid and 3-aminopyridine. The product was isolated as hydrochloride salt in 55% yield.

^{1}HNMR (300 MHz, D_2O): δ 1.34 (s, 3H), 1.54 (s, 3H), 2.60 (d,1H), 3.4 (d, 1H), 3.50 (d, 1H), 3.70 (t, 1H), 3.81 (d, 1H) 4.23 (d, 2H), 7.69 (d, 1H), 7.98 (d, 1H), 8.01 (d, 1H), 8.17(d, 1H), 8.42 (d, 1H), 8.48-8.55 (m, 3H), 9.09 (s,1H), 9.30 (d, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.56 min.

MS ($M+H^+$): 493.2.

EXAMPLE 69: (S)-6,6-Dimethyl-4-{[(pyridin-4-ylmethyl)-carbamoyl]-methyl}-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00238] The desired compound was prepared according to Method F from [(S)-5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid and 4-(aminomethyl)pyridine. The product was isolated as hydrochloride salt in 53% yield.

^{1}HNMR (300 MHz, D_2O): δ 1.34 (s, 3H), 1.49 (s, 3H), 2.64 (d, 1H), 3.03 (d, 1H), 3.48 (d, 1H), 3.72 (t, 1H), 3.74 (d, 1H), 4.15-4.25 (m, 2H), 7.66 (d, 1H), 7.88 (s, 1H), 7.91 (s, 1H), 8.21(d, 1H), 8.44 (d,1H), 8.53 (d, 1H), 8.56 (s, 1H), 8.58 (s, 1H), 9.08 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 1.47 min.

MS ($M+H^+$): 507.3.

EXAMPLE 70: 4-[2-(4-Hydroxymethyl-piperidin-1-yl)-2-oxo-ethyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00239] [5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid (200 mg, 0.48 mmol) and piperidin-4-yl-methanol (111 mg, 0.96 mmol) were dissolved in pyridine (4 mL). The resulting yellow solution was stirred at room temperature for 10 min and then EDC (184 mg, 0.96 mmol) was added in a single portion. The reaction mixture was allowed to stir over night (16 h).

Water (4 mL) was added and the mixture was concentrated under reduced pressure. The resulting residue was partitioned between ethyl acetate (50 mL) and 1 M aqueous potassium carbonate. The aqueous layer was back-extracted with ethyl acetate (3 x 50 mL) and the combined extracts were washed with water and brine, dried over sodium sulfate, filtered, and concentrated. The crude residue was purified by silica gel chromatography (methylene chloride and methanol gradient) to afford pure 4-[2-(4-hydroxymethyl-piperidin-1-yl)-2-oxo-ethyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide as a yellow foam (152 mg, 62%). The bis-HCl salt was prepared by adding 2 equivalents of conc. HCl to an ethanolic solution of the freebase. Concentration, followed by ether trituration afforded the salt as a free-flowing, yellow powder.

^{1}H-NMR (freebase, 300 MHz, $CDCl_3$) δ: 11.03 (d, 1 H), 10.51 (d, 1 H), 8.97 - 8.80 (m, 1 H), 8.47 - 8.27 (m, 2 H), 7.94 - 7.76 (m, 2 H), 4.84 (d, 1 H), 4.16 - 3.77 (m, 3 H), 3.68 - 3.26 (m, 5 H), 3.08 (ddd, 1 H), 2.89 - 2.64 (m, 2 H), 2.50 - 2.37(m, 2 H), 1.98 - 1.70 (m, 3 H), 1.38 (s, 3 H), 1.25 (s, 3 H), 1.19 - 0.97 (m, 1).

MS (NH_4OAc standard conditions, ES+) e/z = 514 $(M+H)^+$

DAD R_t = 1.51 min

EXAMPLE 71: 4-[2-(4-Hydroxy-piperdin-1-yl)-2-oxo-ethyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00240] The desired compound was synthesized using [5-(6-Chloro-9*H*-beta-carbolin-8-ylcarbamoyl)-2, 2-dimethyl-

morpholin-4-yl]-acetic acid and 4-hydroxypiperazine following Method F in 31% yield.

^{1}H-NMR (300MHz, D_2O): δ 8.85 (1H,s), 8.19 (2H, s), 7.82 (1H, s), 7.44 (1H, s), 4.15 (2H, m), 3.9-3.75 (4H, m), 3.6 (2H, m), 3.56 (2H, m), 3.12 (1H, m), 2.9 (3H, m), 2.8 (1H, m), 1.7 (2H, m) 1.34 (3H, s), 1.19 (3H, s)

Retention time (LC, method: ammonium acetate standard): 1.5 min

MS (M+H+): 501

EXAMPLE 72: 4-Diethylcarbamoylmethyl-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00241] The desired compound was made using of [5-(6-Chloro-9*H*-beta-carbolin-8-ylcarbamoyl)-2, 2-dimethyl-morpholin-4-yl]-acetic acid and diethylamine following Method F in 60% yield.

^{1}H-NMR (300MHz, D_2O): δ 8.87 (1H, s), 8.26 (2H, m), 7.8 (1H, s), 7.52 (1H, s), 4.1 (3H, m), 3.8 (2H, m), 3.22 (2H, m), 3.0 (1H, d), 2.7 (1H, d), 1.3 (3H, s), 1.19 (3H, s), 0.09 (6H, m).

Retention time (LC, method: ammonium acetate standard) : 1.96min

MS (M+H+): 472

EXAMPLE 73: 6,6-dimethyl-4-[-2-(4-methyl-piperazin-1-yl)-2-oxo-ethyl]-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00242] The desired compound was prepared using [5-(6-Chloro-9*H*-beta-carbolin-8-ylcarbamoyl)-2, 2-dimethyl-

morpholin-4-yl]-acetic acid and 1-methyl piperazine following Method F in 12% yield.

^{1}H-NMR (DMSO) δ 11.28 (1H, s), 10.35 (1H, s), 9.01 (1H,s), 8.37 (1H, s), 8.21-8.15 (2H, m), 7.9 (1H, s), 3.89 (2H, m), 3.7 (5H, m), 3.1 (2H, m), 2.85 (2H, m), 2.49 (3H, s), 2.4 (3H, m), 1.2 (3H, s), 1.16 (3H, s)

Retention time (LC, method: ammonium acetate standard): 1.34 min.

MS (M+H+): 500

EXAMPLE 74: 4-[2-(2,6-Dimethyl-morpholin-4-yl)-2-oxo-ethyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-b-carbolin-8-yl)-amide

[00243] The desired compound was prepared using [5-(6-Chloro-9*H*-beta-carbolin-8-ylcarbamoyl)-2, 2-dimethyl-morpholin-4-yl]-acetic acid and 2,6-dimethyl morpholine following Method F. Chromatographic purification gave the desired product in 70-80% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 9.31 (s, 1H), 8.75 (d, 1H), 8.55 (d, 1H), 8.37 (d, 1H), 8.14 (m, 1H), 4.73 (m, 2H), 4.96 (m, 1H), 4.37 (m, 2H), 3.69 (m, 2H), 3.57 (m, 4H), 2.82 (t, 1H), 2.47 (t, 1H), 1.50 (d, 3H), 1.42 (d, 3H), 1.18 (m, 6H)

Retention Time (LC, method: formic acid standard): 1.14 min (Diode Array).

MS ($M+H^+$): 514, ($M-H^+$): 512

EXAMPLE 75: 1-{2-[5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetyl}-piperidine-4-carboxylic acid methyl ester

[00244] [5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid (200 mg, 0.48 mmol)

and methyl isonipecotate (137 mg, 130 uL, 0.96 mmol) were dissolved in pyridine (4 mL) and stirred at room temperature 10 min. EDC was added and the reaction mixture was allowed to stir at room temperature over night (16 h). Additional methyl isonipecotate (137 uL, 0.96 mmol) was added and the mixture was stirred an additional 24 h. Water (4 mL) was added and the mixture concentrated. The crude residue was partitioned between ethyl acetate (75 mL) and 1 M aqueous potassium carbonate (50 mL). The aqueous phase was back-extracted with additional ethyl acetate (75 + 50 mL). The combined extracts were washed with water and brine, dried over sodium sulfate, filtered, concentrated and purified by silica gel chromatography (methylene chloride/methanol gradient) to afford the freebase as a yellow foam (150 mg, 57%). The bis-HCl salt was prepared by adding 2 equivalents of conc. HCl to an ethanolic solution of the freebase. Concentration, followed by ether trituration afforded the salt as a free-flowing, yellow powder.

^{1}H-NMR (freebase, 300 MHz, $CDCl_3$) δ 11.04 (d, 1 H), 10.48 (d, 1 H), 8.95 (s, 1 H), 8.48 - 8.31 (m, 2 H), 7.96 - 7.79 (m, 2 H), 4.48 (dd, 1 H), 4.13 - 3.92 (m, 2 H), 3.88 - 2.97 (m, 9 H), 2.84 - 2.35 (m, 3 H), 2.09 - 1.90 (m, 2 H), 1.85 - 1.52 (m, 2 H), 1.38 (s, 3 H), 1.23 (s, 3 H).

MS (NH_4OAc standard conditions, ES+) e/z = 542 $(M+H)^+$

DAD R_t = 1.72 min

EXAMPLE 76: (*S*)-4-[2-(3,3-Dimethyl-morpholin-4-yl)-2-oxo-ethyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9*H*-beta-carbolin-8-yl)-amide-*bis*-hydrochloride salt

[00245] 2,2-Dimethylmorpholine (3.0 g, 26.1 mmol) (prepared according to the procedure of Cottle, D.L., et al: *J. Org. Chem.* 1946, *11*, 286-291) was dissolved in dichloromethane (60 mL). Triethylamine (3.6 mL, 2.66 g, 26.1 mmol) was added and the reaction was cooled to -10°C. Bromoacetyl chloride (2.2 mL, 4.08 g, 26.1 mmol) was added dropwise and the solution was warmed slowly to room temperature. The reaction was concentrated to dryness *in vacuo*, redissolved in ethyl acetate and passed through a plug of silica gel. The eluant was concentrated to a yellow oil (3.45 g, 56%) that was carried to the next step. Retention Time (LC, method: ammonium acetate standard): 1.20 min.
MS ($M+H^+$): 237.

[00246] Intermediate 59 (60 mg, 0.14 mmol) was suspended in dichloromethane (3 mL). A solution of 1M potassium carbonate (0.5 mL) was added. The organic layer was separated, dried over $MgSO_4$ and concentrated to dryness. The free base was dissolved in DMF (1 mL) and stirred at room temperature. 2-Bromo-1-(3,3-dimethyl-morpholin-4-yl)-ethanone (30 mg, 0.13 mmol) was dissolved in DMF (1 mL) and added dropwise. The reaction was stirred for 3 hours at room temperature and concentrated to dryness *in vacuo*. Flash column chromatography (93:7 dichloromethane:methanol) afforded a yellow oil, which was dissolved in 4N HCl/dioxane (2 mL). Concentration *in vacuo* afforded the title compound as a yellow solid (22 mg, 29%).
^{1}HNMR (300 MHz, MeOH-d_4): δ 1.35 (s, 3H), 1.41 (s, 3H), 1.43 (s, 6H), 2.98 (m, 1H), 3.45 (m, 4H), 3.56 (m, 1H), 3.76 (m, 3H), 4.30 (m, 4H), 8.04 (s, 1H), 8.39 (s, 1H), 8.52 (d, 1H), 8.73 (d, 1H), 9.24 (s, 1H)

Retention Time (LC, method: ammonium acetate standard): 1.16 min.

MS ($M+H^+$): 514

EXAMPLE 77: 4-[(*Trans*-4-hydroxy-cyclohexylcarbamoyl)-methyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00247] [5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid (200 mg, 0.48 mmol), *trans*-4-aminocyclohexanol hydrochloride (145 mg, 0.96 mmol) and diisopropylethylamine (124 mg, 167 uL, 0.96 mmol) were dissolved in pyridine (4 mL) and stirred at room temperature10 min. EDC (184 mg) was added and the reaction was stirred at room temperature over night (16 h). Water (2 mL) was added and the resulting mixture was concentrated under reduced pressure. The resulting residue was diluted with 50 mL 1 M aqueous potassium carbonate and extracted with ethyl acetate (75 + 2 x 50 mL). The combined extracts were washed with water and brine, dried over sodium sulfate, filtered and concentrated. The crude residue was purified by silica gel chromatography (methanol / methylene chloride gradient) to afford pure 4-[(*trans*-4-hydroxy-cyclohexylcarbamoyl)-methyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide (148 mg, 59%) as a yellow foam. The bis-HCl salt was prepared by adding 2 equivalents of conc. HCl to an ethanolic solution of the freebase. Concentration, followed by ether trituration afforded the salt as a free-flowing, yellow powder.

^{1}H-NMR (freebase, 300 MHz, $CDCl_3$) δ 11.71 (br s, 1 H), 10.21 (s, 1 H), 9.06 (s, 1 H), 8.45 - 8.27 (m, 2 H), 8.08 -

7.84 (m, 2 H), 6.36 (br s, 1 H), 4.13 - 3.81 (m, 3 H), 3.72 - 3.56 (m, 1H), 3.51 - 3.37 (m, 2 H), 3.03 (d, 1 H), 2.79 - 2.26 (m, 5 H), 2.16 - 1.94 (m, 4 H), 1.54 - 1.30 (m, 5 H), 1.22 (s, 3 H).

MS (NH_4OAc standard conditions, ES+) e/z = 514 $(M+H)^+$

DAD R_t = 1.39 min

EXAMPLE 78: (*S*)-4-[2-((2*R*,5*R*)-2,5-Dimethyl-pyrrolidin-1-yl)-2-oxo-ethyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9*H*-beta-carbolin-8-yl)-amide-*bis*-hydrochloride salt

[00248] (2R,5R)-2,5-Dimethyl-pyrrolidine hydrochloride (270 mg, 2.0 mmol) (prepared using the procedure of Masamune, S., et al. *J. Org. Chem.* 1989, *54*, 1756) was dissolved in dichloromethane (3 mL) and cooled to 0°C. Triethylamine (405 mg, 0.56 mL, 4.0 mmol) was added. Chloroacetyl chloride (226 mg, 0.16 mL, 2.0 mmol) was dissolved in dichloromethane (1 mL) and added dropwise. The mixture was warmed to room temperature and stirred an additional 30 minutes. The reaction was diluted with dichloromethane (5 mL), extracted with 1N HCl and brine, then dried over $MgSO_4$. The organic layer was concentrated to a brown oil. Wt.: 242 mg (82%).

Retention Time (LC, method: ammonium acetate standard): 1.24 min.

MS ($M+H^+$): 176.5. The material was carried to the next step without further purification.

[00249] Intermediate 59 (495 mg, 1.15 mmol) was dissolved in a mixture of acetonitrile (8 mL) and water (2 mL). Potassium carbonate (477 mg, 3.45 mmol) was added and the reaction was warmed to 40 °C. 2-Chloro-1-((2R,5R)-2,5-dimethyl-pyrrolidin-1-yl)-ethanone (242 mg, 1.38 mmol)

(prepared as described above) was dissolved in acetonitrile (1 mL) and added dropwise. The reaction was warmed to 80°C and stirred overnight. The reaction was cooled to 40 °C. Sodium iodide (207 mg, 1.38 mmol) was dissolved in acetone (1 mL) and added in one portion. The reaction was stirred overnight at 40 °C. The reaction was concentrated *in vacuo* and diluted with ethyl acetate (40 mL). The organic layer was extracted twice with water, brine then dried over $MgSO_4$. The organic layer was filtered, and concentrated to an orange foam *in vacuo*. Flash column chromatography (95:5 dichloromethane:methanol) afforded a yellow solid, which was dissolved in 4N HCl/dioxane (1 mL) and concentrated to dryness. Trituration with ether afforded the title compound as a yellow solid (18 mg, 3%).

^{1}H-NMR (300 MHz, MeOH-d_4): δ 1.23 (dd, 6H), 1.38 (s, 3H), 1.48 (s, 3H), 1.65 (m, 2H), 2.23 (m, 2H), 3.18 (m, 1H), 3.50 (m, 1H), 3.66 (m, 1H), 4.18 (m, 2H), 4.35 (m, 4H), 8.15 (s, 1H), 8.42 (s, 1H), 8.55 (d, 1H), 8.78 (d, 1H), 9.29 (s, 1H).

Retention Time (LC, method: ammonium acetate standard): 2.02 min.

MS ($M+H^+$): 498.

EXAMPLE 79: 4-{2-[4-(1-Hydroxy-1-methyl-ethyl)-piperidin-1-yl]-2-oxo-ethyl}-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-b-carbolin-8-yl)-amide.

[00250] A solution of 1-{2-[5-(6-chloro-9H-b-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetyl}-piperidine-4-carboxylic acid methyl ester (62 mg, 0.11 mmol) in a mixture of anhydrous ether and toluene (1 ml: 1ml) was stirred at 0° C under N_2. To this solution was slowly added methlymagnesium bromide (3.0 M in ether,

306μL, 0.917mmol). The reaction mixture was stirred at room temperature overnight, and then quenched by adding saturated aqueous sodium bicarbonate. The resulting mixture was further diluted with water (10 ml) and ethyl acetate (30 ml). The aqueous layer was removed and extracted with ethyl acetate (30x2 ml). The organic layers were combined, washed with brine, dried over magnesium sulfate, filtered and concentrated to afford a yellow solid (85 mg). The residue was purified by HPLC, to afford the pure product (13 mg, 20%).

^{1}H-NMR (300 MHz, $HCDCl_3$): δ 10.94 (d, 1 H), 10.47 (d, 1 H), 8.94 (s; 1 H), 8.41 (d, 1 H), 8.32 (d, 1 H), 7.89 - 7.81 (m, 2H), 4.91 (d, 1 H), 4.04 - 3.91 (m, 2 H); 3.64 - 3.57 (m, 1 H), 3.43 (s, 1 H), 3.37 - 3.31 (m, 1 H), 3.15 - 2.90 (m, 1 H), 2.77 - 2.58 (m, 2 H), 2.47 - 2.41 (m, 1 H), 2.00 - 1.86 (m, 2 H), 1.53 - 1.03 (m, 17 H).

NH_4OAc standard conditions.

DAD R_t = 1.85 min

M+H = 542

EXAMPLE 80: 4-[2-(3,3-Dimethyl-4-oxo-piperidin-1-yl)-2-oxo-ethyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloro-9H-beta-carbolin-8-yl)-amide

[00251] 4-Oxo-piperidine-1-carboxylic acid tert-butyl ester (5 g, 25 mmol) was dissolved in tetrahydrofuran (100 mL) and the resulting solution was cooled to 0 °C. Sodium hydride (60% in mineral oil, 2.10 g, 53 mmol) was added t the cooled solution in a single portion, and the resulting cloudy mixture was allowed to stir 10 min. Methyl iodid was subsequently added and the mixture was allowed to warm to room temperature over several hours. Stirring continued

ver night (12 h). The light orange mixture was concentrated under reduced pressure. The residue was partitioned between ether and water. The aqueous phase was back-extracted with additional ether. The combined extracts were washed with water and brine, dried over sodium sulfate, filtered and concentrated to a pale yellow solid. The solid was triturated with 4% ethyl acetate in hexanes (50 mL) to afford 3,3-dimethyl-4-oxo-piperidine-1-carboxylic acid tert-butyl ester as a cream-colored solid (1.8 g, 32%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.73 (t, 2 H), 3.43 (br s, 2 H), 2.49 (t, 2 H), 1.49 (s, 9 H), 1.13 (s, 6 H).

[00252] The 3,3-dimethyl-4-oxo-piperidine-1-carboxylic acid tert-butyl ester thus prepared (450 mg, 1.97 mmol) was dissolved in methylene chloride (10 mL). Trifluoroacetic acid was added (305 uL) and the resulting solution stirred at room temperature 2 h. Additional trifluoroacetic acid was added (300 uL) and the reaction stirred at room temperature 3 days. The pale yellow solution was concentrated to afford an oily residue, which was triturated with ether. The solids were collected by suction filtration and dried *in vacuo*. 3,3-Dimethyl-piperidin-4-one was isolated and used as its trifluoroacetic acid salt (381 mg, 80%).

^{1}H-NMR (d_6-DMSO, 300 MHz) δ 3.44 - 3.33 (m, 4 H), 2.63 - 2.57 (m, 2 H), 1.11 (s, 6 H).

[00253] [5-(6-Chloro-9H-beta-carbolin-8-ylcarbamoyl)-2,2-dimethyl-morpholin-4-yl]-acetic acid (100 mg, 0.24 mmol), 3,3-dimethyl-piperidin-4-one trifluoroacetatic acid salt (116 mg, 0.48 mmol) and diisopropylethylamine (62 mg, 85 □L) were dissolved in pyridine (3 mL) and stirred 10 min.

EDC (92 mg, 0.48 mmol) was added and the mixture was stirred at room temperature 4 days. Water was added (3 mL) and the quenched reaction was concentrated. The residue was partitioned between ethyl acetate (50 mL) and 1 M aqueous sodium carbonate (50 mL). The aqueous phase was extracted with additional ethyl acetate (50 mL), and the extracts were combined. The extracts were then washed with water and brine, dried over sodium sulfate, filtered, dried and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (chloroform, ethyl acetate, methanol gradient), affording 4-[2-(3,3-dimethyl-4-oxo-piperidin-1-yl)-2-oxo-ethyl]-6,6-dimethyl-morpholine-3-carboxylic acid (6-chloroa-9H-beta-carbolin-8-yl)-amide as a yellow foam (91 mg, 73%). The bis-HCl salt was prepared by adding 2 equivalents of conc. HCl to an ethanolic solution of the freebase. Concentration, followed by ether trituration afforded the salt as a free-flowing, yellow powder.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ 10.67 (s, 1 H), 10.37 (d, 1 H), 8.93 (s, 1 H), 8.41 (d, 1 H), 8.15 (dd, 1 H), 7.82 (d, 1 H), 7.71 (d, 1 H), 4.07 - 3.92 (m, 3 H), 3.83 - 3.89 (m, 6 H), 2.79 - 2.68 (m, 1 H), 2.62 - 2.40 (m, 3 H), 1.41 - 1.36 (m, 3 H), 1.27 - 1.22 (m, 3 H), 1.18 - 1.13 (m, 3 H), 1.07 - 0.99 (m, 3 H).

MS (NH_4OAc standard conditions, ES+) e/z = 526 $(M+H)^+$

DAD R_t = 1.79 min

EXAMPLE 81: 6,6-Dimethyl-4-(2-oxo-2-pyrrolidin-1-yl-ethyl)-morpholine-3-carboxylic acid (6-chloro-4-methyl-9H-b-carbolin-8-yl)-amide

[00254] The desired compound was prepared according to Methods C, E and F from 6-chloro-4-methyl-9H-β-carbolin-8-ylamine (Intermediate 54) and pyrrolidine.
^{1}H-NMR (300 MHz, MeOD-d4) δ 9.17 (s, 1) 8.39 (s, 1) 8.26 (d, 1) 8.17 (d, 1) 4.74 - 4.52 (m, 3) 4.47 - 4.30 (m, 1) 3.80 - 3.52 (m, 3) 3.48 - 3.34 (m, 4) 3.01 (s, 3) 1.76 - 1.55 (m, 4) 1.50 (s, 3) 1.43 (s, 3).
NH_4OAc standard conditions ELSD R_t = 2.01 min.
M+H = 498

Biological Testing

[00255] Compounds of this invention are effective inhibitors of IκB kinase (IKK), and therefore, are useful for treating conditions caused or aggravated by the activity of this kinase. The *in vitro* and *in vivo* IκB kinase inhibitory activities of the compounds of formula I may be determined by various procedures known in the art. The potent affinities for IκB kinase exhibited by the inventive compounds can be measured as an IC_{50} value (in nM), which is the concentration (in nM) of compound required to provide 50% inhibition of IκB kinase.

[00256] Following are examples of assays that can be useful for evaluating and selecting a compound that modulates IKK.

Assay for measuring IκB kinase enzyme inhibition

[00257] An *in vitro* assay for detecting and measuring inhibition activity against IκB kinase complex by candidate pharmacological agents can employ a polypeptide spanning both Ser^{32} and Ser^{36} of IκB (SwissProt Accession No. P25963, Swiss Institute of Bioinformatics, Geneva, Switzerland) and

an agent for detection of the phosphorylated product, *e.g.* a specific antibody binding only to the phosphorylated f rm of the polypeptide, being either monoclonal or polyclonal (e.g., commercially-available anti-phospho-serine^{32} IκB antibodies). In the example of detecting the phosphorylated product by an anti-phosphoserine^{32} IκB antibody, once the antibody-phospho-polypeptide complex is formed, the complex can be detected by a variety of analytical methods (e.g., radioactivity, luminescence, fluorescence, or optical absorbance). For the use of the DELFIA (Dissociation Enhancement Lanthanide Fluorescence Immunoassay) method (time-resolved fluorometry, Perkin Elmer Life and Analytical Sciences Inc., Boston, MA), the complex can be immobilized either onto a biotin-binding plate (e.g., Neutravidin coated plate) and detected with a secondary antibody conjugated to Europium, or onto an antibody-binding plate (e.g., Protein-A coated plate) and detected with biotin-binding protein conjugated to Europium (e.g., Streptavidin-Europium). The level of activity can be correlated with a standard curve using synthetic phosphopeptides corresponding to the substrate polypeptide. How to prepare materials for and conduct this assay are described in more detail below.

Isolation of the IκB kinase complex

[00258] An IκB-α kinase complex was prepared by first diluting 10 ml of HeLa S3 cell-extracts S100 fraction (Lee *et al.* (1997) Cell 88:213-222) with 40 ml of 50 mM HEPES pH 7.5. Then, 40% ammonium sulfate was added and incubated on ice for 30 minutes. The resulting precipitated pellet was rediss lved with 5 ml of SEC buffer (50 mM HEPES pH 7.5, 1

mM DTT, 0.5 mM EDTA, 10 mM 2-glycerophosphate), clarified by centrifugation at 20,000 x g for 15 min., and filtrated through a 0.22 μm filter unit. The sample was loaded onto a 320 ml SUPEROSE-6 gel filtration FPLC column (Amersham Biosciences AB, Uppsala, Sweden) equilibrated with a SEC buffer operated at 2 ml/min flow rate at 4 °C. Fractions spanning the 670-kDa molecular-weight marker were pooled for activation. A kinase-containing pool was then activated by incubation with 100 nM MEKK1Δ(Lee *et al.* (1997) Cell 88:213-222), 250 μM MgATP, 10 mM $MgCl_2$, 5 mM DTT, 10 mM 2-glycerophosphate, 2.5 μM Microcystin-LR, for 45 minutes at 37 °C. The activated enzyme was stored at -80 °C until further use.

Measurement of IκB kinase phospho-transferase activity

[00259] At the per well of a 96 well plate, compounds of various concentrations in 5 μL of 20% DMSO were preincubated for 30 minutes at 25 °C with 40 μL of activated enzyme diluted 1:25 with assay buffer (50 mM Hepes pH 7.5, 5 mM DTT, 10 mM $MgCl_2$, 10 mM 2-glycerophosphate, 2 μM Microcystin-LR, 0.1% Bovine Serum Albumin). 5 μL of peptide substrate (biotin-$(CH_2)_6$-DRHDSGLD(phosphoS)MKD-$CONH_2$) at 200 μM + 500 μM ATP were added to each well and incubated for 1 hour before quenching with 50 μL of 50 mM Hepes pH 7.5, 0.1% BSA, 100 mM EDTA. 5 μL of quenched kinase reaction were transferred to a Protein A plate (Pierce Biotechnology, Inc., Rockford, IL, USA) containing 90 μL of anti-phospho IκB S32/S36 antibody (Cell Signaling Technologies Beverly, MA, USA) at 2 μg/ml. Samples were incubated for 2 hours with shaking. Following 3 washes with PBS + 0.05% Tween20,

90 µL of streptavidin linked europium chelate (Perkin Elmer Life and Analytical Sciences, Boston, MA, USA) at 0.1 µg/ml were added to each well and incubated for 1 hour with shaking. Following 3 washes with PBS + 0.05% Tween20, 100 µL of DELFIA Enhancement Solution (Perkin Elmer Life and Analytical Sciences, Boston, MA, USA) were added to each well. An europium signal was read with an excitation of 330 nM and emission of 615 nM on a Wallac Victor plate reader (Perkin Elmer Life and Analytical Sciences, Boston, MA). As the assay was previously shown to be linear with respect to enzyme concentration and time for the enzyme dilution tested, levels of europium signal were used to determine the inhibition activity of candidate pharmacological agents.

[00260] The compounds of the invention were active inhibitors of the IKK complex. It will be appreciated that compounds of this invention can exhibit IKB kinase inhibitor activities of varying degrees. Following assay procedures such as the in vitro and cell-based assays described herein, the IKB kinase inhibition average IC_{50} values for the inventive compounds were generally below about 10 micromolar, preferably, below about 1.0 micromolar and more preferably below about 100 nanomolar. The inventive compounds were also selective for inhibiting IKK-2 as opposed to IKK-1.

Cellular Assays

Multiple Myeloma (MM) cell lines and patient-derived MM cells isolation

[00261] RPMI 8226 and U266 human MM cells were obtained from American Type Culture C llection (Manassas, VA). All

MM cell lines were cultured in RPMI-1640 containing 10% fetal bovine serum (FBS, Sigma-Aldrich Co., St. Louis, MO), 2 mM L-glutamine, 100 U/mL penicillin (Pen) and 100 µg/mL streptomycin (Strep) (GIBCO brand cell culture products available from Invitrogen Life Technologies, Carlsbad, CA). Patient-derived MM cells were purified from patient bone marrow (BM) aspirates using ROSETTESEP (B cell enrichment kit) separation system (StemCell Technologies, Vancouver, Canada). The purity of MM cells was confirmed by flow cytometry using PE-conjugated anti-CD138 antibody (BD Biosciences, Bedford, MA).

Bone Marrow Stroma Cell cultures

[00262] Bone marrow (BM) specimens were obtained from patients with MM. Mononuclear cells (MNCs) separated by Ficoll-Hipaque density sedimentation were used to established long-term BM cultures as previously described (Uchiyama *et al.*, *Blood* 1993, 82:3712-3720). Cells were harvested in Hank's Buffered Saline Solution (HBSS) containing 0.25% trypsin and 0.02% EDTA, washed, and collected by centrifugation.

Cell Proliferation via measurement of DNA-synthesis rate

[00263] Proliferation was measured as previously described (Hideshima *et al.*, *Blood* 96:2943 (2000)). MM cells (3 x 10^4 cells/well) were incubated in 96-well culture plates (Corning Life Sciences, Corning, NY) in the presence of media or an IKK inhibitor of this invention for 48 h at 37°C. DNA synthesis was measured by [^{3}H]-thymidine ([^{3}H]-TdR, New England Nuclear division of Perkin Elmer Life and Analytical Sciences, Boston, MA) incorporation into

dividing cells. Cells were pulsed with [^{3}H]TdR (0.5 μCi/well) during the last 8 h of 48 h cultures. All experiments were performed in triplicate.

MTT Cell Viability assay

[00264] The inhibitory effect of the present compounds on MM growth was assessed by measuring the reduction of yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) by metabolically active cells (J. Immunol. Methods 174: 311-320, 1994). Cells from 48 h cultures were pulsed with 10 μL of 5 mg/mL MTT to each well for the last 4 h of the 48 h cultures, followed by 100 μL isopropanol containing 0.04N HCl. Absorbance was measured at 570 nm using a spectrophotometer (Molecular Devices Corp., Sunnyvale CA).

NF-κB activation via Electrophoretic Mobility Shift Assay

[00265] Electrophoretic mobility shift analyses (EMSA) were carried out as previously described (Hideshima *et al.*, *Oncogene* 2001, 20:4519). Briefly, MM cells were pre-incubated with an IKK inhibitor of this invention (10 μM for 90 min) before stimulation with TNF-α (5 ng/mL) for 10 to 20 min. Cells were then pelleted, resuspended in 400 μL of hypotonic lysis buffer (20 mM HEPES, pH 7.9, 10 mM KCl, 1 mM EDTA, 0.2% Triton X-100, 1 mM Na_3VO_4, 5 mM NaF, 1 mM PMSF, 5 μg/mL leupeptin, 5 μg/mL aprotinin), and kept on ice for 20 min. After centrifugation (14000g for 5 min) at 4°C, the nuclear pellet was extracted with 100 μL hypertonic lysis buffer (20 mM HEPES, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, 5 mM NaF, 1 mM PMSF, 5 μg/mL leupeptin, 5 μg/mL

aprotinin) on ice for 20 min. After centrifugation (14000g for 5 min) at 4°C, the supernatant was collected as nuclear extract. Double-stranded NF-κB consensus oligonucleotide probe (5'-GGGGACTTTCCC-3', Santa Cruz Biotechnology Inc., Santa Cruz CA) was end-labeled with [(^{32}P]ATP (50 µCi at 222 TBq/mM; New England Nuclear division of Perkin Elmer Life and Analytical Sciences, Boston, MA). Binding reactions containing 1 ng of oligonucleotide and 5 µg of nuclear protein were conducted at room temperature for 20 min in a total volume of 10 µL of binding buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 4% glycerol (v/v), and 0.5 µg poly (dI-dC) (Amersham Biosciences AB, Uppsala, Sweden). For supershift analysis, 1 µg of anti-p65 NF-κB Ab was added 5 min before the reaction mixtures, immediately after addition of radiolabeled probe. The samples were loaded onto a 4% polyacrylamide gel, transferred to Whatman paper (Whatman International, Maidstone, U.K.), and visualized by autoradiography.

Diffuse Large B-Cell Lymphoma (DLBCL) Cell Proliferation assay

[00266] ABC-like (LY3 and Ly10) and GCB-like (Ly7 and Ly19) DLBCL cell lines (Alizadeh et al (2000) Nature 403:503-511; Davis et al. (2001) J. Exp. Med. 194:1861-1874) were maintained in growth medium (GM, Iscove's DMEM+10%FBS) by passaging cells twice per week. Cells were starved overnight in Iscove's DMEM medium + 0.5% FBS overnight before plated in proliferation assay. On the day of the assay, cells were counted and viability was checked

using Trypan Blue staining. For the Ly3 and Ly10 cells, 5000 cell were plated in GM per well in a 96-well plate. The Ly7 and Ly19 cells were plated at 10,000 cells per well. IKK inhibitors were first dissolved in DMSO and then diluted in GM to reach the final concentrations of 80 μM - 0.01 μM. Each concentration was plated in triplicate. Cell viability was determined using a standard WST-1 cell viability assay (Roche Applied Science, Indianapolis, IN).

Human peripheral blood monocyte (PBMC) Cytokine Release Assay

[00267] Human PBMC was purified from normal donor whole blood by Ficoll gradient method. After a PBS wash, PBMC were re-suspended in AIM-V medium. Serially diluted IKK inhibitors of this invention in 100% DMSO was added at 1 μl to the bottom of a 96-well plate and mixed with 180 μl 4.5 X 10^5 PBMC in AIM-V media per well. After preincubating PBMC with inhibitor at 37°C for 40 min, cells were stimulated with 20 μl of either with LPS (100 ng/ml) or with anti-CD3 (0.25 μg/ml) and anti-CD28 (0.25μg/ml) (Pharmingen divisi n of BD Biosciences, Bedford, MA) at 37°C for 5 hours. The supernatants were collected and assessed for IL-1β or TNF-α release using standard commercially available ELISA kits.

Human Chondrocyte Matrix Metalloproteases (MMPs) Release Assay

[00268] Human chondrocyte cell line SW1353 (ATCC, Manassas, VA) was cultured containing 10% fetal bovine serum (Hyclone, Logan, UT), 2 mM L-glutamine(GIBCO brand cell culture pr ducts available from Invitrogen Life Technologies, Carlsbad, CA) and 1% Pen/Strep (GIBCO).

Cells were seeded in 96-well Poly-D-Lysine plate (BD BIOCOAT, Black/Clear bottom, BD Biosciences, Bedford, MA). Serially diluted IKK inhibitors at 1 µl were added to each well of 96-well plates and mixed with 180 µl 4.5 X 10^5 chondrocytes per well. After pre-incubating cells with compounds for 1 hr at 37°C, cells were stimulated with 20 µl IL-1β (10 ng/mL, R&D Systems Inc.) at 37°C for 24 hrs. The supernatants were then collected and assessed for production of matrix metalloproteinases (MMPs) using commercially available ELISA kits.

Human Fibroblast Like Synoviocyte (HFLS) Assay

[00269] HFLS isolated from RA synovial tissues obtained at joint replacement surgery was provided by Cell Applications Inc. (San Diego, CA). IKK inhibitors of the invention were tested for their ability to block the TNF- or IL-1β-induced release of IL-6 or IL-8 from these cells using commercially available ELISA kits. Cell culture conditions and assay methods were described in Aupperle et al., *Journal of Immunology*, 163:427-433 (1999).

Human Cord Blood Derived Mast Cell Assay

[00270] Human cord blood was obtained from Cambrex (Walkersville, MD). Mast cells were differentiated and cultured in a manner similar to that described by Hsieh et al., *J. Exp. Med.*, 193:123-133 (2001). IKK inhibitors of the invention were tested for their ability to block the IgE- or LPS-induced TNFα release using commercially available ELISA kits.

Osteoclast Differentiation and Functional Assays

[00271] Human osteoclast precursors were obtained as cryopreserved form from Cambrex (Walkersville, MD). The cells were differentiated in culture based on instructions from the manufacturer. IKK inhibitors of the invention were tested for their ability to block the differentiation, bone resorption and collagen degradation as described previously (see Khapli, S. M., *Journal of Immunol*, 171:142-151 (2003); Karsdal, M. A., *J Biol Chem*, 278:44975-44987 (2003); and Takami, M., *Journal of Immunol*, 169:1516-1523 (2002)).

Rat Models for Rheumatoid Arthritis

[00272] Certain compounds of this invention were found to be active in one or more rat models for rheumatoid arthritis. Such testing is known in the literature and include a standard rat LPS model as described in Conway et al., "Inhibition of Tumor Necrosis Factor-α (TNF-α) Production and Arthritis in the Rat by GW3333, a Dual Inhibitor of TNF-Converting Enzyme and Matrix Metalloproteinases", *J. Pharmacol. Exp. Ther.* 298(3), 900-908 (2001); a rat adjuvant induced arthritis model as described in Pharmacological Methods in the Control of Inflammation (1989) p 363-380 "Rat Adjuvant Arthritis: A Model of Chronic Inflammation" Barry M. Weichman author f book chapter {Alan R. Liss Inc Publisher}; and a rat collagen induced arthritis model as described in Pharmacological Methods in the Control of Inflammation (1989) p 395-413 "Type II Collagen Induced Arthritis in th Rat" DE Trentham and RA Dynesuis-Trentham authors of book chapter {Alan R. Liss Inc Publisher}. See also, "Animal Models of Arthritis: Relevance to Human Disease" (1999) by

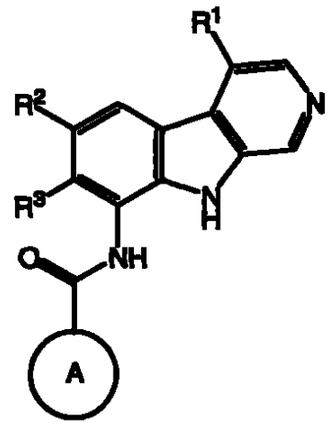
A. Bendele, J. McComb, T. Gould, T. McAbee, G. Sennello, E. Chlipala and M. Guy. Toxicologic Pathology Vol 27 (1) 134-142.

[00273] Based on the results of one or more rat models such as the ones described above, compounds of formula II-C were found to be surprisingly superior compared to other compounds where Ring A is a pyridine ring. Also in the rat models, compounds of formula III-A-a, especially compounds of formula III-A-aa, were found to be surprisingly superior compared to other compounds where Ring A is a morpholine ring.

[00274] While we have described a number of embodiments of this invention, it is apparent that our basic examples may be altered to provide other embodiments, which utilize the compounds and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments, which have been represented by way of example.

We claim:

1. A compound of formula I:



I

r a pharmaceutically acceptable salt thereof, wherein:
Ring A is selected from the group consisting of:

(a) a pyridinyl or pyrimidinyl ring that is substituted by (i) $-CH_2C(O)-G$ and 0-1 R^{6a} or (ii) 1-2 R^{6a}, and

(b) a morpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, pyranyl, tetrahydrofuranyl, cyclohexyl, cyclopentyl or thiomorpholinyl ring that is substituted by (i) $-C(R^9)_3$, -W-G, or -G, (ii) 0-4 R^{6b} and (iii) 0-1 oxo groups on a ring carbon or 0-2 oxo groups on a ring sulfur;

each R^{6a} is independently selected from C_{1-6} aliphatic, halo, alkoxy, or amino;

each R^{6b} is independently selected from C_{1-3} aliphatic or $-N(R^7)_2$, and two R^{6b} on the same or an adjacent carbon optionally are taken together with the intervening carbon(s) to form a 5-6 membered ring having 1-2 ring heteroatoms selected from N, O or S;

W is -Q-, -Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$, or $-C(R^9)_2-[C(R^9)(R^{12})]_2-$;

Q is $-C(R^9)_2-$ or $-C(R^9)_2C(R^9)_2-$;

G is -OH, $-NR^4R^5$, $-N(R^9)CONR^4R^5$, $-N(R^9)SO_2(C_{1-3}$ aliphatic), $-N(R^9)COCF_3$, $-N(R^9)CO(C_{1-6}$ aliphatic), $-N(R^9)CO$(heterocyclyl), $-N(R^9)CO$(heteroaryl), $-N(R^9)CO$(aryl), a 3-7 membered heterocyclyl ring, or a 5-6 membered heteroaryl, wherein each of the heteroaryl, aryl and heterocyclyl moieties of G is optionally substituted by 1-3 R^{10};

R^1 is hydrogen, halo, C_{1-3} aliphatic, amino, cyano, $(C_{1-3}$ alkyl$)_{1-2}$ amino, C_{1-3} alkoxy, $-CONH_2$, $-NHCOCF_3$, or $-CH_2NH_2$;

R^2 is hydrogen, halo, C_{1-3} aliphatic, $-CF_3$;

R^3 is hydrogen, halo, C_{1-6} aliphatic, C_{1-6} haloalkyl, C_{1-6} alkoxy, hydroxy, amino, cyano, or $(C_{1-6}$ alkyl$)_{1-2}$ amino;

R^4 is hydrogen, 3-7 membered heterocyclyl, or C_{1-6} aliphatic;

R^5 is hydrogen, C_{1-6} aliphatic group or a 3-7 membered heterocyclic ring having 1-2 ring heteroatoms selected from N, O, or S, wherein R^5 is optionally substituted by halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

each R^9 is independently selected from hydrogen or C_{1-3} aliphatic;

each R^{10} is independently selected from oxo, $-R^{11}$, $-T-R^{11}$, or $-V-T-R^{11}$;

each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

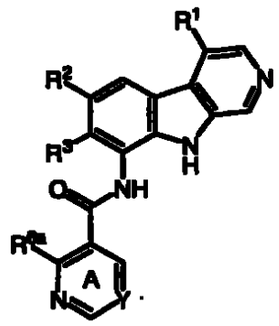
T is a straight or branched C_{1-4} alkylene chain;

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$; and

R^{12} is hydrogen or an amino acid side chain.

2. The compound of claim 1 where Ring A is a 3-pyridinyl or 5-pyrimidinyl ring substituted by 1-2 R^{6a} groups.

3. The compound of claim 2 having formula II-C:



II-C

wherein:

Y is N or CH;

R^1 is hydrogen, halo, C_{1-3} aliphatic, amino, cyano, $(C_{1-3}$ alkyl$)_{1-2}$ amino, C_{1-3} alkoxy, $-CONH_2$, $-NHCOCF_3$, or $-CH_2NH_2$;

R^2 is hydrogen, halo, C_{1-3} aliphatic, $-CF_3$;

R^3 is hydrogen, halo, C_{1-6} aliphatic, C_{1-6} haloalkyl, C_{1-6} alkoxy, hydroxy, amino, cyano, or $(C_{1-6}$ alkyl$)_{1-2}$ amino; and

R^{6a} is selected from C_{1-6} aliphatic or halo.

4. The compound of claim 3 where R^{6a} is methyl.

5. The compound of claim 4 where R^1 is hydrogen, methyl, amino or fluoro; R^2 is hydrogen or halo; and R^3 is hydrogen, halo or C_{1-4} alkoxy.

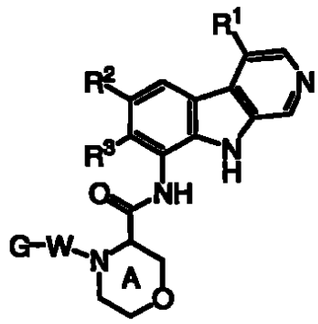
6. The compound of claim 5 where Y is CH.

7. The compound of claim 5 where Y is N.

8. The compound of claim 1 where Ring A is selected from morpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, pyranyl, tetrahydrofuranyl, cyclohexyl, cyclopentyl or thiomorpholinyl and where Ring A is substituted by (i) $-C(R^9)_3$ or -W-G, (ii) 0-4 R^{6b} and (iii) 0-1 oxo groups on a ring carbon or 0-2 oxo groups on a ring sulfur.

9. The compound of claim 8 where the -W-G or $-C(R^9)_3$ substituent on Ring A is ortho to the position where the beta-carboline portion is attached.

10. A compound of formula III-A:



III-A

or a pharmaceutically acceptable salt thereof, wherein:

Ring A is substituted by 0-4 R^{6b};

each R^{6b} is independently selected from C_{1-3} aliphatic or $-N(R^7)_2$, and two R^{6b} on the same or an adjacent carbon optionally are taken together with the intervening carbon(s) to form a 5-6 membered ring having 1-2 ring heteroatoms selected from N, O or S;

W is $-Q-$, $-Q-C(O)-$, $-C(R^9)_2-C(R^9)(R^{12})-$, or $-C(R^9)_2-[C(R^9)(R^{12})]_2-$;

Q is $-C(R^9)_2-$ or $-C(R^9)_2C(R^9)_2-$;

G is $-OH$, $-NR^4R^5$, $-N(R^9)CONR^4R^5$, $-N(R^9)SO_2(C_{1-3}$ aliphatic), $-N(R^9)COCF_3$, $-N(R^9)CO(C_{1-6}$ aliphatic), $-N(R^9)CO$(heterocyclyl), $-N(R^9)CO$(heteroaryl), $-N(R^9)CO$(aryl), a 3-7 membered heterocyclyl ring, or a 5-6 membered heteroaryl, wherein each of the heteroaryl, aryl and heterocyclyl moieties of G is optionally substituted by 1-3 R^{10};

R^1 is hydrogen, halo, C_{1-3} aliphatic, amino, cyano, $(C_{1-3}$ alkyl$)_{1-2}$ amino, C_{1-3} alkoxy, $-CONH_2$, $-NHCOCF_3$, or $-CH_2NH_2$;

R^2 is hydrogen, halo, C_{1-3} aliphatic, $-CF_3$;

R^3 is hydrogen, halo, C_{1-6} aliphatic, C_{1-6} haloalkyl, C_{1-6} alkoxy, hydroxy, amino, cyano, or $(C_{1-6}$ alkyl$)_{1-2}$ amino;

R^4 is hydrogen, 5-6 membered heterocyclyl, or C_{1-6} aliphatic;

R^5 is hydrogen, C_{1-6} aliphatic group or a 5-6 membered heterocyclic ring having 1-2 ring heteroatoms selected from N, O, or S, wherein R^5 is optionally substituted by halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken

together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

each R^9 is independently selected from hydrogen or C_{1-3} aliphatic;

each R^{10} is independently selected from oxo, $-R^{11}$, $-T-R^{11}$, or $-V-T-R^{11}$;

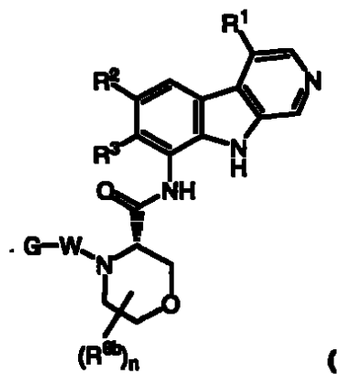
each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain;

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$; and

R^{12} is hydrogen or an amino acid side chain.

11. The compound of claim 10 having the formula (S)-III-A:



(S)-III-A

where n is 0-4 and R^1, R^2, R^3, W, G and R^{6b} are as defined in claim 10.

12. The compound of claim 11 where:

R^1 is hydrogen, halo, methyl or amino;

R^2 is hydrogen, methyl or halo;

R^3 is hydrogen, halo, alkoxy, or $(C_{1-6}$ aliphatic$)_2$ amino;

n is 0-2;

R^{6b} is C_{1-3} aliphatic;

W is -Q-, -Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$, or $-C(R^9)_2-[C(R^9)(R^{12})]_2-$;

Q is $-C(R^9)_2-$ or $-C(R^9)_2C(R^9)_2-$;

G is $-NR^4R^5$, $-N(R^9)C(O)NR^4R^5$, $-N(R^9)SO_2(C_{1-3}$ aliphatic), $-N(R^9)C(O)CF_3$, $-N(R^9)CO(C_{1-6}$ aliphatic), and $-N(R^9)CO$(heterocyclyl), $-N(R^9)CO$(heteroaryl), $-N(R^9)CO$(aryl), a 5-6 membered heterocyclyl ring, or a 5-6 membered heteroaryl, wherein each of the heteroaryl, aryl and heterocyclyl moieties of G is optionally substituted by 1-3 R^{10};

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is hydrogen or a C_{1-6} aliphatic group that is optionally substituted by halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

R^9 is hydrogen;

each R^{10} is independently selected from oxo, R^{11}, $T-R^{11}$, or $V-T-R^{11}$;

each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain;

V is -O-, $-N(R^7)-$, -S-, -S(O)-, $-S(O)_2-$, -C(O)-, or $-CO_2-$; and

R^{12} is hydrogen, C_{1-6} aliphatic, substituted or unsubstituted phenyl, or substituted or unsubstituted benzyl.

13. The compound of claim 12 where:

R^1 is hydrogen, methyl, fluoro or amino;

R^2 is chloro;

R^3 is hydrogen or alkoxy;

n is zero or 2;

R^{6b} is methyl;

W is -Q-, -Q-C(O)- or $-C(R^9)_2-C(R^9)(R^{12})-$;

Q is $-C(R^9)_2-$ or $-C(R^9)_2C(R^9)_2-$;

G is $-NR^4R^5$, $-N(R^9)C(O)NR^4R^5$, $-N(R^9)C(O)CF_3$, $-N(R^9)CO(C_{1-6}$ aliphatic), and $-N(R^9)CO$(heterocyclyl), $-N(R^9)CO$(heteroaryl), a 5-6 membered heterocyclyl ring, or a 5-6 membered heteroaryl, wherein each of the heteroaryl and heterocyclyl moieties of G is optionally substituted by 1-3 R^{10};

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is hydrogen or C_{1-6} aliphatic;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

R^9 is hydrogen;

each R^{10} is independently selected from oxo, R^{11}, $T-R^{11}$, or $V-T-R^{11}$;

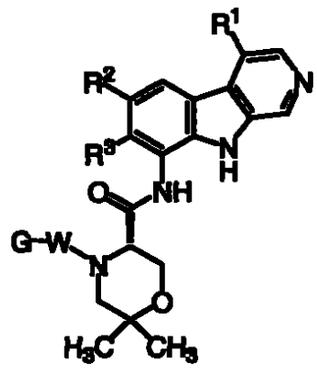
each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain;

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$; and

R^{12} is hydrogen, C_{1-6} aliphatic, phenyl, or benzyl.

14. A compound of formula (*S*)-III-A':



(*S*)-III-A'

r a pharmaceutically acceptable salt thereof, wherein:

R^1 is hydrogen, methyl, fluoro or amino;

R^2 is chloro;

R^3 is hydrogen or alkoxy;

W is $-Q-$, $-Q-C(O)-$ or $-C(R^9)_2-C(R^9)(R^{12})-$;

Q is $-C(R^9)_2-$ or $-C(R^9)_2C(R^9)_2-$;

G is $-NR^4R^5$, $-N(R^9)C(O)NR^4R^5$, $-N(R^9)C(O)CF_3$, $-N(R^9)CO(C_{1-6}$ aliphatic), and $-N(R^9)CO$(heterocyclyl), $-N(R^9)CO$(heteroaryl), a 5-6 membered heterocyclyl ring, or a 5-6 membered heteroaryl, wherein each of the heteroaryl and heterocyclyl moieties of G is optionally substituted by 1-3 R^{10};

R^4 is hydrogen or C_{1-6} aliphatic;

R^5 is hydrogen or C_{1-6} aliphatic;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

R^9 is hydrogen;

each R^{10} is independently selected from oxo, R^{11}, $T-R^{11}$, or $V-T-R^{11}$;

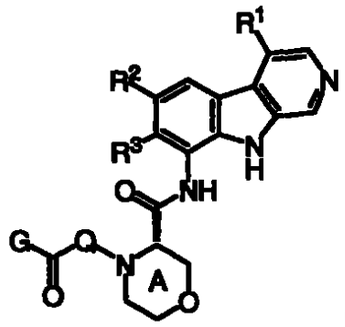
each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

T is a straight or branched C_{1-4} alkylene chain;

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$; and

R^{12} is hydrogen, C_{1-6} aliphatic, phenyl, or benzyl.

15. The compound of claim 14 having the formula (*S*)-III-A-a:



(*S*)-III-A-a

where R^1 is hydrogen, halo, methyl or amino;

R^2 is hydrogen, methyl or halo;

R^3 is hydrogen, halo, alkoxy, or (C_{1-6} aliphatic)$_2$ amino;

Ring A is substituted by 0-2 R^{6b};

R^{6b} is C_{1-3} aliphatic;

Q is $-C(R^9)_2-$ or $-C(R^9)_2C(R^9)_2-$;

G is $-NR^4R^5$ or a substituted or unsubstituted 5-6 membered heterocyclyl ring;

R^4 is hydrogen or C_{1-6} aliphatic;

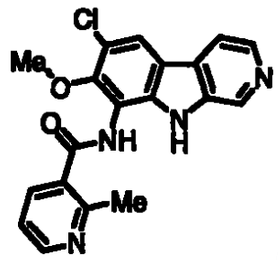
R^5 is hydrogen or a C_{1-6} aliphatic group that is optionally substituted by halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$, or $-N(R^7)C(O)N(R^7)_2$;

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

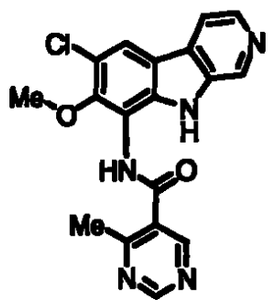
each R^8 is independently selected from C_{1-4} aliphatic; and

each R^9 is independently hydrogen or C_{1-3} aliphatic.

16. A compound selected from the group consisting of:



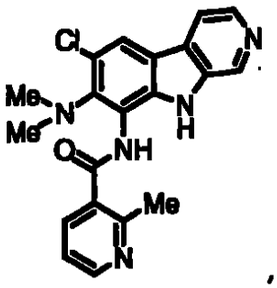
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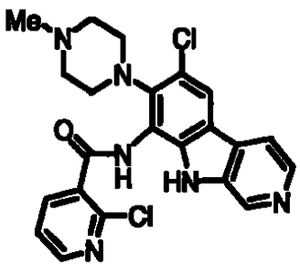
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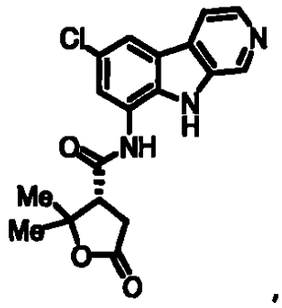
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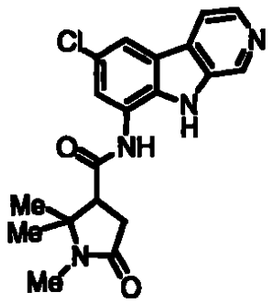
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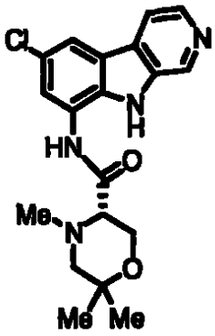
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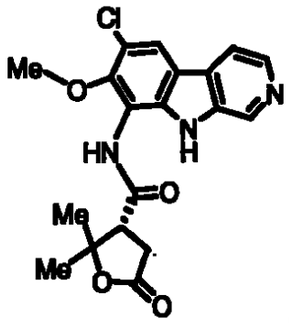
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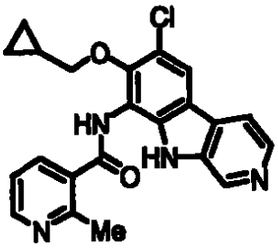
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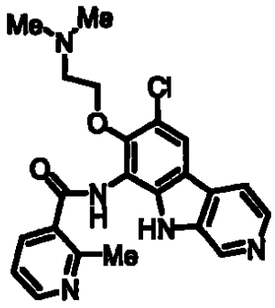
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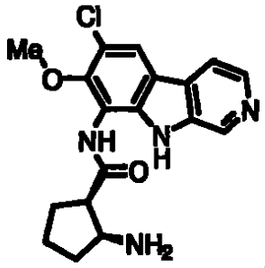
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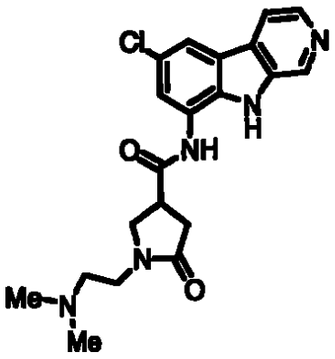
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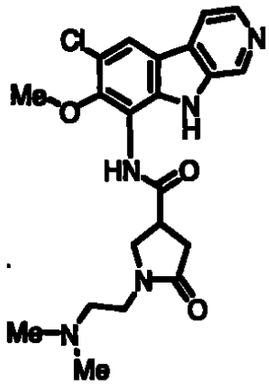
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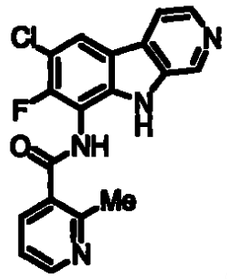
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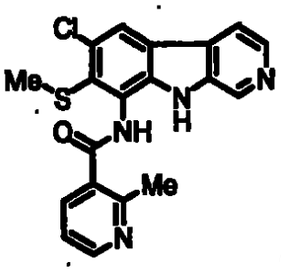
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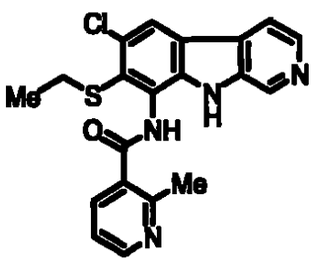
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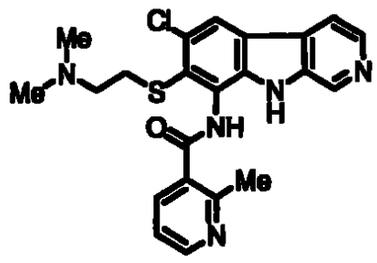
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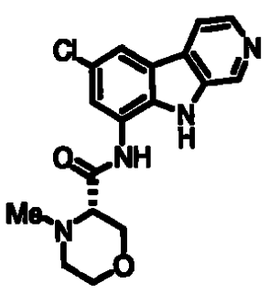
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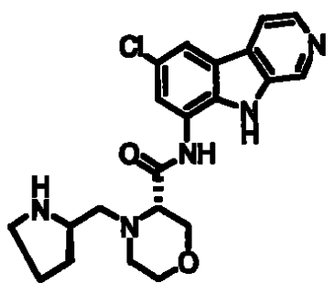
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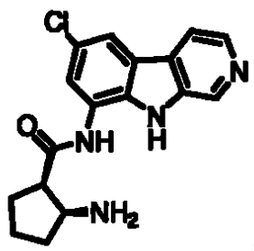
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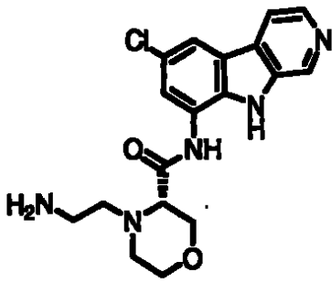
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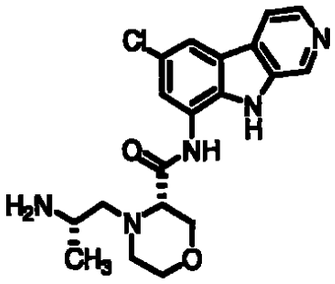
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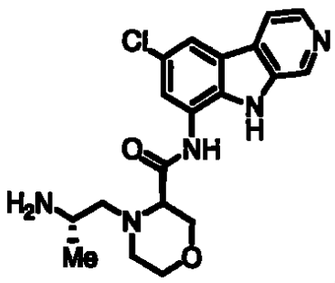
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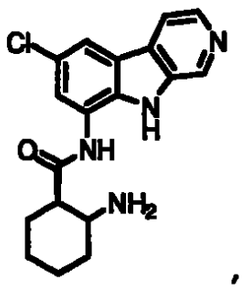
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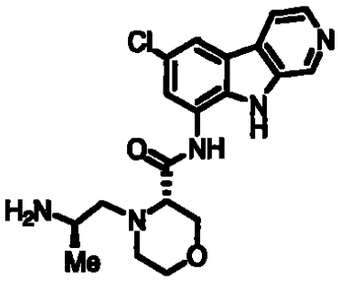
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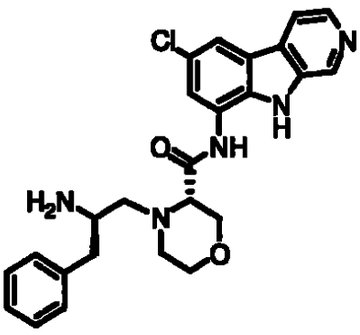
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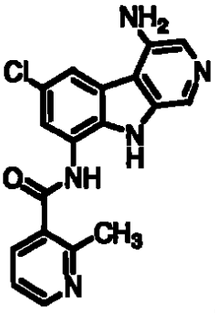
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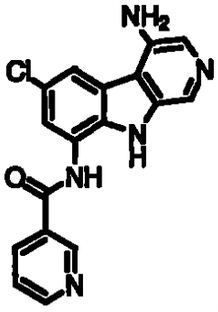
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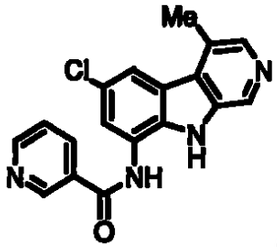
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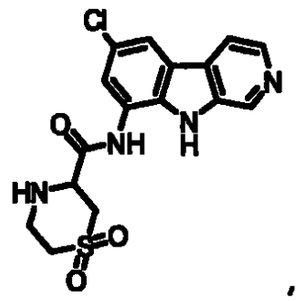
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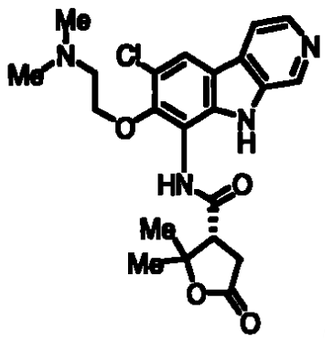
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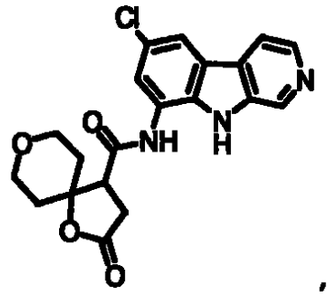
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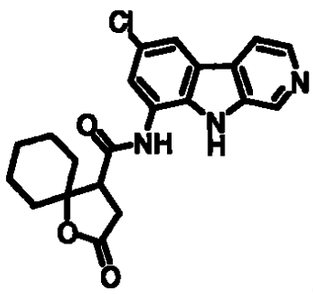
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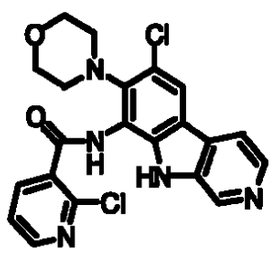
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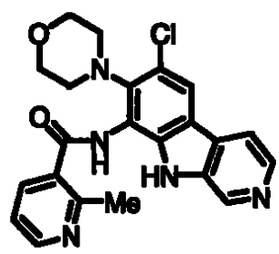
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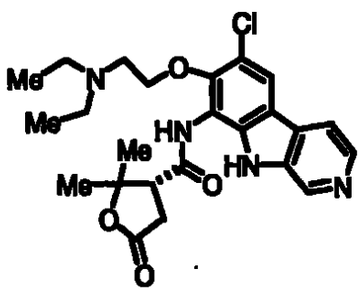
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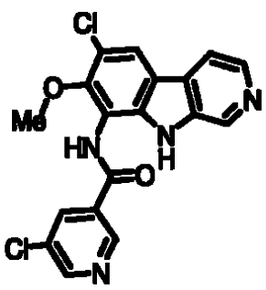
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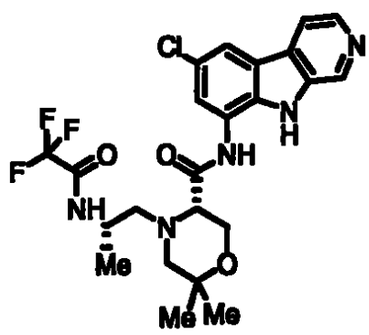
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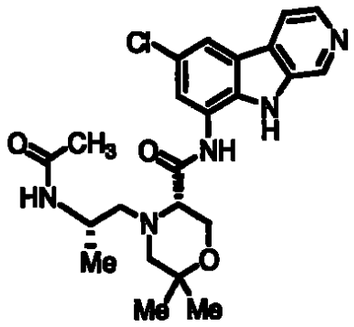
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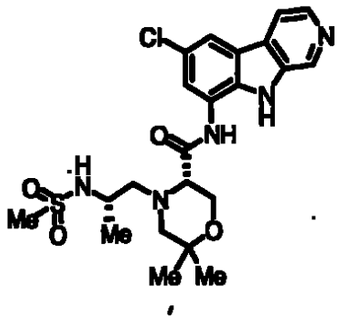
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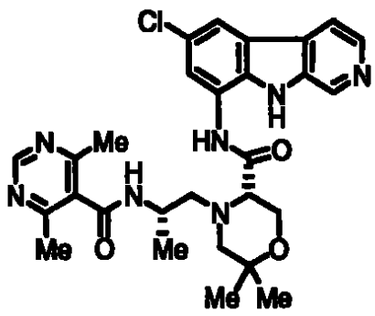
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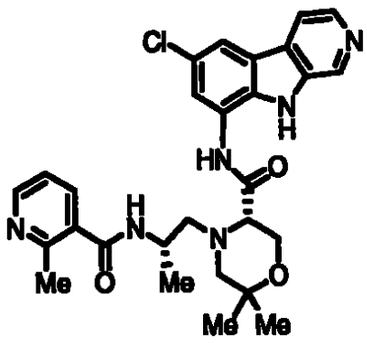
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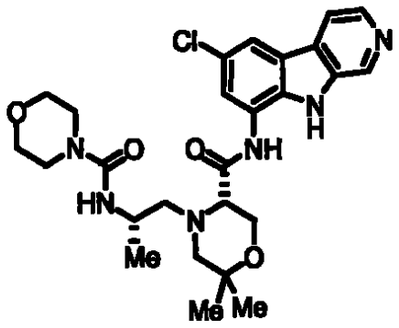
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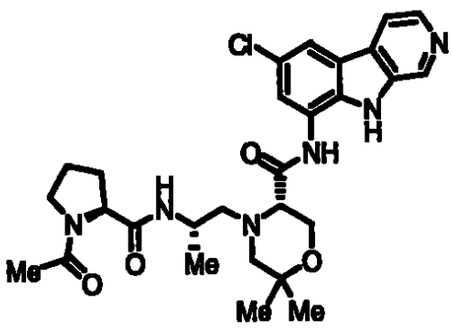
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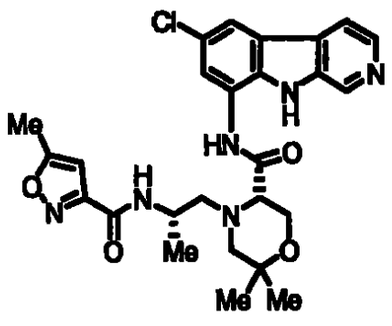
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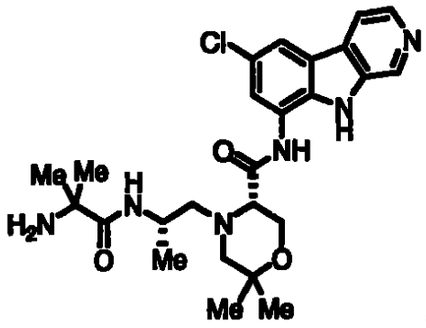
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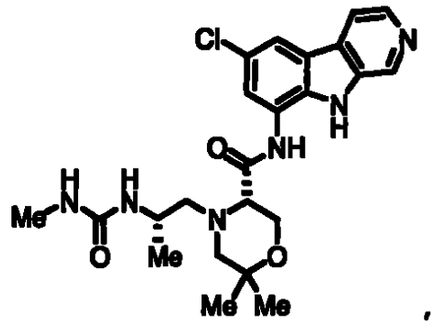
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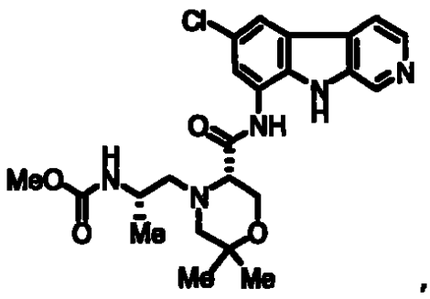
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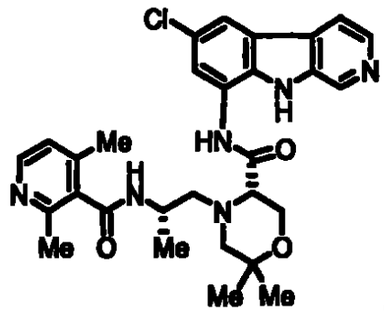
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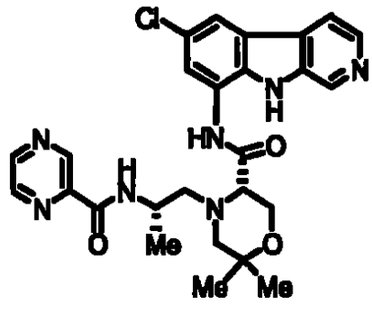
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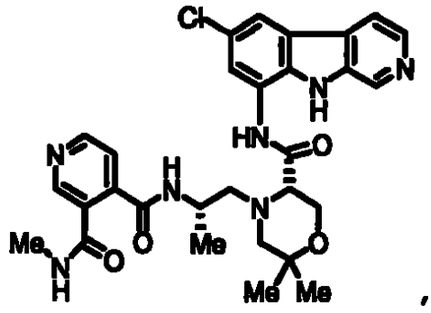
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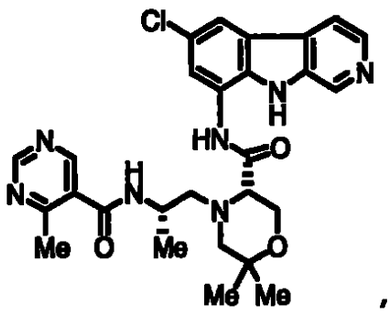
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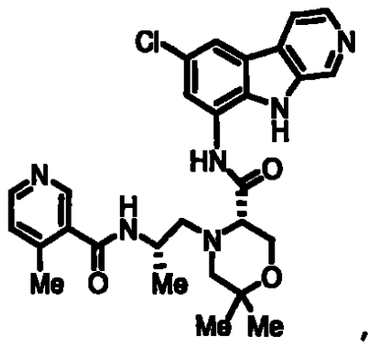
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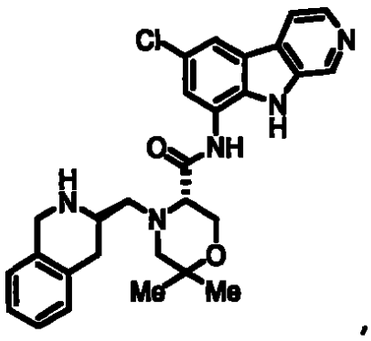
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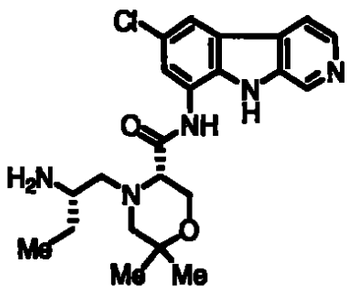
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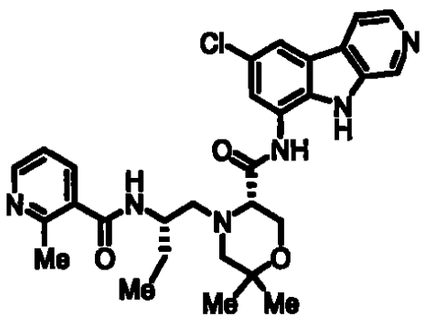
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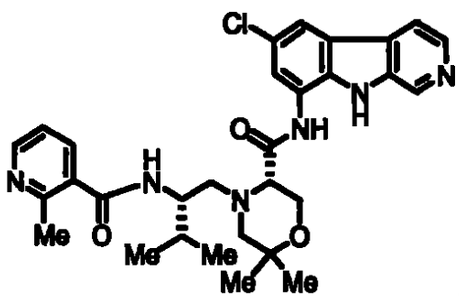
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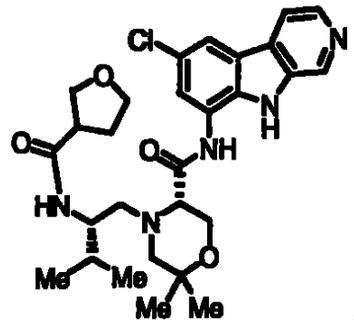
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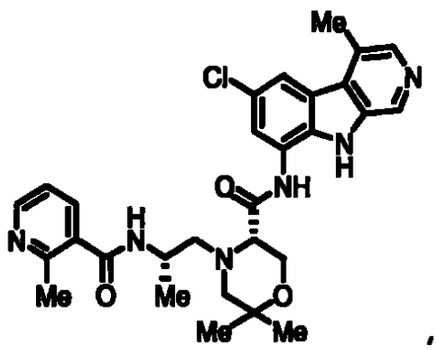
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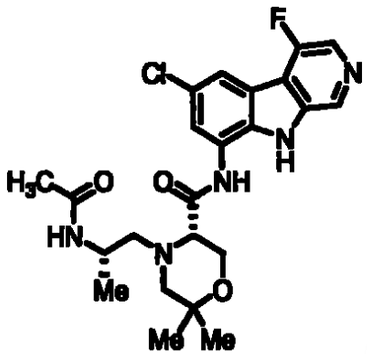
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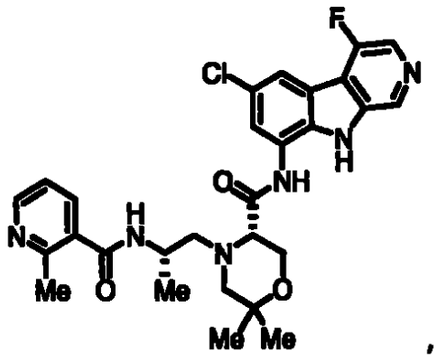
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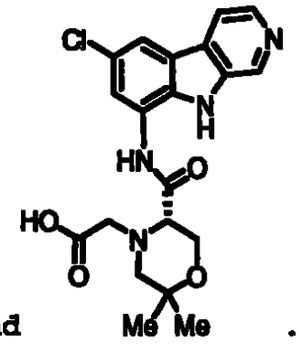
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and

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17. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

18. A pharmaceutical composition comprising a compound of claim 16 and a pharmaceutically acceptable carrier.

19. A method of treating an IKK-mediated disease comprising administering to a patient in need of such treatment a therapeutically effective amount of a compound of claim 1.

20. The method of claim 19 wherein the disease is an inflammatory disease or an immune-related disease.

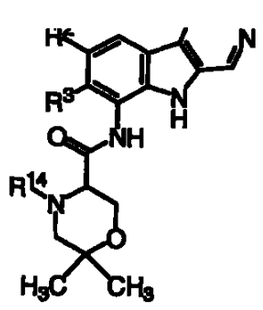
21. The method of claim 19 wherein the disease is selected from the group consisting of rheumatoid arthritis, asthma, psoriasis, psoriatic arthritis, chronic obstructive pulmonary disease, inflammatory bowel disease or multiple sclerosis.

22. The method of claim 19 wherein the disease is cancer.

23. The method of claim 22 wherein the cancer is selected from lymphoma, multiple myeloma, osteolytic bone mestasis, head or neck cancer, lung cancer, prostate cancer or pancreatic cancer.

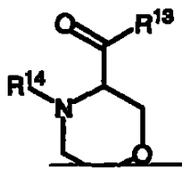
24. The method of claim 23 wherein the cancer is a lymphoma.

25. A method of inhibiting IKK in a patient in need thereof comprising administering to the patient a compound of claim 1.



IV

26. A compound of f rmula 3a:



where R^{14} is an amino protecting group or hydrogen;

R^1 is hydrogen, halo, C_{1-3} aliphatic, amino, cyano, $(C_{1-3}$ alkyl$)_{1-2}$ amino, C_{1-3} alkoxy, $-CONH_2$, $-NHCOCF_3$, or $-CH_2NH_2$;

R^2 is hydrogen, halo, C_{1-3} aliphatic, $-CF_3$; and

R^3 is hydrogen, halo, C_{1-6} aliphatic, C_{1-6} haloalkyl, C_{1-6} alkoxy, hydroxy, amino, cyano, or $(C_{1-6}$ alkyl$)_{1-2}$ amino.

29. The compound of claim 28 that is (*S*)-IV.

each R^7 is independently selected from hydrogen or C_{1-4} aliphatic, or two R^7 on the same nitrogen atom are taken together with the nitrogen to form a 5-6 membered heteroaryl or heterocyclyl ring;

each R^8 is independently selected from C_{1-4} aliphatic;

each R^9 is independently selected from hydrogen or C_{1-3} aliphatic;

each R^{10} is independently selected from oxo, $-R^{11}$, $-T-R^{11}$, or $-V-T-R^{11}$;

each R^{11} is independently selected from C_{1-6} aliphatic, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$, or $-N(R^7)C(O)N(R^7)_2$;

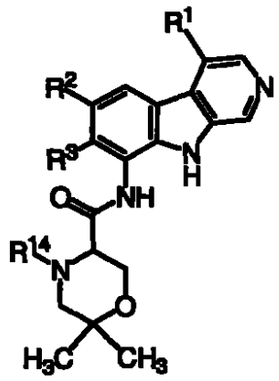
T is a straight or branched C_{1-4} alkylene chain;

V is $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$, or $-CO_2-$; and

R^{12} is hydrogen or an amino acid side chain.

27. The compound of claim 26 that is (*S*)-3a.

28. A compound of formula IV:



IV

where R^{14} is an amino protecting group or hydrogen;

R^1 is hydrogen, halo, C_{1-3} aliphatic, amino, cyano, $(C_{1-3}$ alkyl$)_{1-2}$ amino, C_{1-3} alkoxy, $-CONH_2$, $-NHCOCF_3$, or $-CH_2NH_2$;

R^2 is hydrogen, halo, C_{1-3} aliphatic, $-CF_3$; and

R^3 is hydrogen, halo, C_{1-6} aliphatic, C_{1-6} haloalkyl, C_{1-6} alkoxy, hydroxy, amino, cyano, or $(C_{1-6}$ alkyl$)_{1-2}$ amino.

29. The compound of claim 28 that is (*S*)-IV.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/011080

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D471/04 C07D265/30 A61K31/437

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, EMBASE, WPI Data, BIOSIS, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/68648 A (AVENTIS PHARMA GMBH) 20 September 2001 (2001-09-20) examples 83,118,121	1-29
A,P	CASTRO ALFREDO C. ET AL.: BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 13, no. 14, 2003, pages 2419-2422, XP002295562 the whole document	1-29
A	LEE MICHAEL ET AL.: CANCER LETTERS, vol. 101, no. 1, 1996, pages 53-57, XP002295563 abstract	1-29

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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 September 2004	24/09/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Frelon, D

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Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PALANKI M S S: CURRENT MEDICINAL CHEMISTRY, vol. 9, no. 2, January 2002 (2002-01), pages 219-227, XP001118175 ISSN: 0929-8673 the whole document	1-29
A	HOTTELET M ET AL: IMFLAMMATION RESEARCH, BIRKHAEUSER VERLAG, BASEL, CH, vol. 49, no. Supplement 2, 24 September 2000 (2000-09-24), page S91, XP009023847 ISSN: 1023-3830 abstract	1-29
A	DE 199 51 360 A (AVENTIS PHARMA GMBH) 3 May 2001 (2001-05-03) abstract; claims	1-29
A	WO 03/024936 A (STEALEY MICHAEL A ; VAZQUEZ MICHAEL L (US); CLARE MICHAEL (US); CRICH) 27 March 2003 (2003-03-27) abstract; claims	1-29
A,P	WO 03/039545 A (ADAMS JULIAN ; MILLENNIUM PHARM INC (US)) 15 May 2003 (2003-05-15) abstract; claims	1-29
X	WO 00/09492 A1 (PFIZER PRODUCTS INC., USA) 24 February 2000 (2000-02-24) examples 23-25	26

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/011080

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19-25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/011080

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		BR	9912944 A	08-05-2001
		CA	2340182 A1	24-02-2000
		EP	1104412 A1	06-06-2001
		JP	2003526604 T	09-09-2003
		US	2003181441 A1	25-09-2003

PATENT COOPERATION TREATY

104

RECEIVED
MILLENNIUM PHARMACEUTICALS
SEP 30 2004
DOCKETING DEPARTMENT
DOCKETED BY:

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

MILLENNIUM PHARMACEUTICALS, INC.
Attn. Silverman, Ian Robert
40 Landsdowne Street
Cambridge MA 02139
UNITED STATES OF AMERICA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 24/09/2004
Applicant's or agent's file reference MPI03-043	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2004/011080	International filing date *(day/month/year)* 09/04/2004
Applicant MILLENIUM PHARMACEUTICALS, INC.	

1. [x] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

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

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

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

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

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

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

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

4. **Reminders**

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

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Chantal Meyer

NOTES TO FORM PCT/ISA/220

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

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

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

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

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

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

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

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

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

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

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

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

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

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

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

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

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

BNSDOCID: <XB____ISA220NOTENP4_I_>

NOTES TO FORM PCT/ISA/220 (continued)

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

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

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

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

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

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

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

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

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

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

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

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

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

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

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

EN8DOCID: <XR____ISA220NCENP4_I_>

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
MPI03-043		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2004/011080	09/04/2004	09/04/2003

Applicant

MILLENIUM PHARMACEUTICALS, INC.

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

This International Search Report consists of a total of 5 sheets.

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

1. **Basis of the report**
   a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      [ ] The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. [X] Certain claims were found unsearchable (See Box II).

3. [ ] Unity of invention is lacking (see Box III).

4. With regard to the title,
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regards to the drawings,
   a. the figure of the drawings to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant.
      [ ] as selected by this Authority, because the applicant failed to suggest a figure.
      [ ] as selected by this Authority, because this figure better characterizes the invention.
   b. [ ] none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/011080 168

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D471/04 C07D265/30 A61K31/437

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, EMBASE, WPI Data, BIOSIS, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/68648 A (AVENTIS PHARMA GMBH) 20 September 2001 (2001-09-20) examples 83,118,121	1-29
A,P	CASTRO ALFREDO C. ET AL.: BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 13, no. 14, 2003, pages 2419-2422, XP002295562 the whole document	1-29
A	LEE MICHAEL ET AL.: CANCER LETTERS, vol. 101, no. 1, 1996, pages 53-57, XP002295563 abstract	1-29

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 September 2004	24/09/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Frelon, D

3

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PALANKI M S S: CURRENT MEDICINAL CHEMISTRY, vol. 9, no. 2, January 2002 (2002-01), pages 219-227, XP001118175 ISSN: 0929-8673 the whole document	1-29
A	HOTTELET M ET AL: IMFLAMMATION RESEARCH, BIRKHAEUSER VERLAG, BASEL, CH, vol. 49, no. Supplement 2, 24 September 2000 (2000-09-24), page S91, XP009023847 ISSN: 1023-3830 abstract	1-29
A	DE 199 51 360 A (AVENTIS PHARMA GMBH) 3 May 2001 (2001-05-03) abstract; claims	1-29
A	WO 03/024936 A (STEALEY MICHAEL A ; VAZQUEZ MICHAEL L (US); CLARE MICHAEL (US); CRICH) 27 March 2003 (2003-03-27) abstract; claims	1-29
A,P	WO 03/039545 A (ADAMS JULIAN ; MILLENNIUM PHARM INC (US)) 15 May 2003 (2003-05-15) abstract; claims	1-29
X	WO 00/09492 A1 (PFIZER PRODUCTS INC., USA) 24 February 2000 (2000-02-24) examples 23-25	26

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/011080

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19-25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/011080

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0168648	A	20-09-2001	EP	1134221 A1	19-09-2001
			EP	1209158 A1	29-05-2002
			AU	3741801 A	24-09-2001
			BR	0109161 A	26-11-2002
			CA	2402549 A1	20-09-2001
			CN	1418215 T	14-05-2003
			CZ	20023031 A3	15-01-2003
			EE	200200523 A	15-04-2004
			WO	0168648 A1	20-09-2001
			EP	1268477 A1	02-01-2003
			HU	0300894 A2	28-07-2003
			JP	2003527394 T	16-09-2003
			NO	20024338 A	05-11-2002
			NZ	521386 A	25-06-2004
			PL	357284 A1	26-07-2004
			SK	13082002 A3	01-04-2003
			US	2004110759 A1	10-06-2004
			US	2002099068 A1	25-07-2002
			ZA	200207324 A	07-07-2003
DE 19951360	A	03-05-2001	DE	19951360 A1	03-05-2001
			AU	1272801 A	08-05-2001
			BR	0015026 A	16-07-2002
			CA	2389165 A1	03-05-2001
			CN	1379772 T	13-11-2002
			CZ	20021413 A3	17-07-2002
			EE	200200217 A	16-06-2003
			WO	0130774 A1	03-05-2001
			EP	1261601 A1	04-12-2002
			HR	20020357 A2	29-02-2004
			HU	0203228 A2	28-02-2003
			JP	2003519101 T	17-06-2003
			NO	20021808 A	17-04-2002
			NZ	518587 A	25-06-2004
			PL	354528 A1	26-01-2004
			SK	5432002 A3	06-11-2002
			TR	200201144 T2	21-02-2003
			US	2003119820 A1	26-06-2003
			ZA	200203204 A	23-10-2002
WO 03024936	A	27-03-2003	CA	2460939 A1	27-03-2003
			EP	1427706 A1	16-06-2004
			WO	03024936 A1	27-03-2003
			US	2003114432 A1	19-06-2003
WO 03039545	A	15-05-2003	EP	1443927 A2	11-08-2004
			WO	03039545 A2	15-05-2003
WO 0009492	A1	24-02-2000	AU	4925099 A	06-03-2000
			BR	9912944 A	08-05-2001
			CA	2340182 A1	24-02-2000
			EP	1104412 A1	06-06-2001
			JP	2003526604 T	09-09-2003
			US	2003181441 A1	25-09-2003

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/011080	09.04.2004	09.04.2003

International Patent Classification (IPC) or both national classification and IPC
C07D471/04, C07D265/30, A61K31/437

Applicant
MILLENIUM PHARMACEUTICALS, INC.

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

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office - Gitschiner Str. 103
D-10958 Berlin
Tel. +49 30 25901 - 0
Fax: +49 30 25901 - 840

Authorized Officer

Freion, D

Telephone No. +49 30 25901-312

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/011080

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

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

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/011080

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/011080

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

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

☐ the entire international application,

☒ claims Nos. 19-25

because:

☒ the said international application, or the said claims Nos. 19 to 25 with regard to industrial application relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

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

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/011080

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-16,18,27-29
	No:	Claims	1,2,17,26
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-18,26-29
Industrial applicability (IA)	Yes:	Claims	1-18,26-29
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

Re Item I.

Unclarities and inconsistencies

1. Claims

Formula II-C is claimed in claim 3. Due to the dependency on claim 2 (and consequently on claim 1), definitions of R^1, R^2 and R^3 are unnecessarily repeated.

Formula III-A of claim 10 does not correspond to formula III-A of the description. The compound claimed in claim 10, although clearly part of general formula I, has no exact support in the description. This particular embodiment should be transferred in the specification among the preferred embodiments. A consequence is that the dependence of the following claims is irregular.

Claim 11 appears to find a support in page 16 of the description. But another irregularity is seen in claim 12 where the group R^{12} has not the same meaning as in claim 10 on which claim 12 is said to be dependent. The same remark applies to claim 13.

Claim 14 finds a support in description, page 19.

Claim 16 is directed to compounds of examples 1-31, 39-43, 45-47, 49-63. Compounds of figures Nos. 32-38, 47 are not examplified but disclosed on pages 30, 32 resp.
The figure No. 44 does not correspond to compound of example 44 which shows a morpholine ring in place of a pyran ring. According to the preparation, a morpholine ring should be expected as shown in figure 44.
Example 47, page 126 corresponds to figure No. 48. A second example 47 (?) disclosed on page 130 has no corresponding figure in the description or in claim 16. If the nomenclature is erroneous because of a possibly missing part after "-propionyl-", this second example 47 could correspond to figure No. 47 of page 32 which also appears in claim 16.
According to the preparation, the name of example 71 should probably read "-piperazin-1-yl-" instead of "-piperdin-1-yl-".

The compounds (S)-III-A-a of claim 15 have no support in the description. Moreover the given dependency on claim 14 is erroneous because of several discrepancies in the

substituent definitions.

Support for claim 26 directed to intermediate compounds of formula 3a is partly on page 57 of the description. But R^{15} is there defined as "an organic radical" which is nowhere specified in the specification. The "restricted" definition given in claim 26 in general terms "aliphatic", "aryl", etc, is not more admissible as also lacking support. For an appropriate support, the text of claim 26 should be introduced in the description including correct definitions already disclosed for the unspecific expressions like "aliphatic", "aryl", derivatives thereof and "carboxylic acid and amino protecting groups".

Claims 27, 28 and (implicitely) 29 are supported by the description, pages 58 and 59 with the reservations made above.

2. Description

Definitions given in the description for expressions which do not appear in the claims (like "linker group") are superfluous and may eventually be misleading for the plain understanding of the invention.

Considering the biological testing, essentially assay procedures are given without specific results. Only in the measurement of the IKB kinase phosphotransferase activity description is said that the compounds were active inhibitors of the IKK complex and that the IKB kinase inhibition average IC_{50} values were "generally" below "about" 10 microM. The "inventive compounds" (compounds of the invention or compounds which show an inventive effect ?) are also said to be selective for inhibiting IKK-2 as opposed to IKK-1. There appear mere statements which may not be sufficiently substantiated.

In the test on rat models for rheumatoid arthritis, "certain compounds were found to be active". This test cannot be taken into consideration for the evaluation of an inventive step as not all claimed compounds appear to be concerned. Furthermore, it is said that "compounds of formula II-C were found to be surprisingly superior compared to other compounds where ring A is a pyridine ring". This is quite obscure since, firstly, compounds of formula II-C are compounds wherein ring A is a pyridine ring and, secondly, the said "other compounds" are not defined. Same remark applies to the next sentence about compounds of formula III-A-a.

Re Item III.

Claims 19 to 25 are directed to methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods. They relate to subject-matter considered by this authority to be covered by the provisions of Rule 67.1(iv) PCT.

No opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34 (4) (a)(i) PCT). For the assessment on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treament and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Under the terms of Rule 39.1(iv) PCT, the ISA was not required to carry out a search of such claims, but as indicated in the ISR, the search was carried out and based on the alleged effects of the compounds. Similarly, the IPEA (which is the ISA) is not required to carry out an International preliminary examination of such claims, but as for the ISR, the IPER will be based on the alleged effects of the compounds (Rule 67.1 (iv) PCT).

Re Items V. and VI.

1. **Cited documents**

The following documents are referred to in this communication:

D1 WO 01/68648

D2 LEE MICHAEL ET AL.: CANCER LETTERS, vol. 101, no. 1, 1996, pages 53-57

D3 PALANKI M S S: CURRENT MEDICINAL CHEMISTRY, vol. 9, no. 2, January 2002, pages 219-227

D4 HOTTELET M ET AL: IMFLAMMATION RESEARCH, vol. 49, no. Supplement 2, 24 September 2000 , page S910

D5 DE 199 51 360

D6 WO 03/024936

D7 WO 03/039545 (intermediate document)

D8 CASTRO ALFREDO C. ET AL.: BIOORGANIC & MEDICINAL CHEMISTRY

LETTERS, vol. 13, no. 14, 2003, pages 2419-2422

D9 WO 00/09492

The intermediate documents D7 and D8 are relevant for the purposes of Rules 33.1 c, 64.3 and 70.10 PCT, but since the priority documents are not available at the time of establishing the written opinion, they are not taken into account. It is based on the assumption that all claims enjoy priority rights from the filing date of the priority document(s). If it later turns out that this assumption is not correct, the intermediate document in the International Search Report could become relevant in order to assess whether the claims satisfy the criteria set forth in Article 33(1) PCT.
If the priority date is not valid for the complete claimed subject-matter, this document may become relevant prior art in a possible regional/national phase.

2. Novelty

2.1 Final compounds

D1 destroys the novelty of the final compounds which the disclosure of the examples 83, 118 and 121 which are also claimed for the same biological properties.

Due to the extreme closeness of the prior art, the question arises of which feature should distinguish unambiguously the present subject-matter from the state of the art. The distinguishing feature must be clearly common for all the claimed compounds in order to satisfy the unity criterion (Rule 13 PCT).

2.2 Intermediate compounds

D9 is novelty destroying for the compounds claimed in claim 26 which the disclosure of the examples 23 to 25. It is noted that this is essentially due to the unspecificity of the expressions "carboxylic acid protecting group" or "amino protecting group" (actually they are functional definitions which are not admissible when they can be better defined).

Intermediate compounds of formula IV of claim 28 appear to be novel.

3. Inventive step

3.1 The problem underlying the present application is, according to the description, to provide beta-carbolines useful for treating inflammatory diseases and cancer, and more specifically for treating IKK-2 mediated diseases. D1 discloses novelty destroying compounds which have the same activities.

3.2 Before prosecuting with the substantive examination, the novelty must be clearly established, *i.e.* by at least one characterising feature which complies with the unity requirements.

It is thus necessary that this "novelty feature" be well-defined, since the acknowledgement of the inventive step should be based on an unexpected effect that this particular difference brings to the novel invention (see also item I.2 above the remarks concerning the assays described in the specification).

Citations D2 to D6 belong to the background art which may be useful in the examination process.

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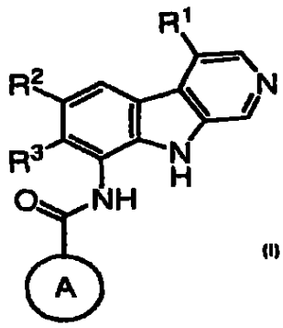
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Para códigos de dos letras y otras abreviaturas, referirse a la "Notas de Guía sobre Códigos y Abreviaturas" que aparece al comienzo de cada número regular de la Gaceta del PCT.

Como se imprimió

(54) Título: BETA-CARBOLINAS ÚTILES PARA TRATAR ENFERMEDADES INFLAMATORIAS



(I)

(57) Extracto: Esta invención proporciona compuestos de beta-carbolinas de la fórmula (I) donde el Anillo A es un anillo sustituido de piridinil, pirimidinil, morfolinil, piperidinil, piperazinil, pirrolidinil, piranil, tetrahidrofuranil, ciclohexil, ciclopentil, o tiomorfolinil y R1, R2 y R3 son como se describieron en la especificación os compuestos son IKK-2 inhibidores que son útiles para tratar enfermedades mediadas de IKK-2 tales como enfermedades inflamatorias y cáncer.

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BETA-CARBOLINAS ÚTILES PARA TRATAR ENFERMEDADES INFLAMATORIAS

Solicitud relacionada

Esta solicitud reivindica el beneficio de la Solicitud de Patente Provisional de Estados Unidos 60/461468 presentada el 9 de abril de 2003.

Campo de la invención

Esta invención se refiere a compuestos de beta-carbolina, composiciones farmacéuticas que los contienen y métodos de uso de las composiciones para tratar enfermedades. Los compuestos son particularmente útiles para tratar enfermedades inflamatorias y cáncer.

Antecedente de la invención

El factor de transcripción (nuclear) NF-κB es un miembro de la familia de la proteína Rel y, típicamente es un heterodímero compuesto de las subunidades p50 y p65. NF-κB está presente de manera constitutiva en el citosol, y se inactiva por su asociación con uno de los inhibidores de la familia IκB. Palombella et al., WO 95/25533, muestra que la vía ubiquitina-proteasoma juega un papel esencial en la regulación de la actividad de NF-κB, siendo responsable del procesamiento de p105 a p50 y la degradación de la proteína inhibidora IκB-a. Chen et al., Cell 84:853 (1996), muestra que antes de la degradación, IκB-a experimenta fosforilación selectiva en los restos de serina 32 y 36 por el complejo multisubunidad IκB quinasa (IKK). IκB-a se fosforila por IKK, que tiene dos subunidades catalíticas, IKK-1 (IκB quinasa a o IKK-a) e IKK-2 (IκB quinasa ß o IKK-ß). Una vez fosforilado, IκB se marca para la ubiquitinación y degradation por el proteasoma 26S, permitiendo el traslado de NF-κB al nucleo, donde se une a secuencias de ADN específicas en los promotores de genes diana y estimula su transcripción. Los inhibidores de IKK pueden bloquear la fosforilación de I?B y sus efectos cadena abajo adicionales, particularmente aquellos asociados con los factores de transcripción NF-κB.

Los productos proteicos de genes bajo el control regulador de NF-κB incluyen citoquinas, quimioquinas, moléculas de adhesión celular, y proteínas que median el crecimiento y el control celular. Es importante observar que, muchas de estas proteínas proinflamatorias también son capaces de funcionar, de un modo autocrino o paracrino, para estimular adicionalmente la activaciób de NF-κB. Además, NF-κB juega un papel en el crecimiento de células normales y mlignas. Además, NF-κB es un factor de transcripción heterodimérico que puede activar una gran cantidad de genes que codifican, entre otros, citoquinas proinflamatorias tales como IL-1, IL-2, TNFα o IL-6. NF-κB está presente en el citosol de células, formando un complejo con su inhibidor IκB de origen natural. La estimulación de células, por ejemplo, por citoquinas, conduce a la fosforilación y degradación proteolítica posterior de IκB. Esta degradación proteolítica conduce a la activación de NF-κB, que posteriormente migra al nucleo de la célula y activa una gran cantidad de genes proinflamatorios.

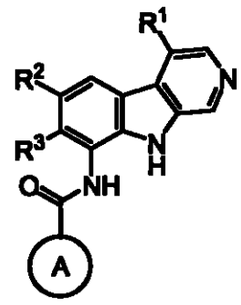
Rinehart et al., Patente de Estados Unidos Nº 4.631.149 (1986), describe compuestos beta-carbolina útiles como agentes antivíricos, antibacterianos y antitumorales.

Ritzeler et al., documento WO 01/68648, describe compuestos beta-carbolina con actividad inhibidora de quinasa I para el uso en el tratamiento de trastornos inflamatorios (por ejemplo, artritis reumatoide), osteoartritis, asma, infarto cardiaco, enfermedad de Alzheimer, trastornos carcinomatosos (potenciación de terapias citotóxicas) y ateroesclerosis.

Debería ser beneficioso proporcionar nuevos inhibidores de IKK que posean buenas propiedades terapéuticas, especialmente para el tratamiento de enfermedades inflamatorias.

Descripción de la invención

Esta invención proporciona compuestos que son útiles para tratar enfermedades mediadas por IKK-2, tales como enfermedades inflamatorias y cáncer. Los compuestos se representan por la fórmula I:



I

donde el Anillo A se selecciona entre el grupo compuesto por:

a) un anillo piridinilo o pirimidinilo que está sustituido con (i) $-CH_2C(O)$-G y 0-1 R^{6a} o (ii) 1-2 R^{6a}, y

b) un anillo morfolinilo, piperidinilo, piperazinilo, pirrolidinilo, piranilo, tetrahidrofuranoilo, ciclohexilo, ciclopentilo o tiomorfolinilo que está sustituido con (i) $-C(R^9)_3$, –W-G o –G, (ii) 0-4 R^{6b} y (iii) 0-1 grupos oxo sobre un carbono del anillo o 0-2 grupos oxo sobre un azufre del anillo;

cada R^{6a} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, alcoxi C_{1-6} o amino;

cada R^{6b} se selecciona independientemente entre un grupo alifático C_{1-3} o $-N(R^7)_2$, y dos R^{6b} sobre el mismo o un carbono adyacente se toman opcionalmente junto con el/los átomo(s) de carbono que intervienen para formar un anillo de 5-6 miembros que tiene 1-2 heteroátomos en el anillo seleccionados entre N, O o S;

W es –Q-, –Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$ o $-C(R^9)_2-[C(R^9)(R^{12})]_2-$;

Q es $-C(R^9)_2-$ o $-C(R^9)_2C(R^9)_2-$;

G es –OH, $-NR^4R^5$, $-N(R^9)CONR^4R^5$, $-N(R^9)SO_2$(un grupo alifático C_{1-3}), $-N(R^9)COCF_3$, $-N(R^9)CO$(un grupo alifático C_{1-6}),

-N(R^9)CO(heterociclilo), -N(R^9)CO(heteroarilo), -N(R^9)CO(arilo), un anillo heterociclilo de 3-7 miembros o un heteroarilo de 5-6 miembros, donde cada uno de los restos heteroarilo, arilo y heterociclilo de G está opcionalmente sustituido con 1-3 R^{10};

R^1 es hidrógeno, halo, un grupo alifático C_{1-3}, amino, ciano, (alquil C_{1-3})$_{1-2}$ amino, alcoxi C_{1-3}, -$CONH_2$, -$NHCOCF_3$ o -CH_2NH_2;

R^2 es hidrógeno, halo, un grupo alifático C_{1-3}, -CF_3;

R^3 es hidrógeno, halo, un grupo alifático C_{1-6}, haloalquilo C_{1-6}, alcoxi C_{1-6}, hidroxi, amino, ciano o (alquil C_{1-6})$_{1-2}$ amino;

R^4 es hidrógeno, heterocicilo de 3-7 miembros o un grupo alifático C_{1-6};

R^5 es hidrógeno, un grupo alifático C_{1-6} o un anillo heterocíclico de 3-7 miembros que tiene 1-2 heteroátomos en el anillo seleccionados entre N, O o S, donde R^5 está opcionalmente sustituido con halo, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$S(O)_2N(R^7)_2$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^8$ o -$N(R^7)C(O)N(R^7)_2$;

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};

cada R^9 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-3};

cada R^{10} se selecciona independientemente entre oxo, -R^{11}, -T-R^{11} o -V-T-R^{11};

cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, -$S(O)_2N(R^7)_2$, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^7$ o -$N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada;

V es –O-, -N(R^7)-, -S-, -S(O)-, -S(O)$_2$-, -C(O)- o -CO_2-; y

R^{12} es hidrógeno o una cadena lateral de aminoácidos.

El término "alifático" como se usa en este documento significa hidrocarburos C_1-C_{12} ramificados-lineales, ramificados o cíclicos que están completamente saturados o que contienen una o más unidades de insaturación pero que no son aromáticos. Por ejemplo, los grupos alifáticos adecuados incluyen grupos alquilo, alquenilo y alquinilo lineales, ramificados o cíclicos, sustituidos o no sustituidos e híbridos de los mismos tales como (cicloalquil)alquilo, (cicloalquenil)alquilo o (cicloalquil)alquenilo. Los términos "alquilo", "alcoxi", "hidroxialquilo", "alcoxialquilo" y "alcoxicarbonilo", usados solos o como parte de un resto mayor, incluyen cadenas lineales y ramificadas que contienen de dos a doce átomos de carbono. Los términos "alquenilo" y "alquinilo", usados solos o como parte de un resto mayor, incluyen cadenas lineales y ramificadas que contienen de dos a doce átomos de carbono. El término "cicloalquilo", usado solo o como parte de un resto mayor, incluye hidrocarburos C_3-C_{12} cíclicos que están completamente saturados o que contienen una o más unidades de insaturación, pero que no son aromáticos.

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

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

Los términos "carbociclo", "carbociclilo", "carbocicio" o "carbocíclico" como se usan en este documento significan un sistema de anillos alifáticos que tiene de tres a catorce miembros. Los términos "carbociclo", "carbociclilo", "carbocicio" o "carbocíclico" tanto saturados como parcialmente insaturados, también se refieren a anillos que están opcionalmente sustituidos. Los términos "carbociclo", "carbociclilo", "carbocicio" o "carbocíclico" también incluyen anillos alifáticos que están condensados a uno o más anillos aromáticos o no aromáticos, tales como en un decahidronaftilo o tetrahidronaftilo, donde el radical o punto de unión está en el anillo un grupo alifático.

El término "arilo" usado solo o como parte de un resto mayor como en "aralquilo", "aralcoxi" o "ariloxialquilo", se refiere a grupos de anillo aromático que tienen de cinco a catorce miembros, tales como fenilo, bencilo, fenetilo, 1-naftilo, 2-naftilo, 1-antracilo y 2-antracilo. El término "arilo" también se refiere a anillos que están opcionalmente sustituidos. El término "arilo" puede usarse de forma intercambiable con el término "anillo arilo ". El grupo "arilo" también incluye sistemas de anillos aromáticos policíclicos en los que un anillo aromático se condensa a uno o más anillos. Los ejemplos incluyen 1-naftilo, 2-naftilo, 1- antracil y 2-antracilo. También se incluye dentro del alcance de término "arilo", tal como se usa en este documento, un grupo en el que un anillo aromático se condensa a uno o más anillos no aromáticos, tal como en un indanilo, fenantridinilo o tetrahidronaftilo, donde el radical o punto de unión está en el anillo aromático.

El término "heterocicio", "heterociclilo" o "heterocíclico" como se usa en este documento incluye sistemas de anillos no aromáticos que tienen de cinco a catorce miembros, preferiblemente de cinco a diez, donde uno o más carbonos del anillo, preferiblemente de uno a cuatro, se reemplazan con un heteroátomo tal como N, O o S. Los ejemplos de anillos heterocíclicos incluyen 3-1H-benzimidazol-2-ona, (1-sustituido)-2-oxo-benzimidazol-3-ilo, 2-tetrahidrofuranoilo, 3-tetrahidrofuranoilo, 2-tetrahidropiranilo, 3-tetrahidropiranilo, 4-tetrahidropiranilo, [1,3]-dioxalanilo, [1,3]-ditiolanilo, [1,3]-dioxanilo, 2-tetrahidrotiofenilo, 3-

tetrahidrotiofenilo, 2-morfolinilo, 3-morfolinilo, 4-morfolinilo, 2-tiomorfolinilo, 3-tiomorfolinilo, 4- tiomorfolinilo, 1-pirrolidinilo, 2-pirrolidinilo, 3-pirrolidinilo, 1-piperazinilo, 2-piperazinilo, 1-piperidinilo, 2-piperidinilo, 3-piperidinilo, 4-piperidinilo, 4- tiazolidinilo, diazolonilo, diazolonilo, 1-ftalimidinilo N-sustituido, benzoxanilo, benzopirrolidinilo, benzopiperidinilo, benzoxolanilo, benzotiolanilo, y benzotianilo. También se incluye dentro del alcance del término "heterociclilo" o "heterocíclico", tal como se usan en este documento, un grupo en el que un anillo no aromático que contiene heteroátomos se condensa a uno o más anillos aromáticos o no aromáticos, tal como en un indolinilo, cromanilo, fenantridinilo o tetrahidroquinolinilo, donde el radical o punto de unión está en el anillo no aromático que contiene heteroátomos. El término "heterociclo", "heterociclilo" o "heterocíclico" tanto saturado como parcialmente insaturado, también se refiere a anillos que están opcionalmente sustituidos.

El término "heteroarilo", usado solo o como parte de un resto mayor como en "heteroaralquilo" o "heteroarilalcoxi", se refiere a grupos de anillo heteroaromático que tienen de cinco a catorce miembros. Los ejemplos de anillos heteroarilo incluyen 2-furanilo, 3-furanilo, 3-furazanilo, N-imidazolilo, 2-imidazolilo, 4-imidazolilo, 5-imidazolilo, 3-isoxazolilo, 4-isoxazolilo, 5-isoxazolilo, 2-oxadiazolilo, 5-oxadiazolilo, 2-oxazolilo, 4-oxazolilo, 5-oxazolilo, 1-pirrolilo, 2-pirrolilo, 3-pirrolilo, 1-pirazolilo, 2-pirazolilo, 3-pirazolilo, 2-piridilo, 3-piridilo, 4-piridilo, 2-pirimidilo, 4-pirimidilo, 5-pirimidilo, 3-piridazinilo, 2-tiazolilo, 4-tiazolilo, 5-tiazolilo, 5-tetrazolilo, 2-triazolilo, 5-triazolilo, 2-tienilo, 3-tienilo, carbazolilo, benzimidazolilo, benzotienilo, benzofuranilo, indolilo, quinolinilo, benzotriazolilo, benzotiazolilo, benzooxazolilo, benzimidazolilo, isoquinolinilo, indazolilo, isoindolilo, acridinilo o benzoisoxazolilo. También se incluye dentro del alcance del término "heteroarilo", tal como se usa en este documento, un grupo en el que un anillo heterocíclico se condensa a uno o más anillos aromáticos o no aromáticos donde el radical o punto de unión está en el anillo heteroaromático. Los ejemplos incluyen tetrahidroquinolinilo, tetrahidroisoquinolinilo, y pirido[3,4-d]pirimidinilo. El término "heteroarilo" también

se refiere a anillos que están opcionalmente sustituidos. El término "heteroarilo" puede usarse de forma intercambiable con el término "anillo heteroarilo " o el término "heteroaromático".

Un grupo arilo (incluyendo aralquilo, aralcoxi, ariloxialquilo y similares) o heteroarilo (incluyendo heteroaralquilo y heteroarilalcoxi y similares) puede contener uno o más sustituyentes. Los ejemplos de sustituyentes adecuados sobre el átomo de carbono insaturado de un grupo arilo, heteroarilo, aralquilo o heteroaralquilo incluyen un halógeno, -R°, -OR°, -SR°, 1,2-metileno-dioxi, 1,2-etilenodioxi, OH protegido (tal como aciloxi), fenilo (Ph), Ph sustituido, -O(Ph), -O(Ph) sustituido, $-CH_2(Ph)$, $-CH_2(Ph)$ sustituido, $-CH_2CH_2(Ph)$, $-CH_2CH_2(Ph)$ sustituido, $-NO_2$, -CN, $-N(R°)_2$, $-NR°C(O)R°$, $-NR°C(O)N(R°)_2$, $-NR°CO_2R°$, $-NR°NR°C(O)R°$, $-NR°NR°C(O)N(R°)_2$, $-NR°NR°CO_2R°$, $-C(O)C(O)R°$, $-C(O)CH_2C(O)R°$, $-CO_2R°$, $-C(O)R°$, $-C(O)N(R°)_2$, $-OC(O)N(R°)_2$, $-S(O)_2R°$, $-SO_2N(R°)_2$, -S(O)R°, $-NR°SO_2N(R°)_2$, $-NR°SO_2R°$, $-C(=S)N(R°)_2$, $-C(=NH)-N(R°)_2$, $-(CH_2)_yNHC(O)R°$, $-(CH_2)_yNHC(O)$ CH(V-R°)(R°); donde cada R° se selecciona independientemente entre hidrógeno, un grupo alifático sustituido o no sustituido, un anillo heteroarilo o heterocíclico no sustituido, fenilo (Ph), Ph sustituido, -O(Ph), -O(Ph) sustituido, $-CH_2(Ph)$ o $-CH_2(Ph)$ sustituido; y es 0-6; y V es un grupo de engarce. Los ejemplos de sustituyentes del grupo un grupo alifático o el anillo fenilo de R° incluyen amino, alquilamino, dialquilamino, aminocarbonilo, halógeno, alquilo, alquilaminocarbonilo, dialquilaminocarbonilo, alquilaminocarboniloxi, dialquilaminocarboniloxi, alcoxi, nitro, ciano, carboxi, alcoxicarbonilo, alquilcarbonilo, hidroxi, haloalcoxi o haloalquilo.

Un grupo alifático o un anillo heterocíclico no aromático puede contener uno o más sustituyentes. Los ejemplos de sustituyentes adecuados sobre el carbono saturado 20 de un grupo alifático o de un anillo heterocíclico no aromático incluyen los que se han indicado anteriormente para el carbono insaturado de un grupo arilo o heteroarilo y los siguientes: =O, =S, =NNHR*, $=NN(R*)_2$, =N-, =NNHC(O)R*, $=NNHCO_2$(alquilo), $=NNHSO_2$(alquilo) o =NR*, donde cada R* se selecciona

independientemente entre hidrógeno, un grupo alifático no sustituido o un grupo alifático sustituido. Los ejemplos de sustituyentes sobre el grupo un grupo alifático incluyen amino, alquilamino, dialquilamino, aminocarbonilo, halógeno, alquilo, alquilaminocarbonilo, dialquilaminocarbonilo, alquilaminocarboniloxi, dialquilaminocarboniloxi, alcoxi, nitro, ciano, carboxi, alcoxicarbonilo, alquilcarbonilo, hidroxi, haloalcoxi o haloalquilo.

Los sustituyentes adecuados sobre el nitrógeno de un anillo heterocíclico no aromático incluyen $-R^+$, $-N(R^+)2$, $-C(O)R^+$, $-CO_2R^+$, $-C(O)C(O)R^+$, $-C(O)CH_2C(O)R^+$, $-SO_2R^+$, $-SO_2N(R^+)_2$, $-C(=S)N(R^+)_2$, $-C(=NH)-N(R^+)_2$, y $-NR^+SO_2R^+$; donde cada R^+ se selecciona independientemente entre hidrógeno, un grupo alifático, un grupo alifático sustituido, fenilo (Ph), Ph sustituido, -O(Ph), -O(Ph) sustituido, $CH_2(Ph)$, $CH_2(Ph)$ sustituido o un anillo heteroarilo o heterocíclico no sustituido. Los ejemplos de sustituyentes sobre el grupo un grupo alifático o el anillo fenilo incluyen amino, alquilamino, dialquilamino, aminocarbonilo, halógeno, alquilo, alquilaminocarbonilo, dialquilaminocarbonilo, alquilaminocarboniloxi, dialquilaminocarboniloxi, alcoxi, nitro, ciano, carboxi, alcoxicarbonilo, alquilcarboniilo, hidroxi, haloalcoxi o haloalquilo.

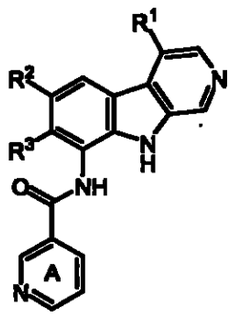
El término "grupo de engarce" o "engarce" significa un resto orgánico que conecta dos partes de un compuesto. Típicamente, los engarces comprenden un átomo tal como oxígeno o azufre, una unidad tal como -NH-, $-CH_2-$, -C(O)-, -C(O)NH- o una cadena de átomos, tal como una cadena alquilideno. La masa molecular de un engarce está típicamente en el intervalo de aproximadamente 14 a 200, preferiblemente en el intervalo de 14 a 96 con una longitud de hasta aproximadamente seis átomos. Los ejemplos de engarces incluyen una cadena alquilideno C_{1-6} saturada o insaturada que está opcionalmente sustituida, y en la que uno o dos carbonos saturados de la cadena se reemplazan opcionalmente con -C(O)-, -C(O)C(O)-, -CONH-, -CONHNH-, $-CO_2-$, -OC(O)-, $-NHCO_2-$, -O-, -NHCONH-, -OC(O)NH-, -NHNH-, $-NHCO-$, -S-, -SO-, $-SO_2-$, -NH-, $-SO_2NH-$ o $-NHSO_2-$.

El término "cadena alquilideno" o "cadena alquileno" se refiere a una cadena de carbonos lineal o ramificada, opcionalmente sustituida que puede estar totalmente saturada o tener una o más unidades de insaturación. Los sustituyentes opcionales son como se ha descrito anteriormente para un grupo alifático.

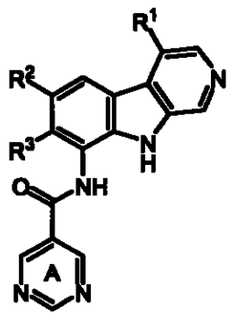
Una combinación de sustituyentes o variables se permite únicamente si tal combinación da como resultado un compuesto estable o químicamente factible. Un compuesto estable o un compuesto químicamente factible es uno en el que la estructura química no se altera sustancialmente cuando se mantiene a una temperatura de 40°C o menos, en ausencia de humedad u otras condiciones Reactivas, durante al menos una semana.

A menos que se indique otra cosa, las estructuras representadas en este documento también pretenden incluir todas las formas químicas de la estructura; es decir, las configuraciones R y S para cada centro asimétrico. Por lo tanto, los isómeros estereoquímicos unitarios así como las mezclas enantioméricas y diastereoméricas de los presentes compuestos están dentro del alcance de la invención. A menos que se indique otra cosa, las estructuras representadas en este documento también pretenden incluir compuestos que difieren únicamente en la presencia de uno o más átomos isotópicamente enriquecidos. Por ejemplo, los compuestos que tienen las presentes estructuras excepto para el reemplazamiento de un átomo de hidrógeno por un deuterio o tritio o el reemplazamiento de un carbono por un carbono enriquecido ^{13}C o ^{14}C están dentro del alcance de esta invención.

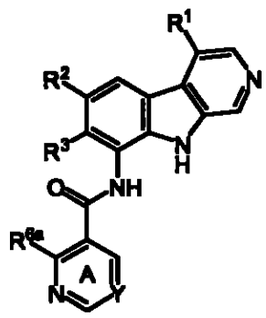
En una realización de la invención, el Anillo A se selecciona entre un anillo piridinilo o pirimidinilo que está sustituido con 1-2 grupos R^{6a}. En esta realización, el Anillo A preferido incluye un anillo 3-piridinilo o 5-pirimidinilo, mostrado a continuación por los compuestos de fórmula II-A y II-B, respectivamente.



II-A



II-B



II-C (Y = N o CH)

Preferiblemente, R^{6a} se selecciona entre halo o un alifático C_{1-6}, tal como cloro o metilo. Cuando R^{6a} es un grupo alifático tal como metilo, una posición favorable para el grupo R^{6a} está en la posición 2 del anillo piridinilo o en la posición 4 del anillo pirimidinilo, como se ha mostrado antes en II-C. Los restos particulares del Anillo A son 2-metil-3-piridinilo y 4-metil-5-pirimidinilo. Se ha descubierto que los compuestos de fórmula II-C en la que R^{6a} es un grupo metilo sorprendentemente son más potentes en los ensayos biológicos para la inhibición de IKK que compuestos análogos que tienen un Anillo A piridina no sustituido, tales como aquellos descritos en la solicitud PCT WO 0168648 mencionada anteriormente de Ritzeler et al.

Los grupos R^1 preferidos son grupos pequeños tales como hidrógeno, metilo, amino y flúor.

Los grupos R^2 preferidos incluyen hidrógeno y halo. Cloro es un grupo R^2 halo preferido.

Los grupos R^3 preferidos incluyen hidrógeno, halo (especialmente cloro) y alcoxi. Los ejemplos de grupos alcoxi adecuados incluyen grupos alcoxi C_{1-4} tales como metoxi, etoxi, propoxi y ciclopropilmetoxi.

En otra realización, el Anillo A se selecciona entre un anillo no aromático de 5-6 miembros que tiene 0-2 heteroátomos en el anillo seleccionados entre nitrógeno, oxígeno y azufre. Éstos se indican generalmente como compuestos de

fórmula III. Los ejemplos de grupos del Anillo A no aromáticos incluyen un anillo morfolinilo, piperidinilo, piperazinilo, pirrolidinilo, piranilo, tetrahidrofuranoilo, ciclohexilo, ciclopentilo y tiomorfolinilo. Preferiblemente, tales anillos no aromáticos están sustituidos con (i) $-C(R^9)_3$ o -W-G, (ii) 0-4 R^{6b} y (iii) 0-1 grupos oxo sobre un carbono del anillo o 0-2 grupos oxo sobre un azufre del anillo. Más preferiblemente, tales anillos no aromáticos están sustituidos con (i) -W-G, (ii) 0-2 R^{6b} y (iii) 0-1 grupos oxo sobre un carbono del anillo o 0-2 grupos oxo sobre un azufre del anillo.

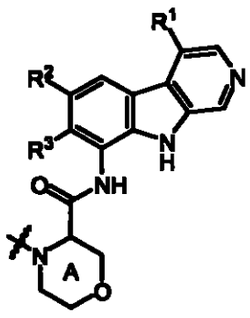
Un G preferido es $-NR^4R^5$ o un anillo heterociclo de 3-7 miembros. Más preferiblemente G es $-NR^4R^5$ o un anillo heterocicilo de 5-6 miembros, donde G está sustituido con 1-2 R^{10}.

Un R^4 preferido es un hidrógeno, anillo heterocicilo de 5-6 miembros o un grupo alifático C_{1-6}, más preferiblemente hidrógeno o un grupo alifático C_{1-6}. R^4 también puede ser un alcoxi C_{1-6}.

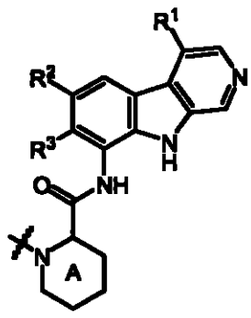
Un R^5 preferido es un hidrógeno, anillo heterocicilo de 5-6 miembros o un grupo alifático C_{1-6}, más preferiblemente hidrógeno o un grupo alifático C_{1-6}.

Diversos compuestos de formula III en la que el Anillo A es un anillo no aromático se muestran en la Tabla 2. Por facilidad de visión, no se muestran los sustituyentes de los compuestos del Anillo A no aromáticos, excepto para el grupo oxo en algunos casos.

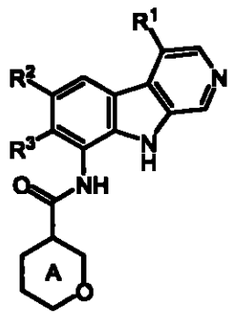
Tabla 2. Compuestos en los que el Anillo A es no aromático



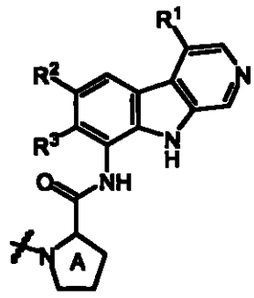
III-A



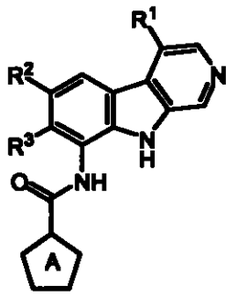
III-B



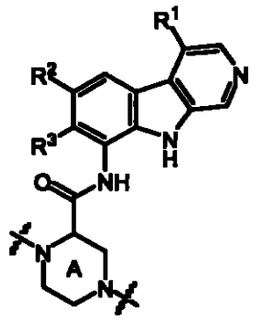
III-C



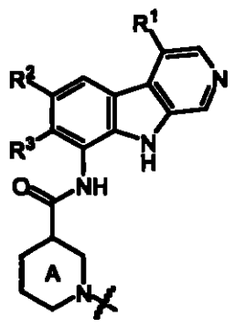
III-D



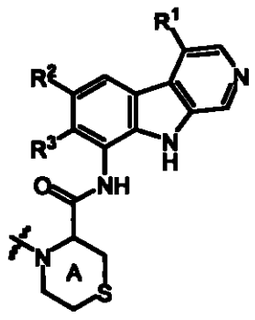
III-E



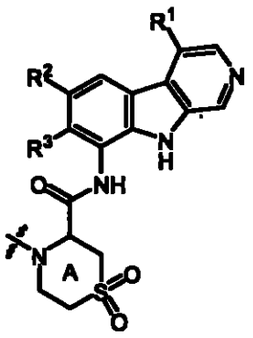
III-F



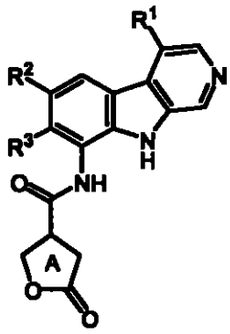
III-G



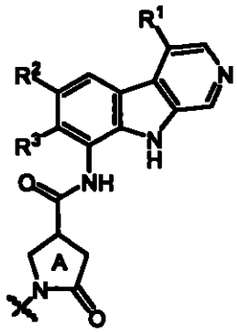
III-H



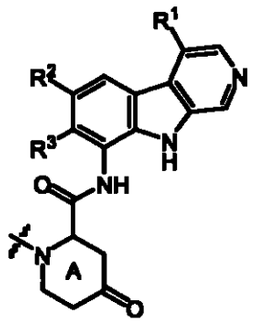
III-J



III-K



III-L



III-M

Cuando el Anillo A es un anillo heterocíclico, no aromático, de seis miembros, una posición favorable para los sustituyentes–W-G y -C(R^9)$_3$ sobre el Anillo A es orto a la posición en la que se une la porción beta-carbolina. Por ejemplo, en los compuestos III-A, III-B, III-D, III-H, III-J y III-M, una posición preferida para la unión de–W-G y -C(R^9)$_3$ está en el nitrógeno del Anillo A o en N-1 en el caso del compuesto III-F.

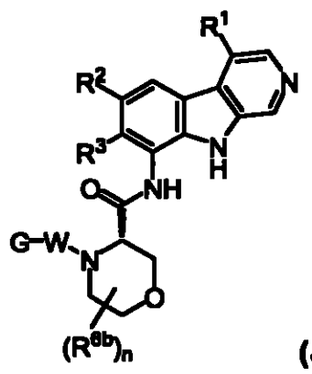
Preferiblemente, W es –Q-, –Q-C(O)-, -C(R^9)$_2$-C(R^9)(R^{12})- o -C(R^9)$_2$-[C(R^9)(R^{12})]$_2$- donde R^9 es hidrógeno. Más preferiblemente, W es –Q-, –Q-C(O)- o -C(R^9)$_2$-C(R^9)(R^{12})-. R^{12} es hidrógeno, un grupo alifático C_{1-6}, fenilo sustituido o no sustituido, bencilo sustituido o no sustituido o una cadena lateral de aminoácidos, particularmente la cadena lateral de un aminoácido neutro. Los ejemplos de aminoácidos neutros particulares incluyen alanina, fenilalanina, valina, leucina, isoleucina, serina, tirosina, ácido aspártico y ácido glutámico.

En una realización, W es Q-C(O)-. En esta realización, un Q preferido es –CH_2- o –CH_2-CH_2-, más preferiblemente –CH_2-.

En otra realización, el Anillo A está sustituido con 0-2 R^{6b}. Un grupo R^{6b} preferido es metilo. Cuando el Anillo A es un anillo de seis miembros no aromático, una realización proporciona compuestos de fórmula III en la que hay dos grupos metilo sobre la posición para del Anillo A para la posición en la que se une la porción beta carbolina. Un ejemplo de esta realización es un compuesto en el que

el Anillo A es un anillo 6,6-dimetil-morfolinilo. Preferiblemente, tales compuestos también están sustituidos con–W-G como se ha descrito anteriormente.

Cuando el Anillo A es un anillo morfolinilo, se ha descubierto que se prefieren los compuestos que tienen la estereoquímica "S" en la posición 3 del anillo morfolina, como se muestra a continuación con los compuestos de fórmula (*S*)-III-A.



(*S*)-III-A

donde n es 0-4 y R^1, R^2, R^3, W, G y R^{6b} son como se han definido anteriormente. Por analogía, se espera que se prefiera la estereoquímica "S" para otros compuestos de Anillo A no aromáticos de seis miembros de fórmula III.

Una realización se refiere a compuestos de fórmula III-A o (*S*)-III-A en la que R^1 es hidrógeno, halo, metilo o amino;

R^2 es hidrógeno, metilo o halo;

R^3 es hidrógeno, halo, alcoxi o (un grupo alifático C_{1-6})$_2$ amino;

El Anillo A está sustituido con 0-2 R^{6b};

R^{6b} es un grupo alifático C_{1-3};

W es –Q-, –Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$ o $-C(R^9)_2-[C(R^9)(R^{12})]_{1-2}-$;

Q es $-C(R^9)_2-$ o $-C(R^9)_2C(R^9)_2-$;

G es $-NR^4R^5$, $-N(R^9)C(O)NR^4R^5$, $-N(R^9)SO_2$(un grupo alifático C_{1-3}), $-N(R^9)C(O)CF_3$, $-N(R^9)CO$(un grupo alifático C_{1-6}), y $-N(R^9)CO$(heterociclilo),

-N(R^9)CO(heteroarilo), -N(R^9)CO(arilo), un anillo heterociclilo de 5-6 miembros o un heteroarilo de 5-6 miembros, donde cada uno de los restos heteroarilo, arilo y heterociclilo de G está opcionalmente sustituido con 1-3 R^{10};

R^4 es hidrógeno o un grupo alifático C_{1-6};

R^5 es hidrógeno o un grupo alifático C_{1-6} que está opcionalmente sustituido con halo, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$S(O)_2N(R^7)_2$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^8$ o -$N(R^7)C(O)N(R^7)_2$;

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};

R^9 es hidrógeno;

cada R^{10} se selecciona independientemente entre oxo, R^{11}, T-R^{11} o V-T-R^{11};

cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, -$S(O)_2N(R^7)_2$, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^7$ o -$N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada;

V es –O-, -$N(R^7)$-, -S-, -S(O)-, -$S(O)_2$-, -C(O)- o -CO_2-; y

R^{12} es hidrógeno, un grupo alifático C_{1-6}, fenilo sustituido o no sustituido o bencilo sustituido o no sustituido.

Otra realización se refiere a compuestos de fórmula III-A o (*S*)-III-A en la que:

R^1 es hidrógeno, metilo, flúor o amino;

R^2 es cloro;

R^3 es hidrógeno o alcoxi;

El Anillo A está sustituido con –W-G y 0-2 R^{6b};

R^{6b} es metilo;

W es –Q-, –Q-C(O)- o -$C(R^9)_2$-$C(R^9)(R^{12})$-;

Q es -$C(R^9)_2$- o -$C(R^9)_2C(R^9)_2$-;

G es $-NR^4R^5$, $-N(R^9)C(O)NR^4R^5$, $-N(R^9)C(O)CF_3$, $-N(R^9)CO$(un grupo alifático C_{1-6}), y $-N(R^9)CO$(heterociclilo), $-N(R^9)CO$(heteroarilo), un anillo heterociclilo de 5-6 miembros o un heteroarilo de 5-6 miembros, donde cada uno de los restos heteroarilo y heterociclilo de G está opcionalmente sustituido con 1-3 R^{10};

R^4 es hidrógeno o un grupo alifático C_{1-6};

R^5 es hidrógeno o un grupo alifático C_{1-6};

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};

R^9 es hidrógeno;

cada R^{10} se selecciona independientemente entre oxo, R^{11}, $T\text{-}R^{11}$ o $V\text{-}T\text{-}R^{11}$;

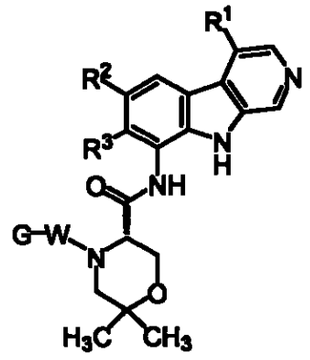
cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$ o $-N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada;

V es $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$ o $-CO_2-$; y

R^{12} es hidrógeno, un grupo alifático C_{1-6}, fenilo o bencilo.

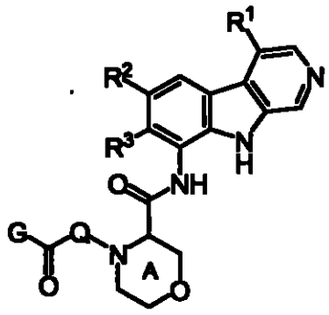
Los compuestos preferidos de fórmula III-A son los compuestos de fórmula (*S*)-III-A':



(*S*)-III-A'

donde R^1, R^2, R^3, W y G son como se han definido anteriormente para (*S*)-III-A.

Otra realización se refiere a compuestos de fórmula III-A-a:



III-A-a

o a una sal farmacéuticamente aceptable de los mismos, donde:

Q es $-CH_2-$, $-CH(R^9)-$ o $-C(R^9)_2-$;

G es $-NR^4R^5$ o a heterocicilo de 3-7 miembros o un anillo heteroarilo que está opcionalmente sustituido con 1-2 R^{10};

R^1 es hidrógeno, halo, un grupo alifático C_{1-3}, amino, ciano, (alquil C_{1-3})$_{1-2}$amino, alcoxi C_{1-3}, (un grupo alifático C_{1-3})-C(O)-, (un grupo alifático C_{1-6})-CO_2- o (un grupo alifático C_{1-3})-C(O)NH-;

R^2 es hidrógeno, halo, un grupo alifático C_{1-3}, alcoxi C_{1-3}, haloalcoxi C_{1-3} o haloalquilo C_{1-3};

R^3 es hidrógeno, halo, un grupo alifático C_{1-6}, haloalquilo C_{1-6}, alcoxi C_{1-6}, hidroxi, amino, ciano o (alquil C_{1-6})$_{1-2}$amino;

R^4 es hidrógeno o un grupo alifático C_{1-6};

R^5 es un grupo alifático C_{1-6} que está opcionalmente sustituido con halo, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^8$ o -$N(R^7)C(O)N(R^7)_2$;

El Anillo A está sustituido con 0-4 R^{6b};

cada R^{6b} se selecciona independientemente entre un grupo alifático C_{1-6};

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};

cada R^9 se selecciona independientemente entre un alifático C_{1-3};

cada R^{10} se selecciona independientemente entre R^{11}, T-R^{11} o V-T-R^{11};

cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^7$ o -$N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada; y

V es –O-, -$N(R^7)$-, -S-, -S(O)-, -$S(O)_2$-, -C(O)- o -CO_2-.

Una realización se refiere a compuestos de fórmula III-A-a en la que:

Q es –CH_2- o -$CH(R^9)$-;

G es –NR^4R^5 o un anillo heterociclilo o heteroarilo de 5-6 miembros que está opcionalmente sustituido con 1-2 R^{10};

R^1 es hidrógeno, halo, alquilo C_{1-2}, amino o (alquil C_{1-2})$_{1-2}$amino;

R^2 es hidrógeno, halo, un grupo alifático C_{1-2}, alcoxi C_{1-2} o haloalquilo C_{1-2};

R^3 es hidrógeno, halo, un grupo alifático C_{1-2}, alcoxi C_{1-2} o haloalquilo C_{1-2};

R^4 es hidrógeno o un grupo alifático C_{1-6};

R^5 es un grupo alifático C_{1-6} que está opcionalmente sustituido con halo, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^8$ o -$N(R^7)C(O)N(R^7)_2$;

El Anillo A está sustituido con 0-2 R^{6b};

cada R^{6b} se selecciona independientemente entre un grupo alifático C_{1-3};

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

R^8 es un grupo alifático C_{1-4};

R^9 se selecciona independientemente entre un alifático C_{1-3};

cada R^{10} se selecciona independientemente entre R^{11}, T-R^{11} o V-T-R^{11};

cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^7$ o -$N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada; y

V es –O-, -$N(R^7)$-, -S-, -S(O)-, -$S(O)_2$-, -C(O)- o -CO_2-.

Otra realización se refiere a compuestos de fórmula III-A-a en la que:

Q es –CH_2- o -$CH(R^9)$-;

G es –NR^4R^5 o un anillo heterociclilo de 5-6 miembros que tiene 1-2 heteroátomos en el anillo seleccionados entre oxígeno o nitrógeno, que está opcionalmente sustituido con 1-2 R^{10};

R^1 es hidrógeno, halo, metilo, amino o (alquil $C_{1-2})_{1-2}$amino;

R^2 es hidrógeno, halo, un grupo alifático C_{1-2} o haloalquilo C_{1-2};

R^3 es hidrógeno, halo o un grupo alifático C_{1-2};

R^4 es hidrógeno o un grupo alifático C_{1-6};

R^5 es un grupo alifático C_{1-6} que está opcionalmente sustituido con halo, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^8$ o -$N(R^7)C(O)N(R^7)_2$;

El Anillo A está sustituido con cero o dos R^{6b};

cada R^{6b} se selecciona independientemente entre un grupo alifático C_{1-3};

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

R^8 es un grupo alifático C_{1-4};

R^9 se selecciona independientemente entre un alifático C_{1-3};

cada R^{10} se selecciona independientemente entre R^{11}, T-R^{11} o V-T-R^{11};

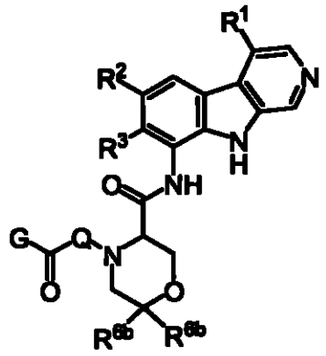
cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^7$ o -$N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada; y

V es –O-, -$N(R^7)$-, -S-, -S(O)-, -$S(O)_2$-, -C(O)- o -CO_2-.

En los compuestos preferidos de fórmula III-A-a, Q es -CH_2-; G se selecciona entre un piperidinilo opcionalmente sustituido, piperazinilo, morfolinilo, pirrolidinilo o -NR^4R^5; R^4 es hidrógeno o un grupo alifático C_{1-6}; y R^5 es un grupo alifático C_{1-6}, heterociclilo de 5-6 miembros o hidroxialquilo C_{1-6}. Se prefieren más los compuestos en los que G está no sustituido o sustituido con 1-2 grupos seleccionados independientemente entre el grupo compuesto por: alquilo C_{1-3}, HO-alquilo, alcoxicarbonilo, mono- o dialquilaminocarbonilo, y HO_2C-alquilo. Para los compuestos de fórmula III-A-a en cada una de las realizaciones anteriores, se prefiere la estereoquímica (*S*) en la posición 3 de morfolina.

Otra realización se refiere a compuestos de fórmula III-A-aa:



III-A-aa

o a una sal farmacéuticamente aceptable de los mismos donde,

Q es $-CH_2-$ o $-CH(R^9)-$;

G es $-NR^4R^5$ o un anillo heterociclilo de 3-7 miembros que está opcionalmente sustituido con 1-2 R^{10};

R^1 es hidrógeno, halo, alquilo C_{1-2}, amino o (alquil C_{1-2})$_{1-2}$amino;

R^2 es hidrógeno, halo, un grupo alifático C_{1-2}, alcoxi C_{1-2} o haloalquilo C_{1-2};

R^3 es hidrógeno, halo, un grupo alifático C_{1-2}, alcoxi C_{1-2} o haloalquilo C_{1-2};

R^4 es hidrógeno o un grupo alifático C_{1-6};

R^5 es un grupo alifático C_{1-6} que está opcionalmente sustituido con halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$ o $-N(R^7)C(O)N(R^7)_2$;

cada R^{6b} se selecciona independientemente entre hidrógeno o a alifático C_{1-6};

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

R^8 es un grupo alifático C_{1-4};

R^9 se selecciona independientemente entre un alifático C_{1-3};

cada R^{10} se selecciona independientemente entre R^{11}, T-R^{11} o V-T-R^{11};

cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, $-OR^7$, $-CN$, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$ o $-N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada; y

V es $-O-$, $-N(R^7)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)-$ o $-CO_2-$.

Otra realización se refiere a compuestos de fórmula III-A-aa en la que:

Q es $-CH_2-$;

G es $-NR^4R^5$ o un anillo heterociclilo de 5-6 miembros que está opcionalmente sustituido con 1-2 R^{10};

R^1 es hidrógeno, halo o metilo;

R^2 es hidrógeno, halo, un grupo alifático C_{1-2}, alcoxi C_{1-2} o haloalquilo C_{1-2};

R^3 es hidrógeno;

R^4 es hidrógeno o un grupo alifático C_{1-6};

R^5 es un grupo alifático C_{1-6} que está opcionalmente sustituido con halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$ o $-N(R^7)C(O)N(R^7)_2$;

cada R^{6b} se selecciona independientemente entre hidrógeno o a alifático C_{1-6};

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

R^8 es un grupo alifático C_{1-4};

cada R^{10} se selecciona independientemente entre R^{11}, $T\text{-}R^{11}$ o $V\text{-}T\text{-}R^{11}$;

cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$ o $-N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada; y

V es –O-, $-N(R^7)$-, -S-, -S(O)-, $-S(O)_2$-, -C(O)- o $-CO_2$-.

Los compuestos preferidos de III-A-aa son compuestos en los que:

Q es $–CH_2$-;

G se selecciona entre un piperidinilo opcionalmente sustituido, piperazinilo, morfolinilo, pirrolidinilo o $-NR^4R^5$;

R^1 es hidrógeno, halo o metilo;

R^2 es halo;

R^3 es hidrógeno;

R^4 es hidrógeno o un grupo alifático C_{1-6};

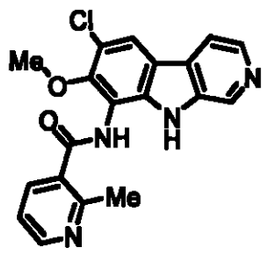
R^5 es alcoxi C_{1-6}, un grupo alifático C_{1-6} o hidroxialquilo C_{1-6};

cada R^{6b} se selecciona independientemente entre hidrógeno o un alifático C_{1-3}. Preferiblemente, cada R^{6b} es hidrógeno o metilo. Para los compuestos de

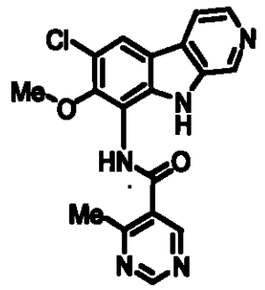
fórmula III-A-aa de cada una de las realizaciones anteriores, se prefiere la estereoquímica (*S*) en la posición tres del Anillo A morfolina.

Los ejemplos de compuestos específicos de formula I se muestra a continuación en las Tablas 3 y 4.

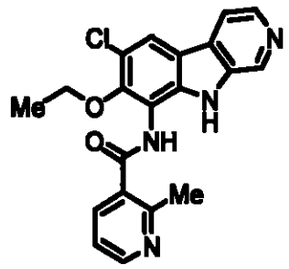
Tabla 3. Ejemplos específicos de compuestos de fórmula I



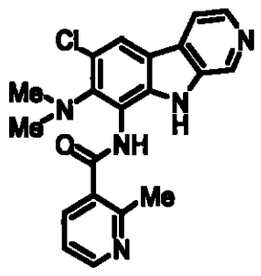
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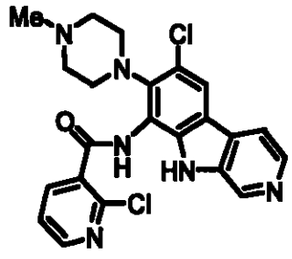
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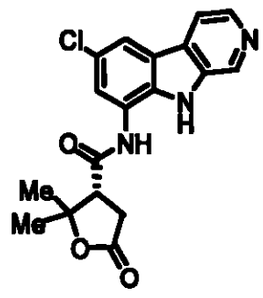
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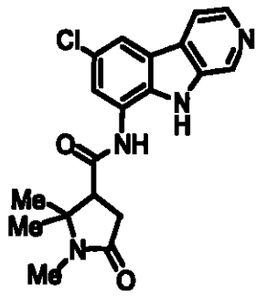
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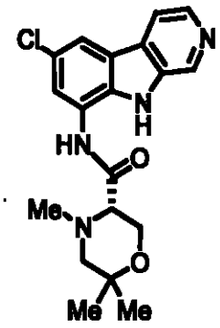
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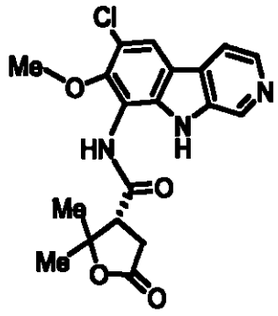
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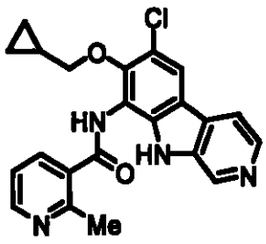
7



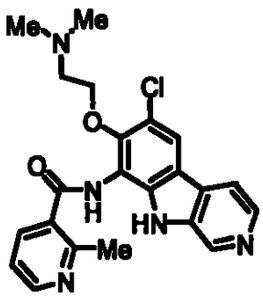
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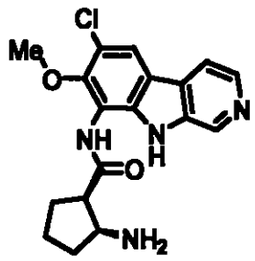
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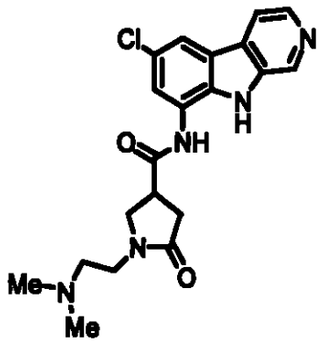
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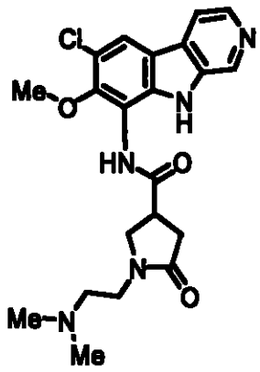
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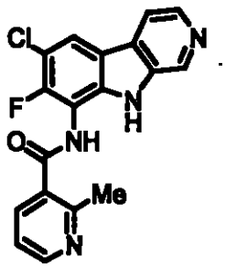
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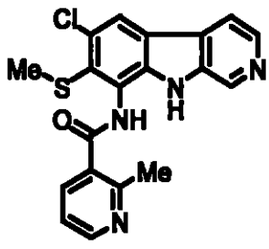
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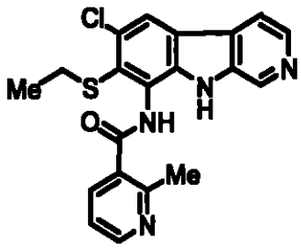
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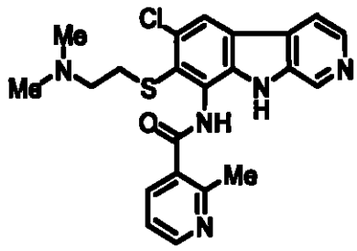
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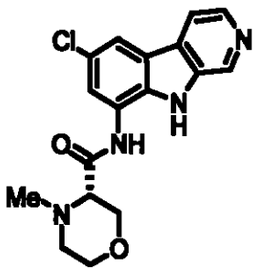
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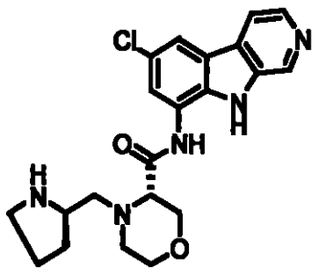
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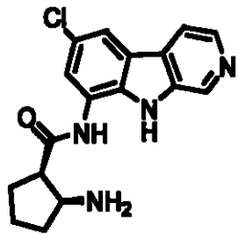
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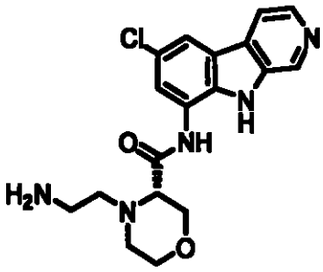
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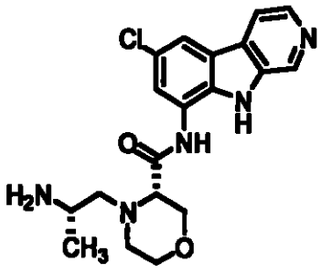
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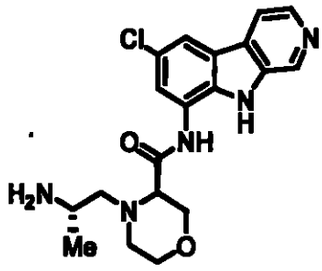
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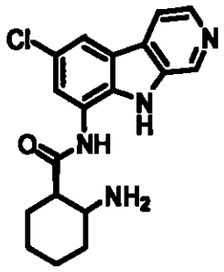
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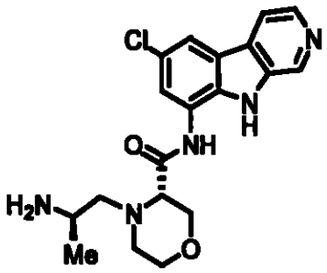
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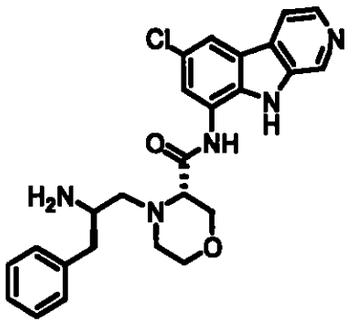
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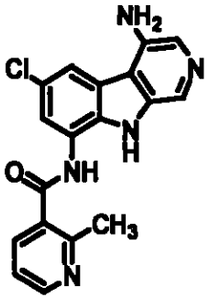
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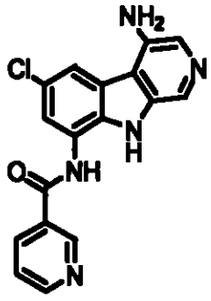
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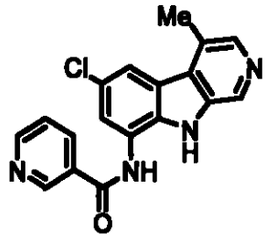
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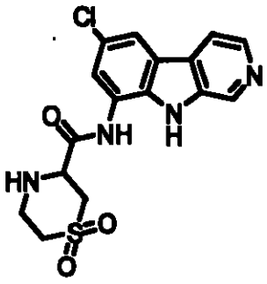
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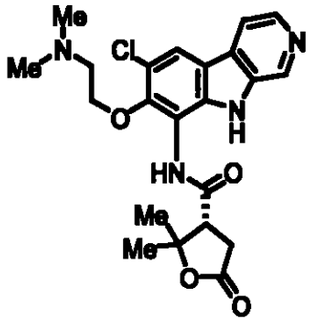
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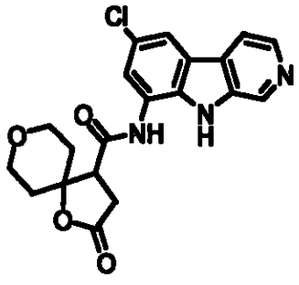
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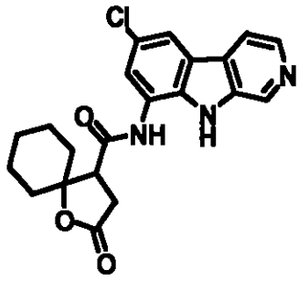
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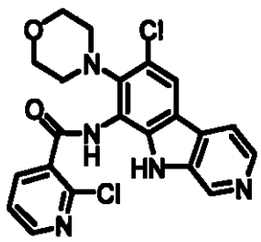
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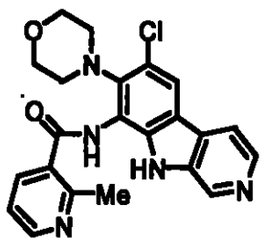
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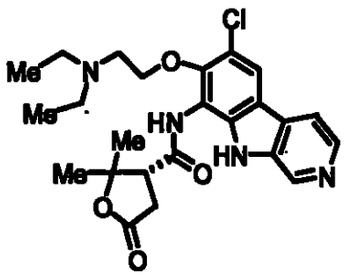
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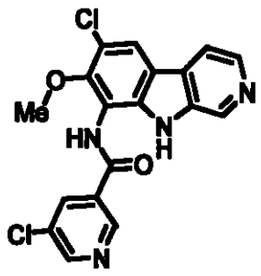
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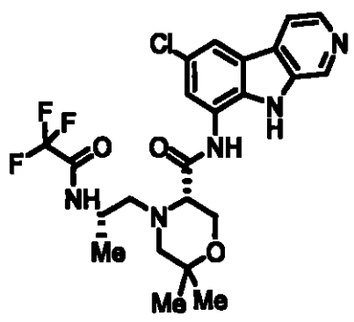
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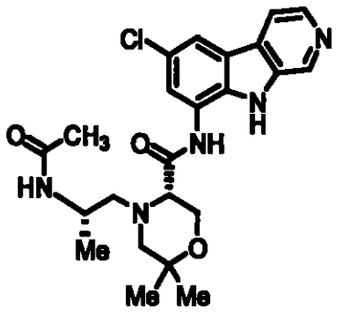
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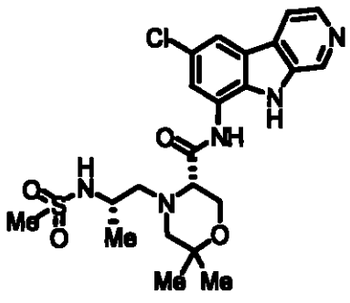
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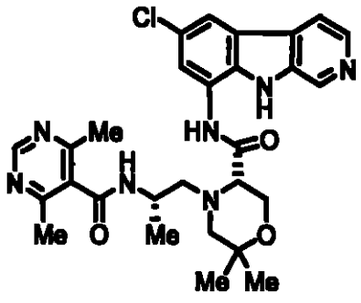
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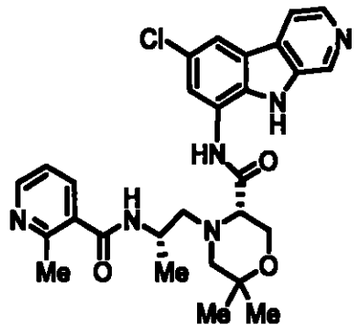
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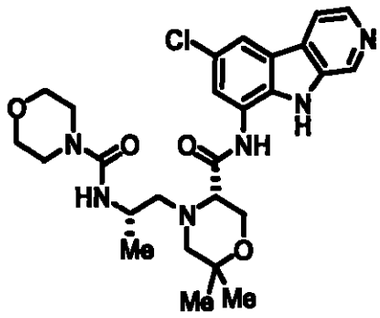
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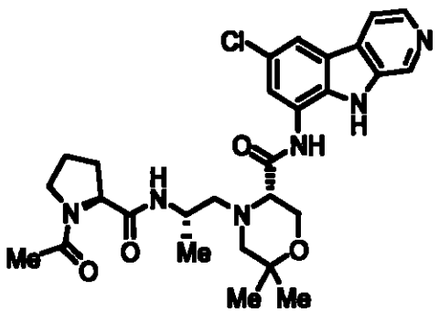
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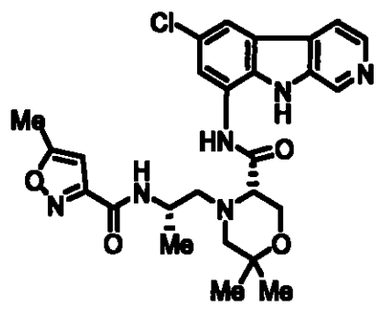
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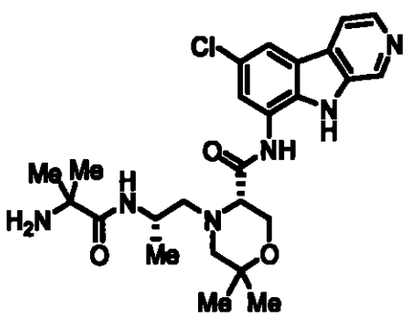
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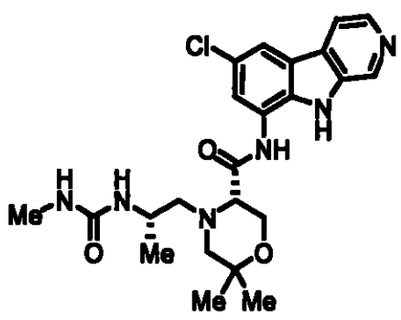
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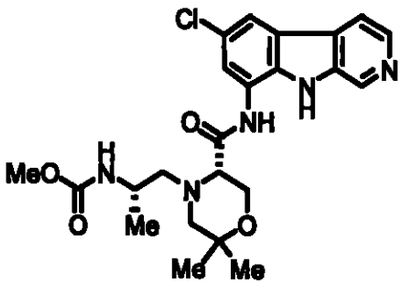
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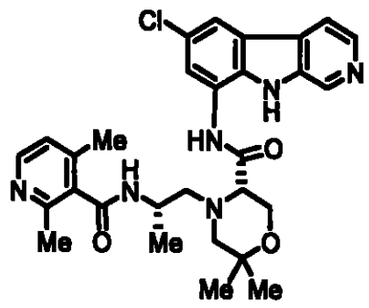
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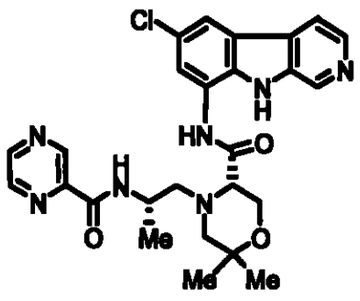
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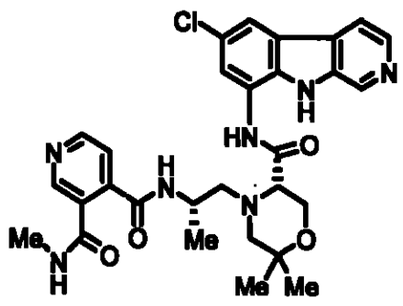
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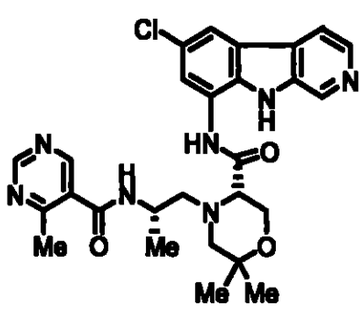
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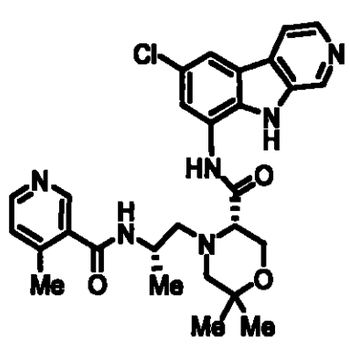
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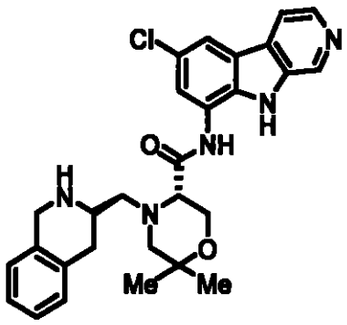
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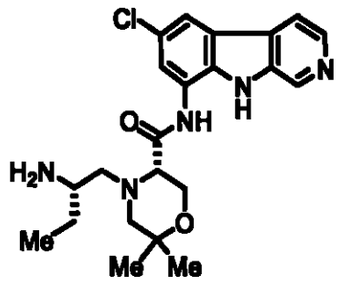
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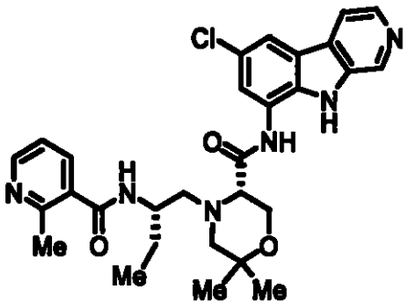
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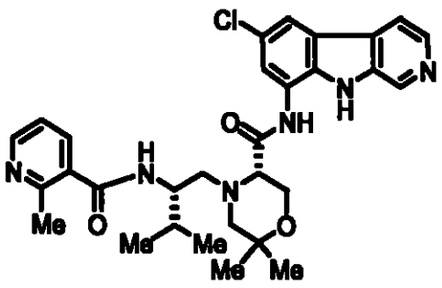
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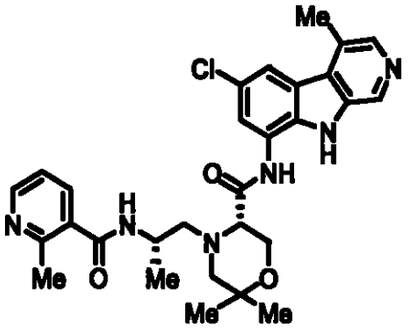
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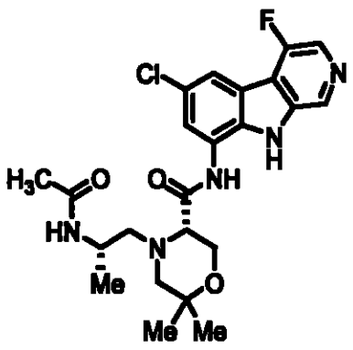
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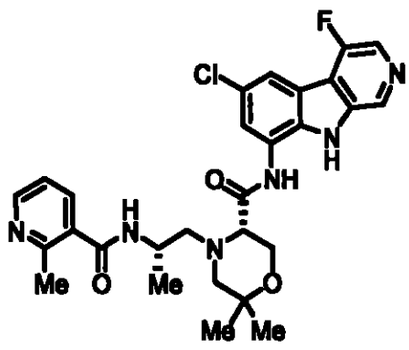
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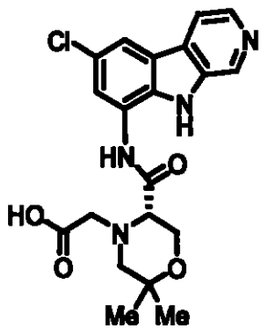
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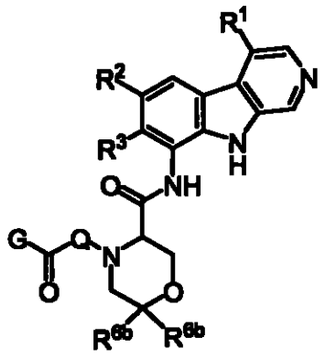
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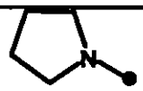
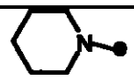
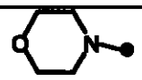
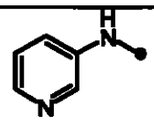
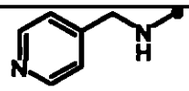
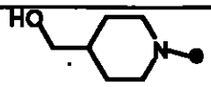
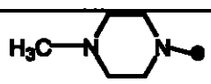
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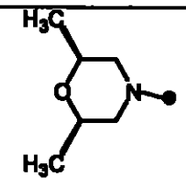
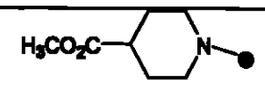
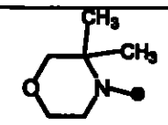
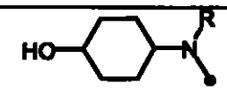
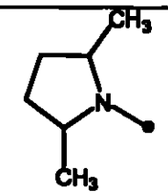
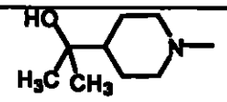
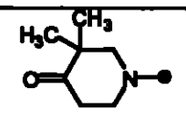
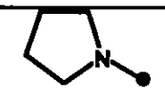
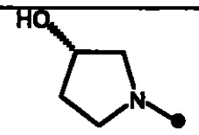
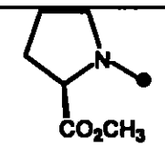
A continuación, la Tabla 4 muestra ejemplos específicos de compuestos de III-A-aa.

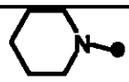
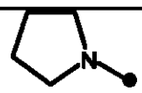
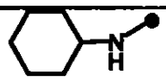
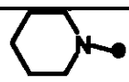
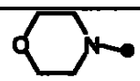
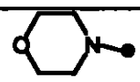
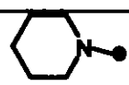
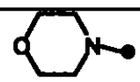
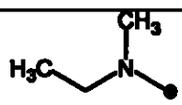
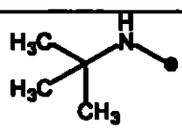
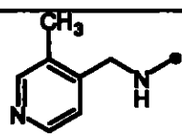
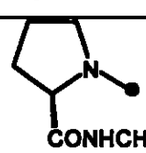
Tabla 4. Ejemplos específicos de compuestos de fórmula III-A-a

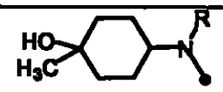
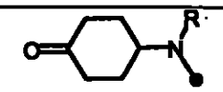
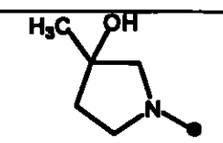
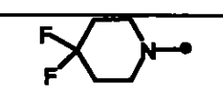
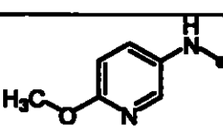
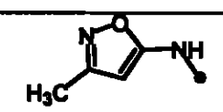
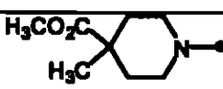
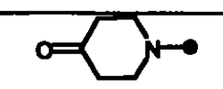


III-A-aa

Nº	R^1	R^{6b}/R^{6b}	Q	G
64	H	CH_3/CH_3	CH_2	
65	H	CH_3/CH_3	CH_2	
66	H	CH_3/CH_3	CH_2	
67	H	CH_3/CH_3	CH_2	$HO\text{-}CH_2CH_2N(CH_3)\text{-}$
68	H	CH_3/CH_3	CH_2	
69	H	CH_3/CH_3	CH_2	
70	H	CH_3/CH_3	CH_2	
71	H	CH_3/CH_3	CH_2	
72	H	CH_3/CH_3	CH_2	$Et_2N\text{-}$
73	H	CH_3/CH_3	CH_2	

N°	R^1	R^{6b}/R^{6b}	Q	G
74	H	CH_3/CH_3	CH_2	
75	H	CH_3/CH_3	CH_2	
76	H	CH_3/CH_3	CH_2	
77	H	CH_3/CH_3	CH_2	
78	H	CH_3/CH_3	CH_2	
79	H	CH_3/CH_3	CH_2	
80	H	CH_3/CH_3	CH_2	
81	CH_3	CH_3/CH_3	CH_2	
82	H	CH_3/CH_3	CH_2	
83	H	CH_3/CH_3	CH_2	

N°	R^1	R^{db}/R^{db}	Q	G
84	CH_3	H/H	CH_2	
85	CH_3	H/H	CH_2	
86	H	CH_3/CH_3	CH_2	
87	CH_3	CH_3/CH_3	CH_2	
88	CH_3	CH_3/CH_3	CH_2	
89	F	CH_3/CH_3	CH_2	
90	F	CH_3/CH_3	CH_2	
91	H	CH_3/CH_3	*R*-CH(CH_3)	
92	H	CH_3/CH_3	CH_2	
93	H	CH_3/CH_3	CH_2	
94	H	CH_3/CH_3	CH_2	Me_2N-
95	H	CH_3/CH_3	CH_2	(CH_3O)(CH_3)N-
96	H	CH_3/CH_3	CH_2	
97	H	CH_3/CH_3	CH_2	

Nº	R^1	R^{6b}/R^{6b}	Q	G
98	H	CH_3/CH_3	CH_2	
99	H	CH_3/CH_3	CH_2	
100	H	CH_3/CH_3	CH_2	
101	H	CH_3/CH_3	CH_2	
102	H	CH_3/CH_3	CH_2	
103	H	CH_3/CH_3	CH_2	
104	H	CH_3/CH_3	CH_2	
105	H	CH_3/CH_3	CH_2	
106	H	CH_3/CH_3	CH_2	

En base a sus propiedades inhibidoras de IκB quinasa y otras propiedades farmacológicas, los ejemplos de compuesto números 1-30, 39-62 y 64-106 son preferidos. Son más preferidos los ejemplos de compuesto números 1, 2, 7, 10, 11, 13, 16, 17, 19-27, 39-62, 64-106.

Los compuestos de la presente invención pueden administrarse a seres humanos u otros mamíferos por una diversidad de vías, incluyendo formas de dosificación oral e inyecciones (intravenosa, intramuscular, intraperitoneal, subcutánea, y similares). Otras numerosas formas de dosificación que contienen

compuestos de la invención pueden formularse fácilmente por un especialista el la técnica, utilizando los excipientes (o vehículos) farmacéuticos apropiados como se define a continuación.

Los ejemplos de excipientes (o vehículos) farmacéuticamente aceptables y métodos de fabricación para diversas composiciones pueden encontrarse en A. Gennaro (ed.), Remington: The Science and Practice of Pharmacy, 20ª Edición, Lippincott Williams & Wilkins, Baltimore, MD (2000), que se incorpora en su totalidad como referencia en este documento. Los excipientes (o vehículos) farmacéuticamente aceptables incluyen agentes aromatizantes, colorantes o pigmentos de calidad farmacéutica, disolventes, co-disolventes, sistemas tampón, tensioactivos, conservantes, agentes edulcorantes, agentes de viscosidad, cargas, lubricantes, emolientes, disgregantes, aglutinantes y resinas.

Pueden usarse agentes aromatizantes convencionales, tales como los descritos en Remington's Pharmaceutical Sciences, 18ª Ed., Mack Publishing Co., páginas 1288-1300 (1990), que se incorpora en su totalidad como referencia en este documento. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 2% de agentes aromatizantes.

También pueden usarse colorantes y/o pigmentos convencionales, tales como los descritos en the Handbook of Pharmaceutical Excipients, por the American Pharmaceutical Association & the Pharmaceutical Society of Great Britain, páginas 81-90 (1986), que se incorpora en su totalidad como referencia en este documento. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 2% de colorantes y/o pigmentos.

Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0,1 a un 99,9% de disolvente o disolventes. Un disolvente preferido es agua. Los co-disolventes preferidos incluyen etanol, glicerina, propilenglicol, polietilenglicol, y similares. Las composiciones farmacéuticas de la invención pueden incluir de aproximadamente un 0 a un 50% de co-disolventes.

Los sistemas tampón preferidos incluyen ácido acético, bórico, carbónico, fosfórico, succínico, maleico, tartárico, cítrico, acético, benzoico, láctico, glicérico, glucónico, glutárico y glutámico y sus sales de sodio, potasio y amonio. Los tampones particularmente preferidos son ácido fosfórico, tartárico, cítrico y acético y sales de los mismos. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 5% de un tampón.

Los tensioactivos preferidos incluyen ésteres de ácidos grasos de polioxietilensorbitano, monoalquil éteres de polioxietileno, monoésteres de sacarosa y ésteres y éteres de lanolino, sales alquilsulfato y sales de sodio, potasio y amonio de ácidos grasos. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 2% de tensioactivos.

Los conservantes preferidos incluyen fenol, ésteres de alquilo de ácido parahidroxibenzoico, ácido o-fenilfenol benzoico y sales del mismo, ácido bórico y sales del mismo, ácido sórbico y sales del mismo, clorobutanol, alcohol bencílico, timerosal, acetato y nitrato fenilmercúrico, nitromersol, cloruro de benzalconio, cloruro de cetilpiridinio, metil parabeno y propil parabeno. Los conservantes particularmente preferidos son las sales de ácido benzoico, cloruro de cetilpiridinio, metil parabeno y propil parabeno. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 2% de conservantes.

Los edulcorantes preferidos incluyen sacarosa, glucosa, sacarina, sorbitol, manitol y aspartamo. Los edulcorantes particularmente preferidos con sacarosa y sacarina. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 5% de edulcorantes.

Los agentes de viscosidad preferidos incluyen metilcelulosa, carboximetilcelulosa sódica, hidroxipropil-metilcelulosa, hidroxipropilcelulosa, alginato sódico, carbómero, povidona, goma arábiga, goma guar, goma de xantano y tragacanto. Los agentes de viscosidad particularmente preferidos son

metilcelulosa, carbómero, goma de xantano, goma guar, povidona, carboximetilcelulosa sódica, y silicato de magnesio y aluminio. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 5% de agentes de viscosidad.

Las cargas preferidas incluyen lactosa, manitol, sorbitol, fosfato cálcico tribásico, fosfato cálcico dibásico, azúcar comprimible, almidón, sulfato cálcico, dextro celulosa y celulosa microcristalina. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 75% de cargas.

Los lubricantes/emolientes preferidos incluyen estearato de magnesio, ácido esteárico y talco. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 7%, preferiblemente, de aproximadamente un 1 a un 5% de lubricantes/emolientes.

Los disgregantes preferidos incluyen almidón, glicolato sódico de almidón, crospovidona y croscarmelosa sódica y celulosa microcristalina. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 20%, preferiblemente, de aproximadamente un 4 a un 15% de disgregantes.

Los aglutinantes preferidos incluyen goma arábiga, tragacanto, hidroxipropilcelulosa, almidón pregelatinizado, gelatina, povidona, hidroxipropilcelulosa, hidroxipropilmetilcelulosa, metilcelulosa, soluciones de azúcar, tales como sacarosa y sorbitol, y etilcelulosa. Las composiciones farmacéuticas de la invención generalmente contienen de aproximadamente un 0 a un 12%, preferiblemente, de aproximadamente un 1 a un 10% de aglutinantes.

Los agentes adicionales conocidos por un formulador especialista pueden combinarse con los compuestos de la invención para crear una forma de dosificación sencilla. Como alternativa, pueden administrarse por separado los agentes adicionales a un mamífero como parte de una forma de dosificación múltiple.

Para preparar composiciones farmacéuticas que contienen los compuestos de la invención, los excipientes o vehículos inertes farmacéuticamente aceptables pueden ser sólidos o líquidos. Las preparaciones en forma sólida incluyen polvos, comprimidos, gránulos dispersables, cápsulas, obleas y supositorios. Generalmente, los polvos y comprimidos pueden estar compuestos de aproximadamente un 5 a un 95 por ciento en peso de ingrediente activo. Los vehículos sólidos apropiados son conocidos en la técnica, por ejemplo, carbonato de magnesio, estearato de magnesio, talco, azúcar y lactosa. Los comprimidos, polvos, obleas y cápsulas pueden usarse como formas de dosificación sólidas apropiadas para administración oral. Los ejemplos de vehículos farmacéuticamente aceptables y métodos de fabricación para diversas composiciones pueden encontrarse en Remington's Pharmaceutical Sciences, 18ª Ed., Mack Publishing Co. (1990).

Las preparaciones en forma líquida incluyen soluciones, suspensiones y emulsiones. Las preparaciones en forma líquida habituales incluyen agua y soluciones de agua-propilenglicol para inyección parenteral o adición de edulcorantes y opacificantes para soluciones, suspensiones y emulsiones orales. Las preparaciones en forma líquida también pueden incluir soluciones para administración intranasal.

Las preparaciones en aerosol apropiadas para inhalación incluyen soluciones y sólidos en forma de polvo, que pueden combinarse con un vehículo farmacéuticamente aceptable, tal como un gas comprimido inerte (por ejemplo, nitrógeno).

También se incluyen preparaciones en forma sólida que pueden convertirse, poco antes de su uso, en preparaciones en forma líquida para administración oral o parenteral. Dichas formas líquidas incluyen soluciones, suspensiones y emulsiones.

Los compuestos de la invención también pueden suministrarse por vía transdérmica. Las composiciones transdérmicas pueden tomar forma de cremas,

lociones, aerosoles y emulsiones y pueden incluirse en un parche transdérmico de un matriz o tipo reserva como es convencional en la técnica para este propósito.

El modo preferido de administración de los compuestos de la invención es oral. Preferiblemente, la preparación farmacéutica está en una forma de dosificación unitaria. En dicha forma, la preparación se subdivide en dosis unitarias de tamaño apropiado que contienen cantidades apropiadas del componente activo, por ejemplo, una cantidad eficaz para conseguir el propósito deseado.

La cantidad de ingrediente (compuesto) activo en una dosis unitaria de preparación puede variarse o ajustarse de aproximadamente 0,01 a 4.000 mg, preferiblemente, de aproximadamente 0,01 a 1.000 mg, más preferiblemente, de aproximadamente 0,01 a 500 mg, e incluso más preferiblemente, de aproximadamente 0,01 a 250 mg, de acuerdo con la aplicación particular. Un régimen de dosificación diaria recomendado típico para administración oral habitualmente variará de aproximadamente 0,02 a 2.000 mg/día, en una a cuatro dosis divididas. Por comodidad, la dosificación diaria total puede dividirse y administrarse en partes durante el día según se requiera. Típicamente, las composiciones farmacéuticas de la invención se administrarán de aproximadamente 1 a 5 veces por día, o como alternativa, en forma de infusión continua. Dicha administración puede usarse para terapia crónica o aguda. La cantidad de ingrediente activo que puede combinarse con materiales excipientes o de vehículo para producir una forma de dosificación sencilla variará dependiendo del hospedador tratado y el modo particular de administración. Una preparación típica habitualmente contendrá de aproximadamente un 5 a un 95% de compuesto activo (p/p). Preferiblemente, dichas preparaciones contendrán de aproximadamente un 20 a un 80 % en peso de compuesto activo.

Los excipientes o vehículos farmacéuticamente aceptables empleados junto con los compuestos de la invención se usan a una concentración suficiente para proporcionar una relación tamaño a dosificación práctica. Los excipientes o

vehículos farmacéuticamente aceptables, en total, pueden comprender de aproximadamente un 0,1 a un 99,9% en peso de las composiciones farmacéuticas de la invención, preferiblemente, de aproximadamente un 20 a un 80% en peso.

Después de la mejora de una afección de un paciente, puede administrarse una dosis de mantenimiento de un compuesto, composición o combinación de la invención, si es aplicable. Posteriormente, la dosificación o frecuencia de administración, o ambas, pueden reducirse, en función de los síntomas, a un nivel al que se retenga la afección mejorada. Cuando los síntomas se han aliviado al nivel deseado, debe terminar el tratamiento. Los pacientes pueden, sin embargo, necesitar tratamiento intermitente en una base de larga duración tras cualquier reaparición de síntomas de enfermedad.

Los regímenes de dosificación o tratamiento específicos para cualquier paciente particular pueden variarse y dependerán de una diversidad de factores, incluyendo la actividad del compuesto específico empleado, la edad, peso corporal, estado de salud general, sexo y dieta del paciente, el tiempo de administración, la velocidad de excreción, la combinación de fármaco específica, la gravedad y transcurso de los síntomas que se están tratando, la disposición del paciente a la afección que se está tratando y el juicio del médico que está tratando. La determinación del régimen de dosificación apropiado para una situación particular pertenece a la especialidad de la técnica. La cantidad y frecuencia de la administración de compuestos de la invención o sus sales farmacéuticamente aceptables pueden regularse de acuerdo con el juicio del médico que está atendiendo, en base a los factores mencionados anteriormente. Como un especialista en la técnica apreciará, pueden necesitarse dosis más bajas o más altas que las mencionadas anteriormente.

Se entiende que los compuestos de la invención proporcionan tratamiento eficaz de una diversidad de enfermedades, síntomas y trastornos, particularmente, aquellos que son de naturaleza inflamatoria o relacionada con el sistema inmune, incluyendo un tiempo razonable de aparición después de la administración, y una

duración razonable después de la administración. Aunque los alimentos, dieta, afecciones pre-existentes, alcohol y otras afecciones sistémicas podrían prolongar el tiempo de retardo de un fármaco de la invención para funcionar después de su administración, se entiende que las dosificaciones óptimas darán como resultado un tratamiento de fármaco eficaz en y durante una cantidad de tiempo razonable.

Los compuestos de la invención pueden existir en formas no solvatada así como solvatada, incluyendo formas hidratadas. En general, las formas solvatadas, con disolventes farmacéuticamente aceptables, tales como agua, etanol y similares, son equivalentes a las formas no solvatadas para propósitos de esta invención.

Los compuestos de la invención pueden formar sales farmacéuticamente aceptables con ácidos orgánicos e inorgánicos. Los ejemplos de ácidos apropiados para la formación de sales son ácido clorhídrico, sulfúrico, fosfórico, acético, cítrico, malónico, salicílico, málico, fumárico, succínico, ascórbico, maleico, metanosulfónico y otros ácidos minerales y carboxílicos bien conocidos por los especialistas en la técnica. Las sales se preparan poniendo en contacto las formas de base libre con una cantidad suficiente del ácido deseado para producir una sal de un modo convencional. Las formas de base libre pueden regenerarse tratando la sal con una solución básica de dilución acuosa apropiada, tal como hidróxido sódico, carbonato potásico, bicarbonato amónico o sódico de disolución acuosa. Las formas de base libre pueden diferir algo de sus formas salinas respectivas en ciertas propiedades físicas, tales como solubilidad en disolventes polares, pero las sales son equivalentes de otro modo a sus formas respectivas de base libre para propósitos de la invención.

A causa de sus propiedades farmacológicas, los compuestos de acuerdo con la invención son apropiados para el tratamiento y terapia profilácticos de enfermedades, trastornos y síntomas que implican actividad aumentada de IkB kinasa. Estos incluyen, por ejemplo, inflamación articular (por ejemplo, artritis reumatoide (RA), espondilitis reumatoide, artritis gotosa, artritis traumática, artritis

por rubéola, artritis psoriática, osteoartritis, y otras afecciones artríticas), sinovitis aguda, tuberculosis, aterosclerosis, degeneración muscular, caquexia, síndrome de Reiter, endotoxaemia, sepsis, choque séptico, choque endotóxico, sepsis por gram negativas, gota, síndrome de choque tóxico, enfermedades inflamatorias pulmonares (por ejemplo, asma, síndrome de distrés respiratorio agudo, enfermedad pulmonar obstructiva crónica, silicosis, sarcoidosis pulmonar, y similares), enfermedades de resorción ósea, lesiones por reperfusión, carcinosis, leucemia, sarcomas, tumores de nódulo linfático, carcinosis de piel, linfoma, apoptosis, reacción de injerto contra hospedador, enfermedad de injerto contra hospedador (GVHD), rechazo de aloinjerto y lepra.

Además, los compuestos de la invención pueden usarse en el tratamiento de enfermedades, síntomas y trastornos relacionados con el sistema inmune, por ejemplo, infecciones, tales como infecciones víricas (por ejemplo, VIH, citomegalovirus (CMV), gripe, adenovirus, el grupo de virus de Herpes, y similares), infecciones parasitarias (por ejemplo, malaria, tal como malaria cerebral), e infecciones por levaduras y fúngicas (por ejemplo, meningitis fúngica). Además, los compuestos de la invención pueden ser útiles para tratar fiebre y mialgias debidas a infección, síndrome de inmunodeficiencia adquirida (SIDA), complejo relacionado con SIDA (ARC), caquexia debida a infección o malignidad, caquexia debida a SIDA o cáncer, formación de tejido queloide o cicatrizado, piresis, diabetes, y enfermedades inflamatorias del intestino (IBD) (por ejemplo, enfermedad de Crohn y colitis ulcerativa). Los compuestos de la invención también son útiles en el tratamiento de enfermedades o lesiones del cerebro en las que se ha implicado la sobre-expresión de TNF-a, tales como esclerosis múltiple (MS), lesión cerebral isquémica, por ejemplo, infarto cerebral (apoplejía) y traumatismo craneal. Los compuestos de la invención también son útiles en el tratamiento de psoriasis, enfermedad de Alzheimer, trastornos carcinomatosos (potenciación de terapias citotóxicas), infarto cardiaco, enfermedad pulmonar obstructiva crónica (COPD) y síndrome de distrés respiratorio agudo (ARDS).

En una realización, los compuestos de esta invención son útiles para tratar cáncer, especialmente para tratar cánceres donde la actividad de IKK es anormalmente alta. Los tipos de cáncer que pueden tratarse incluyen linfoma, tal como difuso de células B grandes, primario de células B mediastinales, y de células del manto; mieloma múltiple; metástasis ósea osteolítica; cáncer de células escamosas de la cabeza y el cuello; cáncer de próstata; cáncer pancreático y cáncer de pulmón mastocístico. En una realización, los compuestos son útiles para linfoma ABC. Para el tratamiento de cáncer, los compuestos pueden usarse en forma de agente sencillo o en combinación con otros agentes que se sabe que son útiles para el tratamiento de cáncer. Los ejemplos de dichos agentes distintos incluyen bortezomib; capecitibina; gemcitabina; irinotecano; fludarabina; 5-fluorouracilo o 5-fluorouracilo/leucovorina; taxanos, incluyendo, por ejemplo, paclitaxel y docetaxel; agentes de platino, incluyendo, por ejemplo, cisplatino, carboplatino, y oxaliplatino; antraciclinas, incluyendo, por ejemplo, doxorrubicina y doxorrubicina liposomal pegilada; mitoxantrona; dexametasona; vincristina; etopósido; prednisona; talidomida; Herceptina; temozolomida; y agentes alquilantes tales como melfalán, clorambucilo, y ciclofosfamida.

Los compuestos de fórmula (I) son especialmente útiles para tratar enfermedades, trastornos y síntomas inflamatorios y relacionados con el sistema inmune, más especialmente, las inflamatorias tales como RA, asma, IBD, psoriasis, COPD y MS. Se apreciará que los presentes compuestos son útiles para tratar enfermedades, trastornos o síntomas relacionados con la actividad de NF-?B, TNF-a, y otras enzimas en vías donde se sabe que IKK modula la actividad.

Los compuestos de esta invención también son útiles para tratar una enfermedad, síntoma o trastorno asociado con el hueso en el que hay un déficit o deficiencia de hueso – como resultado de formación de hueso nuevo disminuida o un aumento en la resorción ósea o una combinación de ambos. Los ejemplos específicos incluyen osteoporosis, enfermedad periodontal, osteomielitis, artritis reumatoide, pérdida de asepsia en la articulación y lesiones osteolíticas

(típicamente relacionadas con cáncer). Se sabe que la artritis reumatoide, que está caracterizada por inflamación de las articulaciones, también está asociada a la destrucción del cartílago y el hueso. Además, se ha informado de que un inhibidor de IKK proporcionó inhibición de pérdida de cartílago y ósea en un modelo murino de artritis inducida por colágeno. Véase, McIntyre et al., *Arthritis & Rheumatism* (2003), 48(9), 2652-2659.

La osteoporosis es un amplio término aplicado a varias enfermedades distintas en las que hay masa ósea disminuida. Estas incluyen osteoporosis primaria (por ejemplo, osteoporosis post-menopáusica, osteoporosis senil y osteoporosis juvenil) y osteoporosis secundaria. Los ejemplos de osteoporosis secundaria serían aquellos asociados a enfermedades crónicas (por ejemplo, enfermedad renal crónica, insuficiencia hepática, mala absorción gastrointestinal, inmobilización crónica y enfermedades inflamatorias crónicas, incluyendo artritis reumatoide, osteoartritis, enfermedad periodontal y pérdida de asepsis de articulación prostética), enfermedades relacionadas con disfunción endocrina (por ejemplo, diabetes, hipertiroidismo, hiperparatiroidismo, hipogonadismo e hipopituitarismo), síntomas relacionados con fármacos y sustancias (por ejemplo, corticosteroides, heparina, anticonvulsivos, alcohol e inmunosupresores), y trastornos hematológicos (por ejemplo, enfermedad metastásica, mieloma, leucemia, enfermedad de gaucher y anemia). Se ha informado de que la inhibición de IkB directamente o la vía de NF-kB indirectamente es útil para el tratamiento de osteoporosis y osteoartritis. Véanse, por ejemplo, las solicitudes PCT Nº WO 2003104219, WO 2003103658, WO 2003029242, WO 2003065972, y WO 9965495, Por consiguiente, esta invención también proporciona un método para tratar o prevenir la pérdida ósea en un paciente en necesidad del mismo, que comprende administrar al paciente un compuesto de esta invención. También se proporciona un método para generar formación ósea en un paciente que comprende administrar un compuesto de esta invención.

Otra realización de la invención proporciona un método para inhibir la activación de la expresión de genes dependiente de NF-?B asociada a la inhibición de la actividad catalítica de IKK y/o fosforilación de I?B, que comprende administrar a un paciente en necesidad del mismo una cantidad del compuesto de acuerdo con la reivindicación 1 o la sal farmacéuticamente aceptable o solvato del mismo, o una composición farmacéutica del mismo, que es eficaz para inhibir la actividad catalítica de IKK y/o la fosforilación de I?B, inhibiendo de este modo la activación de la expresión de genes dependiente de NF-?B.

En una realización de la invención, se proporciona un método para tratar o prevenir un trastorno, síntoma o enfermedad inflamatoria o fisiológica relacionada con el sistema inmune en un paciente en necesidad de dicho tratamiento, que comprende administrar al paciente una cantidad de al menos un compuesto de acuerdo con la reivindicación 1, o la sal farmacéuticamente aceptable o solvato del mismo, o una composición farmacéutica del mismo, que es eficaz para tratar o prevenir el trastorno, síntoma o enfermedad inflamatoria o fisiológica relacionada con el sistema inmune. Preferiblemente, la enfermedad, trastorno o síntoma inflamatorio es artritis reumatoide, asma, psoriasis, artritis psoriática, enfermedad pulmonar obstructiva crónica (COPD), enfermedad inflamatoria del intestino o esclerosis múltiple.

La invención comprende un compuesto que tiene fórmula (I), un método para fabricar un compuesto de la invención, un método para fabricar una composición farmacéutica a partir de al menos un compuesto de la invención y al menos un vehículo o excipiente farmacéuticamente aceptable, y un método para usar uno o más compuestos de la invención para tratar una diversidad de trastornos, síntomas y enfermedades, particularmente las que son de naturaleza inflamatoria o relacionada con el sistema inmune. Los compuestos de la invención y sus sales farmacéuticamente aceptables y composiciones neutras pueden formularse junto con un excipiente o vehículo farmacéuticamente aceptable y la composición resultante puede administrarse *in vivo* a mamíferos, tales como

primates, por ejemplo, chimpancés y seres humanos (por ejemplo, hombres y mujeres) y animales (por ejemplo, perros, gatos, vacas, caballos, y similares), para tratar una diversidad de trastornos, síntomas y enfermedades. Además, los compuestos de la invención pueden usarse para preparar un medicamento que es útil para tratar una diversidad de trastornos, síntomas y enfermedades.

Aunque se pueden usar uno o más compuestos de la invención en una aplicación de monoterapia para tratar un trastorno, enfermedad o síntoma, también pueden usarse en terapia de combinación, en la que el uso de un compuesto de la invención o composición (agente terapéutico) se combina con el uso de uno o más agentes terapéuticos diferentes para tratar el mismo y/o diferentes tipos de trastornos, síntomas y enfermedades. La terapia de combinación incluye la administración de los agentes terapéuticos de manera concurrente o secuencial. Como alternativa, los agentes terapéuticos pueden combinarse en una composición que se administra al paciente.

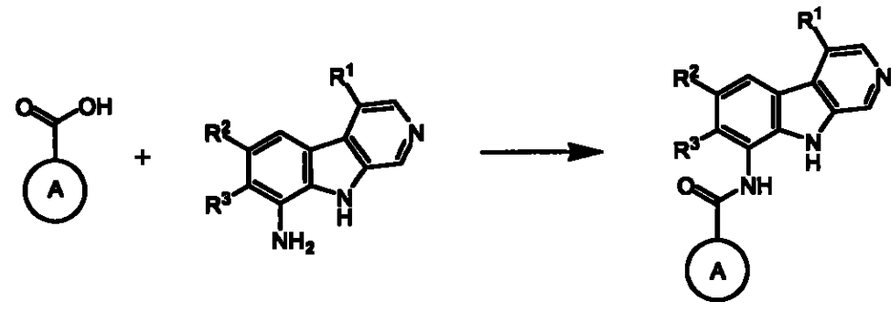
En una realización, los compuestos de esta invención se usan en combinación con otros agentes terapéuticos, tales como inhibidores de IKK, otros agentes útiles en el tratamiento de afecciones asociadas a NF-?B y TNF-a, y agentes útiles para tratar otros trastornos, síntomas y enfermedades. En particular, los agentes que inducen apoptosis, tales como agentes que interrumpen el ciclo celular o la función mitocondrial son útiles en combinación con los inhibidores de IKK de esta invención. Los agentes ejemplares para combinación con los inhibidores de IKK incluyen agentes antiproliferativos (por ejemplo, metotrexato) y los agentes descritos en la Publicación de Solicitud de Patente de Estados Unidos Nº US2003/0022898, página 14, párrafo [0173-0174], que se incorpora en este documento en su totalidad. En algunas realizaciones, un compuesto de la invención se administra junto con un agente terapéutico seleccionado entre el grupo compuesto por agentes citotóxicos, radioterapia, e inmunoterapia. Los ejemplos no limitantes de agentes citotóxicos apropiados para su uso en combinación con los inhibidores de IKK de la invención incluyen capecitibina;

gemcitabina; irinotecano; fludarabina; 5-fluorouracilo o 5-fluorouracilo/leucovorina; taxanos, incluyendo, por ejemplo, paclitaxel y docetaxel; agentes de platino, incluyendo, por ejemplo, cisplatino, carboplatino, y oxaliplatino; antraciclinas, incluyendo, por ejemplo, doxorrubicina y doxorubicina liposomal pegilada; mitoxantrona; dexametasona; vincristina; etopósido; prednisona; talidomida; herceptina; temozolomida; y agentes alquilantes tales como melfalán, clorambucilo, y ciclofosfamida. Se entiende que pueden asumirse otras combinaciones mientras que permanezcan en el alcance de la invención.

Otro aspecto más de esta invención es proporcionar un kit que comprenda recipientes separados en un envase único, donde los compuestos farmacéuticos de la invención, composiciones y/o sales de los mismos se usan en combinación con vehículos farmacéuticamente aceptables para tratar trastornos, síntomas y enfermedades donde IkB quinasa juega un papel.

Los compuestos de esta invención pueden prepararse por métodos conocidos por los especialistas en la técnica para compuestos análogos, como se ilustra por los esquemas generales a continuación, y como referencia a los ejemplos preparativos mostrados a continuación.

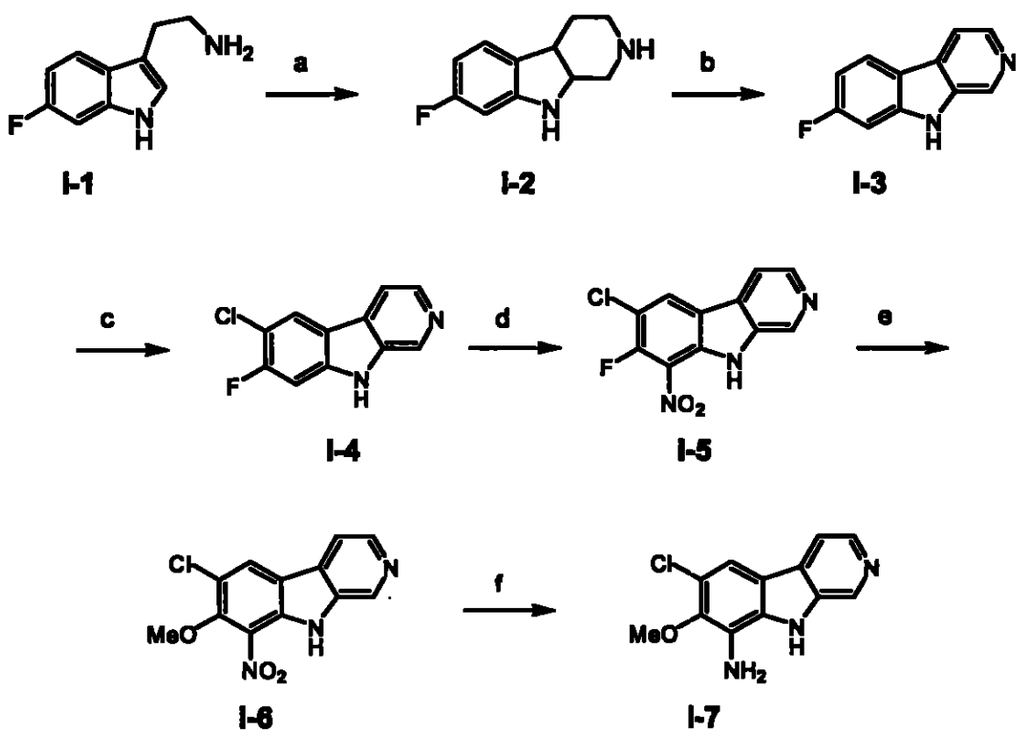
Esquema I



1a 2a I

El Esquema I anterior muestra una vía general para obtener compuestos de fórmula I. Un Anillo A ácido carboxílico **1a** puede acoplarse con la amino beta-carbolina deseada **2a** para proporcionar I. Muchos intermedios **1a** que son útiles para preparar compuestos de esta invención están fácilmente disponibles a partir de materiales de partida y métodos químicos conocidos, especialmente en vista de los ejemplos sintéticos detallados en este documento. Los Esquemas II-IV describen vías para fabricar diversos intermedios de β-carbolina **2a**.

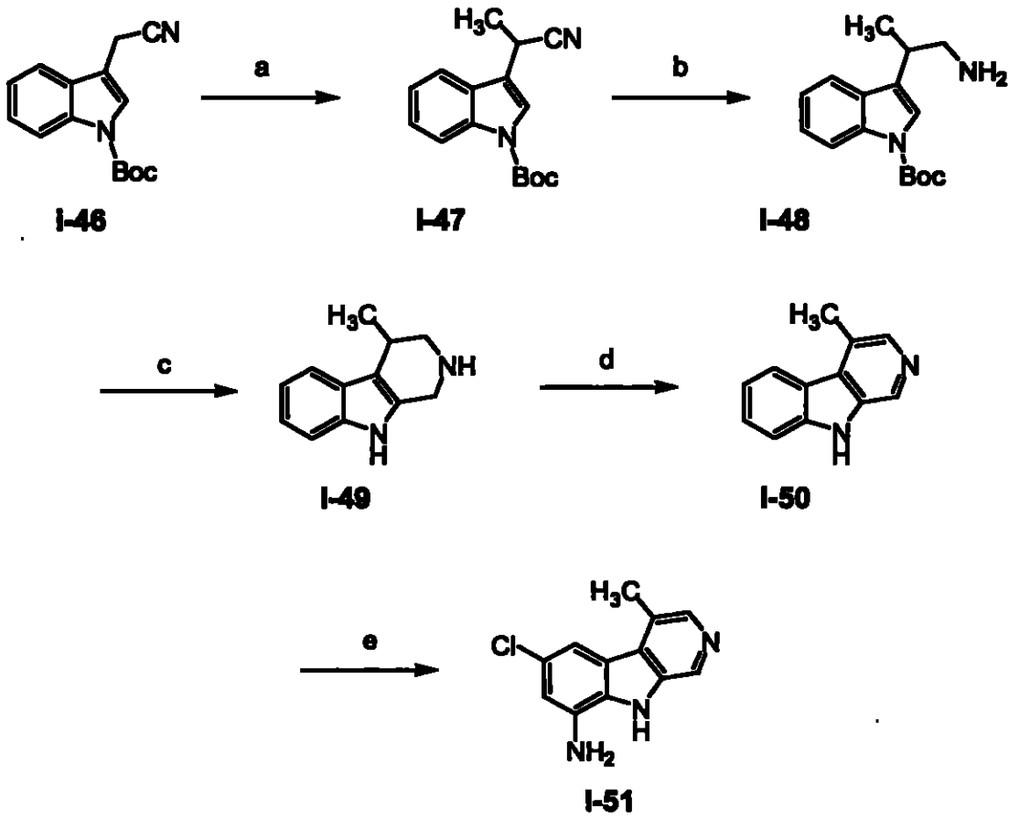
Esquema II



Etapas: (a) (i) $HCOCO_2H$ (ii) HCl (b) Pd/C (c) NCS (d) $NaNO_2$ (e) NaOMe (f) Pt/H_2

El Esquema II anterior muestra una vía para fabricar un resto beta-carbolina en el que R^1 es hidrógeno, R^2 es cloro y R^3 es alcoxi. Mientras que el esquema es ejemplifica para que R^3 sea metoxi, se apreciará por un especialista en la técnica que las beta-carbolinas que tienen otros grupos alcoxi R^3 pueden obtenerse reemplazando NaOMe en la etapa (e) por otros alcóxidos sódicos o metálicos.

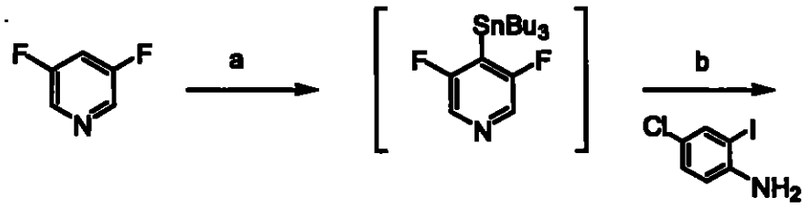
Esquema III



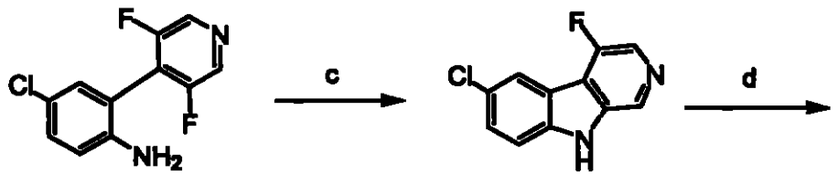
Etapas: (a) NaHMDS, MeI (b) H_2, Níquel Raney, PtO (c) i. TFA ii. $CHOCO_2H$ iii. HCl (d) Pd/C, xilenos, 160°C (e) i. NCS, HCl 1 N ii. $NaNO_2$, TFA iii. H_2, Pt

El Esquema III anterior muestra una vía para preparar un intermedio de beta-carbolina en el que R^1 es un alquilo tal como metilo, R^2 es un halo tal como cloro y R^3 es hidrógeno. Un especialista en la técnica entenderá cómo puede modificarse el esquema anterior para obtener un grupo alquilo R^1 diferente de metilo o un grupo halo R^2 diferente de cloro.

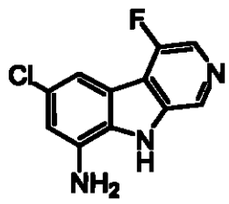
Esquema IV



I-63



I-64 I-65

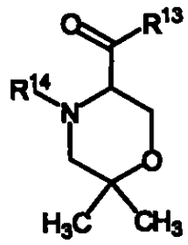


I-66

Etapas: (a) i. LDA, THF, -78C, ii. $SnBu_3Cl$ (b) $Pd(PPh_3)_2Cl_2$ al 5%, CuI al 10%, DMF, reflujo durante una noche (c) 3 equiv. de NHMDS, THF, ta durante una noche (d) i. TFA/$NaNO_3$, ii. $NaHCO_3$ saturado, iii. Pt(S) al 5%, NH_4O_2CH

El Esquema IV anterior muestra una vía para fabricar un intermedio de beta-carbolina en el que R^1 es flúor, R^2 es cloro y R^3 es hidrógeno. Se apreciará que una sencilla modificación de este esquema permitirá la preparación de otros intermedios. Por ejemplo, puede introducirse otro grupo R^2 reemplazando la 4-cloro-2-yodoanilina en la etapa (b) con otra 2-yodoanilina que tenga un sustituyente diferente de cloro en la posición 4.

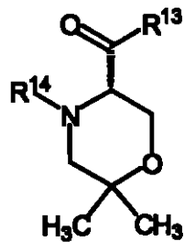
Un intermedio particularmente útil para fabricar los compuestos de fórmula III-A-aa es el intermedio 3a:



3a

en el que R^{13} es halo, OH, OR^{15} o un grupo protector de ácido carboxílico; R^{14} es un grupo protector de amino, hidrógeno o -W-G como se ha definido anteriormente; y R^{15} es un radical orgánico. Los grupos protectores de amino se conocen bien en la técnica. Los ejemplos de grupos protectores de amino adecuados incluyen grupos alcoxicarbonilo tales como t-butoxicarbonilo (t-BOC) y grupos bencilo tales como bencilo y para-metoxibencilo. El grupo ácido carboxílico de la posición 3 del anillo puede protegerse en forma de cualquier grupo éster estable tal como un éster simple de alquilo o arilo tal como un éster de metilo, etilo, bencilo o pentafluorofenilo. En una realización, R^{14} es –W-G y R^{13} es –OH, halo o un grupo protector de ácido carboxílico. Se describen con detalle diversos grupos protectores en Protecting Groups in Organic Synthesis, Theodora W. Greene y Peter G. M. Wuts, 3ª edición, 1999, publicado por John Wiley y Sons.

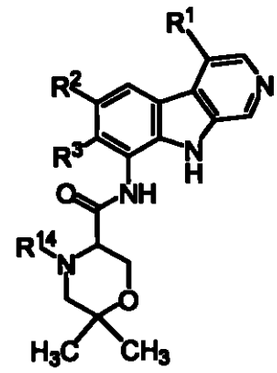
Un enantiómero preferido del intermedio 3a es (*S*)-3a:



(*S*)-3a

donde R^{13} y R^{14} son como se han descrito anteriormente.

El intermedio 3a o (*S*)-3a, como el ácido carboxílico o una forma activada del mismo (tal como el cloruro de ácido), puede acoplarse con una amino-beta-carbolina apropiada como se ha indicado en el Esquema I anterior. Cuando R^{14} es un grupo protector de amino, la reacción de acoplamiento de amida proporciona otros intermedios útiles, mostrados a continuación como compuestos de fórmula IV:

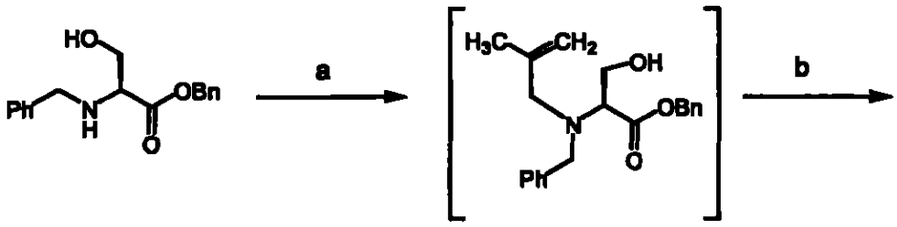


IV

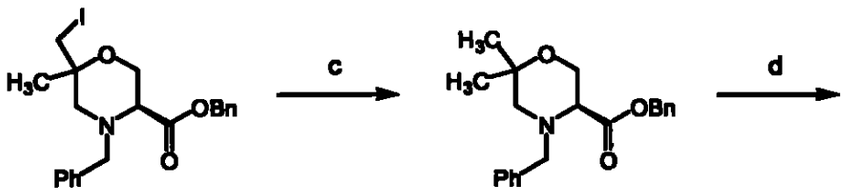
donde R^{14} es un grupo protector de amino y R^1, R^2 y R^3 son como se han descrito anteriormente. Se apreciará por un especialista en la técnica que ciertos compuestos de fórmula III-A-aa (en la que cada R^{6b} es metilo) pueden prepararse a partir de compuestos de fórmula IV retirando el grupo protector de R^{14} y después uniendo la porción –W-G usando métodos conocidos. Como alternativa, los

compuestos de fórmula III-A-aa pueden prepararse construyendo primero el intermedio **3a** en el que R^{14} es -W-G y R^{13} es un ácido carboxílico o un derivado del mismo. Después, la reacción de acoplamiento de amida con una amino-beta-carbolina apropiada proporciona directamente los compuestos de fórmula III-A-aa.

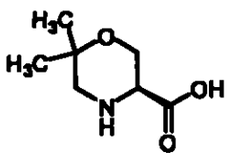
Esquema V



I-16



I-17 **I-18**



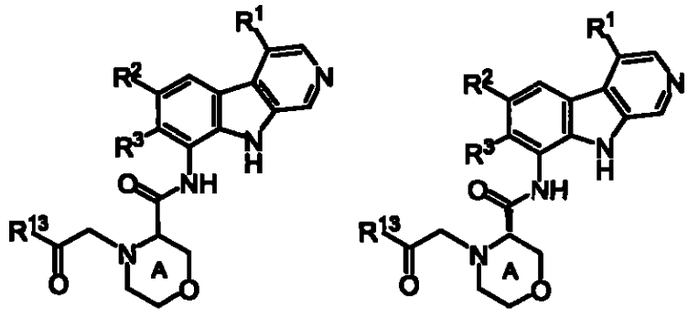
I-19

Etapas: (a) K_2CO_3, KI, 3-Bromo-2-metil-propeno, MeCN (b) I_2, $NaHCO_3$, MeCN (c) Bu_3SnH, AIBN, tolueno, 110°C (d) H_2, $Pd(OH)_2$ al 20%/C, AcOH al 10%/MeOH

El Esquema V anterior muestra una vía para fabricar intermedios de fórmula **3a**, incluyendo el **I-19** desprotegido. La protección y desprotección selectivas de

los grupos amino y ácido carboxílico de I-19 para proporcionar diversos intermedios 3a estará dentro del conocimiento de un especialista en la técnica.

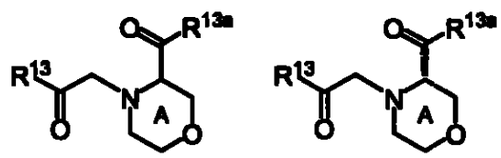
Otro intermedio útil para fabricar compuestos de fórmula III-A-aa es un compuesto de fórmula V, preferiblemente (*S*)-V:



V (*S*)-V

donde R^{13} es halo otro grupo saliente, OH, OR^{15} o un grupo protector de ácido carboxílico, R^{15} es un radical orgánico tal como un alifático C_{1-6}, arilo o bencilo, el Anillo A tiene 0-2 o 0-4 R^{6b}, y R^1, R^2 R^3, y R^{6b} son como se han definido anteriormente.

Otro intermedio útil para fabricar compuestos de esta invención es VI, preferiblemente (*S*)-VI:



VI (*S*)-VI

donde uno de R^{13} y R^{13a} es OH o un grupo saliente tal como halo y el otro es OR^{15} o un grupo protector de ácido carboxílico, R^{15} es un radical orgánico tal como un

alifático C_{1-6}, arilo o bencilo, el Anillo A tiene 0-2, y R^{6b} es como se ha definido anteriormente.

Ejemplos de síntesis

En los métodos de preparación se usan las siguientes abreviaturas se usan: TA o ta es temperatura ambiente; h, h o h es hora u horas; min es minutos; TFA es ácido trifluoroacético; DMSO es dimetilsulfóxido; NCS es N-clorosuccinimida; EDCI es clorhidrato de 1-(3-dimetilaminopropil)-3-etilcarbodiimida; EtOAc es acetato de etilo; DIEA es diisopropiletilamina; DCM es diclorometano; DDQ es dicloro diciano benzoquinona; mCPBA es ácido meta-cloroperbenzoico; MeOH es metanol; EtOH es etanol; MeCN es acetonitrilo; TLC es cromatografía de capa fina; AIBN es azobisisobutironitrilo; NH_4OAc es acetato amónico; NaOAc es acetato sódico; Et_2O es éter dietílico; AcOH es ácido acético; y DMF es dimetilformamida. TBTU es tetrafluoroborato de N,N,N',N'-tetrametil-o-(benzotriazol-1-il)uranio.

INTERMEDIO 1: ácido 7-fluoro-2,3,4,9-tetrahidro-1H-ß-carbolina-1-carboxílico

A 10 g (46,6 mmol) de clorhidrato de 6-fluorotriptamina disponible en el mercado se le añadieron 50 ml de tampón acetato 1 M (pH 4,4) para dar una suspensión que se agitó a temperatura ambiente (TA). Después, a la suspensión agitada se le añadió en una porción una suspensión de ácido glioxílico monohidrato (1,1 equiv., 51,28 mmol, 4,72 g) en acetato de etilo. La suspensión se agitó durante una noche (16 h) a TA y el sólido precipitado se recogió por filtración y se lavó con H_2O y acetato de etilo. Después, la muestra se secó al vacío para dar un sólido amarillo claro con rendimiento cuantitativo.

^1H RMN (300 MHz, ácido acético-d_4): d 3,04 (m, 2H), 3,56 (m, 1H), 3,83 (m, 1H), 6,80 (m, 1H), 7,13 (dd, 1H), 7,34 (dd, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,17 min.

MS ($M+H^+$): 235,0.

INTERMEDIO 2: 7-fluoro-2,3,4,9-tetrahidro-1H-ß-carbolina

Se suspendió ácido 7-fluoro-2,3,4,9-tetrahidro-1H-ß-carbolina-1-carboxílico (5 g, 21,36 mmol) en 130 ml de HCl 3 N en un matraz de fondo redondo de 500 ml y se calentó a reflujo durante una noche (16 h) con agitación. Después de la refrigeración, precipitó un sólido pardo claro, que se recogió por filtración y se lavó con H_2O. Después, la sal obtenida por filtración anterior se disolvió en metanol caliente (200 ml) y se trató con K_2CO_3 3 M (5–10 ml) de tal forma que el pH es de aproximadamente 9, a esta mezcla se le añadieron 100 ml de H_2O y que después se dejó en agitación a TA. El metanol se evaporó en un evaporador rotatorio para dar una suspensión blanca acuosa de la base libre deseada, que se recogió por filtración (3,2 g, rendimiento de 79%).

^{1}H RMN (300 MHz, metanol-d_4): d 2,73 (t, 2H), 3,11 (t, 2H), 3,94 (s, 2H), 6,73 (m, 1H), 6,94 (m, 1H), 7,30 (dd, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,25 min.

MS ($M+H^+$): 191,1.

INTERMEDIO 3: 7-fluoro-9H-ß-carbolina

Se suspendió 7-fluoro-2,3,4,9-tetrahidro-1H-ß-carbolina (3,5 g, 18,42 mmol) en xilenos (60 ml) en un matraz de fondo redondo de 250 ml equipado con un condensador que se abrió a la atmósfera y se calentó. A esta mezcla de reacción caliente se le añadió Pd/C (al 10% en peso, 0,2 equiv., 700 mg) y la mezcla se calentó a reflujo en xilenos durante una noche (12-14 horas). Después, la solución se filtró a través de una capa de celite y después el filtrado recogido se evaporó en un evaporador rotatorio para dar el producto deseado en forma de un sólido pardo/castaño (3,0 g, rendimiento de 88%).

^{1}H RMN (300 MHz, DMSO-d_6): d 7,10 (m, 1H), 7,37 (dd, 1H), 8,10 (d, 1H), 8,28 (dd, 1H), 8,35 (dd, 1H), 8,89 (s, 1H), 11,74 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,88 min.

MS ($M+H^+$): 187,1.

INTERMEDIO 4: 6-cloro-7-fluoro-9H-ß-carbolina

Se suspendió 7-fluoro-9H-ß-carbolina (2,15 g, 11,58 mmol) en 100 ml de HCl 1 N. A esta mezcla se le añadió NCS (1,85 g, 13,89 mmol, 1,2 equiv.) y la mezcla resultante se agitó a TA durante una noche. Después, la mezcla de reacción se filtró para dar un sólido amarillo claro (2,1 g, rendimiento de 83%).

^{1}H RMN (300 MHz, DMSO-d_6): d 7,86 (d, 1H), 8,64 (d, 1H), 8,79 (d, 1H), 8,91 (d, 1H), 9,33 (s, 1H), 13,05 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,19 min.

MS ($M+H^+$): 221,1.

INTERMEDIO 5: 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina

Se recogió 6-cloro-7-fluoro-9H-ß-carbolina (2,1 g, 9,54 mmol) en un matraz de fondo redondo (250 ml) y se añadió $NaNO_3$ (1,136 g, 13,36 mmol, 1,4 equiv.). Después, al matraz se le añadió TFA (48 ml) y la mezcla resultante se calentó a reflujo durante una noche. Después, el TFA se retira en un evaporador rotatorio. La suspensión resultante se suspende en agua (50 ml) y se sonica minuciosamente. Después, la suspensión resultante se filtra para dar un sólido amarillo (2,0 g, rendimiento de 80%).

^{1}H RMN (300 MHz, DMSO-d_6): d 8,21 (d, 1H), 8,46 (d, 1H), 9,04 (m, 2H), 12,55 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,24 min.

MS ($M+H^+$): 266,2.

INTERMEDIO 6: 6-cloro-7-metoxi-8-nitro-9H-ß-carbolina

Se añadió metanol (0,462 ml, 11,4 mmol) a una suspensión en agitación de NaH (684 mg, 17,1 mmol) en DMF (10 ml) en una atmósfera de argón. La solución resultante se dejó en agitación a TA durante 20 min. A la solución en agitación se

le añadió 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina (500 mg, 1,9 mmol) y la mezcla resultante se dejó en agitación a TA. Después de la adición de H_2O, precipitó un sólido pardo que se filtró para dar la 6-cloro-7-metoxi-8-nitro-9H-ß-carbolina deseada (510 mg, rendimiento de 97%).

^{1}H RMN (300 MHz, DMSO-d_6): d 4,02 (s, 3H), 8,52 (d, 1H), 8,60 (d, 1H), 9,05 (s, 1H), 9,12 (s, 1H), 12,78 (a, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,28 min.

MS ($M+H^+$): 278.

INTERMEDIO 7: 6-cloro-7-metoxi-9H-ß-carbolina-8-ilamina

Se suspendió 6-cloro-7-metoxi-8-nitro-9H-ß-carbolina (510 mg, 1,84 mmol) en 50 ml de metanol y se añadieron 100 mg de Pd/C (al 10%). El matraz se equipó con un globo de hidrógeno y la mezcla de reacción se agitó durante una noche a TA. Después de la filtración a través de una capa de celite y la evaporación del metanol, se obtuvo un sólido pardo oscuro. Este residuo se suspendió en metanol (15 ml) y se añadió, con agitación vigorosa, a una solución de $NaHCO_3$ saturado (100 ml). El sólido pardo claro que precipitó se recogió por filtración y se secó minuciosamente al vacío para dar el producto deseado (512 mg, rendimiento cuantitativo).

^{1}H RMN (300 MHz, metanol-d_4): d 3,90 (s, 3H), 7,63 (s, 1H), 8,11 (d, 1H), 8,27 (d, 1H), 8,84 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,12 min.

MS ($M+H^+$): 248.

INTERMEDIO 8: 6-cloro-9H-ß-carbolina-8-ilamina

El compuesto diana se preparó de acuerdo con los procedimientos indicados para el *Intermedio 1* al *Intermedio 7* donde el material de partida para el *Intermedio 1* fue triptamina no sustituida. Una síntesis alternativa para 6-cloro-9H-□-carbolina-8-ilamina se describe en la página 34, ejemplo 15 de la Publicación de

Solicitud PCT Nº WO 01/68648 A1, que se incorpora en este documento en su totalidad.

MÉTODO A: PROCEDIMIENTO DE ACOPLAMIENTO PARA β-CARBOLINAS 6,7,8-SUSTITUIDAS

Se recogieron 9H-ß-carbolina 6,7,8-sustituida (1 mmol), EDCI (1,6 mmol) y el ácido carboxílico apropiado (1,2 mmol) en un matraz de fondo redondo y se suspendieron en piridina (5 ml). La mezcla resultante se calentó a 60ºC durante una noche. Después, la piridina se retiró por evaporación rotatoria y se añadió una solución al 5% de Na_2CO_3. El sólido resultante que precipitó se recogió por filtración. La purificación cromatográfica dio el producto deseado.

MÉTODO B: PROCEDIMIENTO DE ACOPLAMIENTO PARA β-CARBOLINAS 6,8-SUSTITUIDAS

Se recogieron 9H-β-carbolina 6,8-sustituida (1,0 mmol), EDCI (1,6 mmol) y el ácido carboxílico (1,2 mmol) para acoplarse en un matraz de fondo redondo y se suspendieron en piridina (5 ml). La mezcla resultante se agitó durante una noche. Después, la piridina se retiró por evaporación rotatoria y se añadió una solución al 5% de Na_2CO_3. El sólido resultante que precipitó se recogió por filtración. La purificación cromatográfica dio el producto deseado.

EJEMPLO 1: N-(6-cloro-7-metoxi-9H-ß-carbolin-8-il)-2-metil-nicotinamida

Se recogieron 6-cloro-7-metoxi-9H-ß-carbolin-8-ilamina (100 mg, 0,4 mmol), EDCI (125 mg, 0,64 mmol) y ácido 2-metil-nicotínico (66 mg, 0,48 mmol) en un matraz de fondo redondo y se suspendieron en piridina (2 ml). La mezcla resultante se calentó a 80ºC durante una noche. Después, la piridina se retiró por evaporación rotatoria y se añadió una solución al 5% de Na_2CO_3. El sólido

resultante que precipitó se recogió por filtración. La purificación cromatográfica dio el producto deseado con un rendimiento de 50-70%.

^{1}H RMN (300 MHz, DMSO-d_6): d 2,71 (s, 3H), 3,89 (s, 3H), 7,45 (dd, 1H), 8,15 (d, 1H), 8,21 (d, 1H), 8,38 (d, 1H), 8,45 (s, 1H), 8,61 (d, 1H), 8,92 (s, 1H), 10,33 (s, 1H), 11,57 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,77 min.

MS ($M+H^+$): 367,1.

EJEMPLO 2: (6-cloro-7-metoxi-9H-beta-carbolin-8-il)-amida del ácido 4-metil-pirimidin-5-carboxílico

El compuesto deseado se preparó de acuerdo con el *Método A* a partir de 6-cloro-7-metoxi-9H-beta-carbolin-8-ilamina y ácido 4-metil-5-pirimidin-carboxílico con un rendimiento de 80%.

^{1}H RMN (300 MHz, DMSO-d_6): d 2,75 (s, 3H), 3,92 (s, 3H), 8,19 (d, 1H), 8,41 (d, 1H), 8,50 (s, 1H), 8,96 (s, 1H), 9,20 (s, 1H), 9,25 (s, 1H), 10,63 (s, 1H), 11,67 (s, 1H).

Tiempo de retención (LC, método: patrón de ácido fórmico): 0,95 min.

MS ($M+H^+$): 368.

INTERMEDIO 9: 6-cloro-7-etoxi-8-nitro-9H-ß-carbolina

Se añadió etóxido sódico (232 mg, 3,4 mmol) a una solución de 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina (200 mg, 0,76 mmol) en DMSO (4 ml) y la mezcla de reacción se dejó en agitación durante una noche. La mezcla de reacción se diluyó con agua y el pH de la solución se ajustó a aproximadamente 4 mediante la adición de HCl 1 N. La solución acuosa se extrajo (3 x) con EtOAc. Las capas de EtOAc combinadas se secaron y se evaporaron. El producto bruto se purificó por cromatografía ultrarrápida para dar el producto deseado con un rendimiento de 40-60%.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,44 (t, 3H), 4,24 (c, 2H), 8,21 (d, 1H), 8,46 (d, 1H), 8,91 (s, 1H), 9,02 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,37 min.

MS ($M+H^+$): 291,9.

INTERMEDIO 10: 6-cloro-7-etoxi-9H-ß-carbolin-8-ilamina

Se suspendió 6-cloro-7-etoxi-8-nitro-9H-ß-carbolina (160 mg, 0,55 mmol) en 4 ml de metanol y se añadieron 25 mg de Pd/C (10%). El matraz se equipó con un globo de hidrógeno y la mezcla de reacción se agitó durante una noche a TA. Después de la filtración a través de una capa de celite y la evaporación del metanol, se obtuvo un sólido pardo oscuro y se determinó que era la 6-cloro-7-ciclopropilmetoxi-9H-ß-carbolin-8-ilamina deseada (80 mg, 55%).

^{1}H RMN (300 MHz, Metanol-d_4/$CDCl_3$): d 1,26 (t, 3H), 3,91 (c, 2H), 7,34 (s, 1H), 7,71 (d, 1H), 8,02 (s, 1H), 8,56 (s, 1H).

Tiempo de retención (LC, método: patrón de ácido fórmico): 1,16 min.

MS ($M+H^+$): 262,0.

EJEMPLO 3: N-(6-cloro-7-etoxi-9H-ß-carbolin-8-il)-2-metil-nicotinamida

El compuesto deseado se preparó de acuerdo con el *Método A* a partir de 6-cloro-7-etoxi-9H-ß-carbolin-8-ilamina y ácido 2-metilnicotínico con un rendimiento de 40%.

^{1}H RMN (300 MHz, MeOH-d_4): d 1,39 (t, 3H), 2,76 (s, 3H), 4,14 (c, 2H), 7,43 (dd, 1H), 8,05 (d, 1H), 8,26 (m, 3H), 8,55 (d, 1H), 8,79 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,98 min.

MS ($M+H^+$): 381,3.

INTERMEDIO 11: 6-cloro-7-(N,N)-dimetilamino-8-nitro-9H-ß-carbolina

Se añadió clorhidrato de N,N-dimetilamina (278 mg, 3,4 mmol) a una solución en agitación de 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina (300 mg, 1,13

mmol) en DMSO (8 ml) en una atmósfera de argón. Esto se siguió de la adición de DIEA (0,83 ml, 4,65 mmol) y la mezcla de reacción se calentó a 60°C durante una noche. Después de dejar enfriar la mezcla de reacción a TA, se añadió agua y precipitó un sólido naranja oscuro. El sólido se filtró, se lavó con agua y se secó para dar el producto deseado (230 mg, rendimiento de 70%).

^{1}H RMN (300 MHz, DMSO-d_6): d 4,06 (s, 6H), 7,23 (d, 1H), 7,53 (d, 1H), 7,66 (s, 1H), 8,05 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,41 min.

MS ($M+H^+$): 290,9.

INTERMEDIO 12: 6-cloro-7-(N,N)-dimetilamino-9H-ß-carbolina-8-ilamina

Se suspendió 6-cloro-7-(N,N)-dimetilamino-8-nitro-9H-ß-carbolina (828 mg, 2,86 mmol) en 30 ml de metanol y se añadieron 166 mg de Pd/C (10%). El matraz se equipó con un globo de hidrógeno y la mezcla de reacción se agitó durante una noche a temperatura ambiente. Después de la filtración a través de una capa de celite y la evaporación del metanol, se obtuvo un sólido pardo oscuro y se determinó que era la 6-cloro-7-(N,N)-dimetilamino-9H-ß-carbolina-8-ilamina deseada (500 mg, rendimiento de 67%).

^{1}H RMN (300 MHz, DMSO-d_6): d 2,80 (s, 6H), 5,42 (s, 2H), 7,45 (s, 1H), 7,95 (d, 1H), 8,23 (d, 1H), 8,86 (d, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,32 min.

MS ($M+H^+$): 261,1.

EJEMPLO 4: N-(6-cloro-7-7-(N,N)-dimetilamino-9H-ß-carbolin-8-il)-2-metil-nicotinamida

El compuesto deseado se preparó de acuerdo con el *Método A* a partir de 6-cloro-7-(N,N)-dimetilamino-9H-ß-carbolina-8-ilamina y ácido 2-metilnicotínico con un rendimiento de 40-60%.

^{1}H RMN (300 MHz, Metanol-d_4/$CDCl_3$): d 2,82 (s, 3H), 2,94 (s, 6H), 7,33 (m, 1H), 8,02 (m, 3H), 8,34 (d, 1H), 8,61 (d, 1H), 8,95 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,29 min.

MS ($M+H^+$): 380,3.

INTERMEDIO 13: 6-cloro-7-(4-metil-piperazin-1-il)-8-nitro-9H-ß-carbolina

A una solución en DMSO (4 ml) de 200 mg (0,755 mmol) de 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina se le añadieron 1-metilpiperazina (226 mg, 2,26 mmol) y DIEA (400 mg, 3,09 mmol) mediante una jeringa. La reacción se dejó en agitación a TA durante una noche. Después de la adición de agua, precipitó un sólido naranja. El sólido se filtró, se lavó y se secó para dar 236 mg (rendimiento de 91%) de la 6-cloro-7-(4-metil-piperazin-1-il)-8-nitro-9H-ß-carbolina deseada.

^{1}H RMN (300 MHz, DMSO-d_6): d 2,24 (s, 3H), 2,48 (m, 4H), 3,13 (m, 4H), 8,13 (d, 1H), 8,40 (d, 1H), 8,73 (s, 1H), 8,94 (s, 1H), 12,05 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,72 min.

MS ($M+H^+$): 346.

INTERMEDIO 14: 6-cloro-7-(4-metil-piperazin-1-il)-8-amino-9H-ß-carbolina

Se suspendieron 236 mg de 6-cloro-7-(4-metil-piperazin-1-il)-8-nitro-9H-ß-carbolina en 100 ml de metanol y se añadió Pd al 10%/C (48 mg) en atmósfera de argón. El matraz se lavó abundantemente con hidrógeno (3 x) y la mezcla de reacción se agitó en una atmósfera de hidrógeno a TA durante una noche. La mezcla de reacción se filtró y el Pd/C se retiró usando una capa de celite. La mezcla de reacción se evaporó para retirar el disolvente y se purificó por cromatografía ultrarrápida para dar 119 mg (rendimiento de 55%) de la 6-cloro-7-(4-metil-piperazin-1-il)-8-amino-9H-□-carbolina deseada.

^{1}H RMN (300 MHz, metanol-d_4): d 2,39 (s, 3H), 2,46 (m, 2H), 2,87 (m, 4H), 3,83 (m, 2H), 7,50 (s, 1H), 7,97 (d, 1H), 8,24 (d, 1H), 8,78 (s, 1H).

Tiempo de retención (LC, método: acetato amónico polar): 1,32 min.

MS ($M+H^+$): 316.

EJEMPLO 5: 2-cloro-N-[6-cloro-7-(4-metil-piperazin-1-il)-9H-ß-carbolin-8-il]-nicotinamida

El compuesto deseado se preparó de acuerdo con el *Método A* a partir de 6-cloro-7-(4-metil-piperazin-1-il)-8-amino-9H-ß-carbolina y ácido 2-cloro-nicotínico con un rendimiento de 25%.

^1H RMN (300 MHz, DMSO-d_6): d 2,21 (s, 3H), 2,33 (m, 2H), 2,54(m, 2H), 3,24 (m, 4H), 7,73 (dd, 1H), 8,12 (d, 1H), 8,35 (d, 1H), 8,37 (s, 1H), 8,48 (dd, 1H), 8,61 (dd, 1H), 8,91 (s, 1H), 10,40 (s, 1H), 11,33 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,46 min.

MS ($M+H^+$): 455.

INTERMEDIO 15: Resolución de ácido rac-terébico

Se disolvió ácido terébico en una mezcla 10:1 de EtOAc-MeOH [10 g de ácido terébico (17,7 g de sal) en 550 ml] y se calentó a 50-55°C, seguido de la adición de (S)-(-)-a-metil-bencilamina. La mezcla de reacción se agitó durante 2 minutos y después se dejó a TA durante 15 minutos. Después, la mezcla de reacción se sembró con sal enriquecida (preparada a menor escala usando 3 ciclos de recristalización), se sonicó durante 10-15 segundos y se dejó a TA durante una noche. El sólido se retiró por filtración, se lavó con EtOAc y se secó al vacío. La recristalización se realizó en la misma mezcla disolvente re-disolviendo la sal (24 mg/ml). Después, esta mezcla se calentó a suave reflujo durante un corto periodo de tiempo (quedaron algunos cristales en suspensión). La mezcla se dejó a TA durante una noche. El sólido se procesó como se ha descrito anteriormente.

El exceso enantiomérico se determinó de manera bruta usando RMN de protones de la amida correspondiente obtenida del acoplamiento de TBTU.

Regeneración de ácido (R)-(+)-terébico: La sal se disolvió en agua (320 mg/ml), se calentó a 65°C y se añadieron 1,2 equivalentes de HCl acuoso 6 M. Después, la mezcla de reacción se dejó a 4°C durante una noche. El sólido se retiró por filtración, se lavó con pequeñas porciones de agua fría y se secó a alto vacío (rendimiento de 25-30%).

^{1}H RMN (300 MHz, DMSO-d_6): d 1,30 (s, 3H), 1,52 (s, 3H), 2,74 (dd, 1H), 2,85 (dd, 1H), 3,25 (t, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 0,33 min.

MS ($M+H^+$): 159,0.

EJEMPLO 6: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 2,2-dimetil-5-oxo-tetrahidro-furan-3-carboxílico

El compuesto deseado se preparó de acuerdo con el *Método B* a partir de 6-cloro-9H-ß-carbolina-8-ilamina y ácido (R)-terébico (*Intermedio 15*) con un rendimiento de 80-90%.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,44 (s, 3H), 1,59 (s, 3H), 2,91 (dd, 1H), 3,08 (dd, 1H), 3,38 (dd, 1H), 7,85 (m, 1H), 8,17 (d, 1H), 8,25 (d, 1H), 8,39 (d, 1H), 9,05 (s, 1H), 10,26 (s, 1H), 11,72 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,22 min.

MS ($M+H^+$): 358,3.

EJEMPLO 7: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 1,2,2-trimetil-5-oxo-pirrolidina-3-carboxílico

El compuesto deseado se preparó de acuerdo con el *Método B* a partir de 6-cloro-9H-ß-carbolina-8-ilamina y ácido 1,2,2-trimetil-5-oxo-pirrolidina-3-carboxílico con un rendimiento de 68%.

^{1}H RMN (DMSO-d6, 300 MHz) d 1,23 (s, 3H), 1,45 (s, 3H), 2,59 (dd, 1H),2,66(s, 3H), 2,70 (dd, 1H), 3,17 (t, 1H), 7,91 (m, 1H), 8,18 (d, 1H), 8,24 (d, 1H), 8,40 (d, 1H), 9,07 (s, 1H), 10,16 (s, 1H), 11,32 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,17 min.
MS ($M+H^+$): 371,3.

INTERMEDIO 16: éster bencílico de N-bencil-serina

A una mezcla de éster bencílico de L-serina-HCl (2,3 g), benzaldehído (1,05 equiv.) y acetato sódico (1 equiv.) en metanol se le añadió cianoborohidruro sódico (1,0 equiv.). La mezcla resultante se agitó a temperatura ambiente durante 15 h y después se repartió entre éter y bicarbonato sódico acuoso saturado. La fase orgánica separada se extrajo con HCl 1 M (3 x). Los extractos acuosos combinados se lavaron con éter, se basificaron con K_2CO_3 acuoso 4,5 M y se extrajeron con éter. Los extractos orgánicos combinados se lavaron con salmuera, se secaron sobre sulfato sódico y se concentraron a sequedad para dar 2,32 g del compuesto deseado (sólido ceroso, rendimiento de 81%).

^1H RMN (300 MHz, $CDCl_3$): d 3,48 (dd, 1H), 3,64 (dd, 1H), 3,74 (d, 1H), 3,80 (dd, 1H), 3,88 (d, 1H), 5,18 (s, 2H), 7,25-7,39 (m, 10H).

MS ($M+H^+$): 286.

INTERMEDIO 17: éster bencílico del ácido 4-bencil-6-yodometil-6-metil-morfolin-3-carboxílico

A una solución de éster bencílico de N-bencil-serina (*Intermedio 16*, 6,35 g) en 90 ml de MeCN a temperatura ambiente se le añadieron 3-bromo-2-metil-propeno (5,6 ml), KI (740 mg) y K_2CO_3 (7,7 g). La mezcla de reacción se agitó a temperatura ambiente durante 72 h. Se añadió 1 ml de 3-bromo-2-metil-propeno y la mezcla de reacción se agitó durante 15 h más. Sólo quedó una pequeña cantidad de material de partida basándose en la TLC (1:1; EtOAc-hexano). A la mezcla resultante se le añadieron 11,2 g de yodo. Después de 4 h de agitación, la TLC (EtOAc al 10%/hexano) mostró la completa conversión. La mezcla de reacción se repartió en éter (300 ml) y $Na_2S_2O_3$ 0,5 M (100 ml). La fase orgánica separada se lavó sucesivamente con agua, $NaHCO_3$ saturado y salmuera, se secó

sobre $MgSO_4$ y se concentró a sequedad. El residuo se purificó sobre sílice (EtOAc al 5%/Hexano) para dar 6,65 g (aceite amarillento, rendimiento de 64%, mezcla aproximadamente 4:1) del compuesto éster bencílico del ácido 4-bencil-6-yodometil-6-metil-morfolin-3-carboxílico. Componente principal: ^1H RMN (300 MHz, $CDCl_3$): d 1,22 (s, 3H), 2,50 (d, 1H), 3,16-3,36 (m, 3H), 3,75-3,95 (m, 4H), 4,06 (dd, 1H), 5,16 (d, 1H), 5,21 (d, 1H), 7,28-7,36 (m, 10H).

MS ($M+H^+$): 466.

INTERMEDIO 18: éster bencílico del ácido 4-bencil-6,6-dimetil-morfolin-3-carboxílico

A una solución de éster bencílico del ácido 4-bencil-6-yodometil-6-metil-morfolin-3-carboxílico (*Intermedio 17*, 1,23 g) e hidruro de tributilestaño (1,8 ml, 2,5 equiv.) en 11 ml de tolueno a suave reflujo se le añadió durante 1,5 h una solución de AIBN en tolueno (25 mg/1 ml). La mezcla se dejó enfriar y se concentró a sequedad. El residuo se repartió en HCl 1 M al 15% en acetonitrilo y hexano. La fase de hexano separada se extrajo dos veces con la solución de acetonitrilo. Las soluciones de acetonitrilo combinadas se lavaron dos veces con hexano y se concentraron. El residuo se repartió en éter y K_2CO_3 1 M. La fase de éter separada se lavó sucesivamente con $Na_2S_2O_3$ 0,4 M y salmuera, se secó sobre $MgSO_4$ y se concentró. El residuo se purificó sobre sílice (EtOAc al 7,5%/hexano) para dar 760 mg (aceite, rendimiento de 85%) del compuesto éster bencílico del ácido 4-bencil-6,6-dimetil-morfolin-3-carboxílico.

^1H RMN (300 MHz, $CDCl_3$): d 1,21 (s, 3H), 1,24 (s, 3H), 2,12 (d, 1H), 2,84 (d, 1H), 3,29 (t, 1H), 3,58 (d, 1H), 3,95-4,05 (m, 2H), 5,15 (d, 1H), 5,21 (d, 1H), 7,28-7,36 (m, 10H).

MS ($M+H^+$): 340.

INTERMEDIO 19: ácido 6,6-dimetil-morfolin-3-carboxílico

A una solución de éster bencílico del ácido 4-bencil-6,6-dimetil-morfolin-3-carboxílico (*Intermedio 18*, 1,25 g) en 40 ml de AcOH al 10%/MeOH (en atmósfera de nitrógeno) se le añadieron 250 mg de $Pd(OH)_2$ al 20% sobre carbono. La mezcla de reacción se purgó con hidrógeno (globo) y se agitó a temperatura ambiente durante 72 h. A la mezcla gris resultante se le añadieron 4 ml de agua para facilitar la disolución. El catalizador se retiró por filtración y el filtrado se concentró a sequedad. El residuo se co-evaporó con etanol (2 x) y después se trituró con EtOAc. El sólido blanco generado se retiró por filtración y se secó a alto vacío para dar 559 mg de ácido 6,6-dimetil-morfolin-3-carboxílico (rendimiento de 95%).

^{1}H RMN (300 MHz, D_2O): d 1,35 (s, 3H), 1,38(s, 3H), 3,11 (d, 1H), 3,32 (d, 1H), 3,81-3,87 (m, 1H), 4,05 (t a, 1H), 4,17 (d a, 1H).

MS ($M+H^+$): 160.

INTERMEDIO 20: ácido 4,6,6-trimetil-morfolin-3-carboxílico

A una suspensión de ácido 6,6-dimetil-morfolin-3-carboxílico (*Intermedio 19*, 540 mg) en 17 ml de etanol (en atmósfera de nitrógeno) se le añadieron 100 mg de Pd al 10% sobre carbono y 830 µl (~3 equiv.) de formaldehído acuoso al 37%. La mezcla se purgó con hidrógeno (globo) y se agitó a temperatura ambiente durante 5 h. A la mezcla gris resultante se le añadieron 4 ml de agua y 4 ml de metanol para facilitar la disolución. El catalizador se retiró por filtración y el filtrado se concentró a sequedad. El residuo se trituró con EtOAc. El sólido blanco generado se retiró por filtración y se secó a alto vacío para dar 574 mg de ácido 4,6,6-trimetil-morfolin-3-carboxílico (rendimiento de 97%).

^{1}H RMN (300 MHz, D_2O): d 1,35 (s, 3H), 1,43(s, 3H), 2,94(s, 3H), 3,08 (d, 1H), 3,41 (d, 1H), 3,68 (dd, 1H), 3,96 (t, 1H), 4,14 (dd, 1H).

MS ($M+H^+$): 174.

EJEMPLO 8: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4,6,6-trimetil-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el *Método B* a partir de 6-cloro-9H-ß-carbolina-8-ilamina y ácido 4,6,6-trimetil-morfolin-3-carboxílico con un rendimiento de 61%.

^{1}H RMN (DMSO-d6, 300 MHz) d 1,15 (s, 3H), 1,39 (s, 3H), 1,98 (d, 1H), 2,26 (s, 3H), 2,72 (d, 1H), 2,80 (dd, 1H), 3,79(m, 2H), 7,91 (s, 1H), 8,03-8,08 (m, 2H), 8,22 (d, 1H), 8,97 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,33 min.

MS ($M+H^+$): 373,2.

EJEMPLO 9: (6-cloro-7-metoxi-9H- ß-carbolin-8-il)-amida del ácido 2,2-dimetil-5-oxo-tetrahidro-furan-3-carboxílico

El compuesto deseado se preparó de acuerdo con el *Método A* a partir de 6-cloro-7-metoxi-9H-ß-carbolina-8-ilamina y ácido (R)-terébico con un rendimiento de 60-80%.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,48 (s, 3H), 1,61 (s, 3H), 3,03 (m, 2H), 3,51 (m, 1H), 3,86 (s, 3H), 8,16 (m, 1H), 8,37 (m, 1H), 8,43 (s, 1H), 8,94 (s, 1H), 10,10 (s, 1H), 11,33 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,94 min.

MS ($M+H^+$): 388.

INTERMEDIO 21: 6-cloro-7-ciclopropilmetoxi-8-nitro-9H-ß-carbolina

Se añadió ciclopropilmetil alcohol (0,921 ml, 11,4 mmol) a una suspensión en agitación de NaH (455 mg, 11,4 mmol) en DMF (20 ml) en una atmósfera de argón. La solución resultante se dejó en agitación a TA durante 20 min. A la solución en agitación resultante se le añadió 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina (500 mg, 1,9 mmol) y la mezcla resultante se dejó en agitación a TA. Después de

la adición de H_2O, precipitó un sólido pardo que se filtró para dar la 6-cloro-7-ciclopropilmetoxi-8-nitro-9H-ß-carbolina deseada (510 mg, 85%).

^{1}H RMN (300 MHz, DMSO-d_6): d 0,35 (m, 2H), 0,59 (m, 2H), 1,32 (m, 1H), 4,04 (d, 2H), 8,21 (d, 1H), 8,46 (d, 1H), 8,90 (s, 1H), 9,02 (s, 1H), 12,32 (a, 1H)

Tiempo de retención (LC, método: patrón de acetato amónico): 2,63 min.

MS ($M+H^+$): 318.

INTERMEDIO 22: 6-cloro-7-ciclopropilmetoxi-9H-ß-carbolin-8-ilamina

Se suspendió 6-cloro-7-ciclopropilmetoxi-8-nitro-9H-ß-carbolina (510 mg, 1,61 mmol) en 12 ml de metanol y se añadieron 100 mg de Pd/C (al 10%). El matraz se equipó con un globo de hidrógeno y la mezcla de reacción se agitó durante una noche a TA. Después de la filtración a través de una capa de celite y la evaporación del metanol, se obtuvo un sólido pardo oscuro. Este residuo se suspendió en metanol (10 ml) y se añadió, con agitación vigorosa, a una solución de $NaHCO_3$ saturado (100 ml). El sólido pardo claro que precipitó se recogió por filtración y se secó minuciosamente al vacío para dar la 6-cloro-7-ciclopropilmetoxi-9H-ß-carbolin-8-ilamina deseada (371 mg, rendimiento de 80%).

^{1}H RMN (300 MHz, metanol-d_4): d 0,36 (m, 2H), 0,61 (m, 2H), 1,37 (m, 1H), 3,88 (d, 2H), 7,58 (s, 1H), 7,96(d, 1H), 8,23 (d, 1H), 8,76 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,28 min.

MS ($M+H^+$): 288.

EJEMPLO 10: N-(6-cloro-7-ciclopropilmetoxi-9H-ß-carbolin-8-il)-2-metil-nicotinamida

El compuesto deseado se preparó de acuerdo con el *Método A* a partir de 6-cloro-7-ciclopropilmetoxi-9H-ß-carbolin-8-ilamina y ácido 2-metilnicotínico con un rendimiento de 40-60%.

^{1}H RMN (300 MHz, MeOH-d_4): d 0,29 (m, 2H), 0,55 (m, 2H), 1,29 (m, 1H), 2,78 (s, 3H), 3,95 (m, 2H), 7,46 (dd, 1H), 8,08 (m, 1H), 8,30 (m, 3H), 8,59 (m, 1H), 8,81 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,18 min.

MS ($M+H^+$): 405.

INTERMEDIO 23: 6-cloro-7-(N,N)-dimetilaminoetoxi-8-nitro-9H-ß-carbolina

Se añadió N,N-dimetilaminoetil alcohol (6,0 equiv.) a una suspensión en agitación de NaH (6,0 equiv.) en DMF en una atmósfera de argón. La solución resultante se dejó en agitación a TA durante 20 min. A la solución en agitación resultante se le añadió 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina (1,0 equiv.) y la mezcla resultante se dejó en agitación a TA. Después de la adición de H_2O, precipitó un sólido pardo que se filtró para dar la 6-cloro-7-(N,N)-dimetilaminoetoxi-8-nitro-9H-β-carbolina deseada (rendimiento cuantitativo).

^{1}H RMN (300 MHz, DMSO-d_6): d 2,23 (s, 6H), 2,74 (t, 2H), 4,28 (t, 2H), 8,21(d, 1H), 8,46 (d, 1H), 8,90 (s, 1H), 9,02 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,26 min.

MS ($M+H^+$): 335.

INTERMEDIO 24: 6-cloro-7-(N,N)-dimetilaminoetoxi-9H-ß-carbolin-8-ilamina

Se suspendió 6-cloro-7-(N,N)-dimetilaminoetoxi-8-nitro-9H-ß-carbolina (500 mg, 1,5 mmol) en 12 ml de metanol y se añadieron 100 mg de Pd/C (al 10%). El matraz se equipó con un globo de hidrógeno y la mezcla de reacción se agitó durante una noche a TA. Después de la filtración a través de una capa de celite y la evaporación del metanol, se obtuvo un sólido pardo oscuro. El residuo se suspendió en metanol (10 ml) y se añadió, con agitación vigorosa, a una solución de $NaHCO_3$ saturado (100 ml). El sólido que precipitó se recogió por filtración y se

secó minuciosamente al vacío para dar la 6-cloro-7-(N,N)-dimetilaminoetoxi-9H-ß-carbolina-8-ilamina deseada (380 mg, 83%).

^{1}H RMN (300 MHz, metanol-d_4): d 2,43 (s, 6H), 2,84 (t, 2H), 4,11 (t, 2H), 7,47 (s, 1H), 7,88 (d, 1H), 8,20 (d, 1H), 8,72 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,34 min.

MS ($M+H^+$): 305.

EJEMPLO 11: N-[6-cloro-7-(2-dimetilamino-etoxi)-9H-ß-carbolin-8-il]-2-metil-nicotinamida

El compuesto deseado se preparó de acuerdo con el *Método A* a partir de 6-cloro-7-(N,N)-dimetilaminoetoxi-9H-ß-carbolina-8-ilamina y ácido 2-metilnicotínico con un rendimiento de 40-60%.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,92 (s, 6H), 2,49 (m, 2H), 2,68 (s, 3H), 4,25 (m, 2H), 7,50 (dd, 1H), 8,16 (m, 2H), 8,38 (d, 1H), 8,42 (s, 1H), 8,66 (m, 1H), 9,00 (s, 1H), 11,27 (s, 1H), 11,78 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,52 min.

MS ($M+H^+$): 424.

EJEMPLO 12: (6-cloro-7-metoxi-9H-ß-carbolin-8-il)-amida del ácido 2-amino-ciclopentanocarboxílico

Se hicieron reaccionar 6-cloro-7-metoxi-9H-ß-carbolina-8-ilamina y ácido 2-terc-butoxicarbonilamino-ciclopentanocarboxílico usando el *Método A*. A este producto se le añadieron ~5 ml de HCl 4 N/dioxano y la mezcla resultante se dejó en agitación a TA. La reacción se siguió por LC-MS hasta que se completó. Se dejó que la evaporación retirase todo el disolvente, lo que dio una sal HCl bruta. Después, el producto deseado se purificó por HPLC preparativa.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,68 (m, 1H), 1,84 (m, 2H), 2,06 (m, 2H), 2,20 (m, 1H), 3,46 (m, 1H), 3,67 (m, 1H), 3,89 (s, 3H), 8,24 (d, 2H), 8,61 (d, 1H), 8,72 (s, 1H), 8,78 (d, 1H), 9,19 (s, 1H), 10,58 (s, 1H), 13,20 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,54 min.
MS ($M+H^+$): 359.

INTERMEDIO 25: ácido 1-(2-dimetilamino-etil)-5-oxo-pirrolidina-3-carboxílico

Una mezcla de ácido itacónico disponible en el mercado y N,N-dimetiletilendiamina se calentó a 160°C durante aproximadamente 20-25 minutos. La mezcla se dejó enfriar a 100°C y después se diluyó con MeOH para prevenir la solidificación. El producto se obtuvo con un rendimiento de 56% después de la cristalización en MeOH/EtOAc.

^{1}H RMN (300 MHz, D_2O): d 2,63 (dd, 1H), 2,80 (dd, 1H), 2,95 (s, 6H), 3,15-3,25 (m, 1H), 3,32-3,44 (m, 1H), 3,44-3,76 (m, 4H), 3,82-3,94 (m, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 0,13 min.
MS ($M+H^+$): 201,0.

EJEMPLO 13: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 1-(2-dimetilamino-etil)-5-oxo-pirrolidina-3-carboxílico

Se preparó de acuerdo con el *Método B* a partir de 6-cloro-9H-ß-carbolina-8-ilamina y ácido 1-(2-dimetilamino-etil)-5-oxo-pirrolidina-3-carboxílico con un rendimiento de 70–80% en forma de la sal di-HCl.

^{1}H RMN (300 MHz, D_2O): d 2,97 (dd, 1H), 3,03 (s, 6H), 3,08 (dd, 1H), 3,51 (t, 2H), 3,76-3,89 (m, 3H), 3,95 (dd, 1H), 4,01 (dd, 1H), 7,75 (d, 1H), 8,24 (d, 1H), 8,45 (dd, 1H), 8,52 (dd, 1H), 9,10 (s a, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 0,99 min.
MS ($M+H^+$): 400.

EJEMPLO 14: (6-cloro-7-metoxi-9H-ß-carbolin-8-il)-amida del ácido 1-(2-dimetilamino-etil)-5-oxo-pirrolidina-3-carboxílico

Se preparó de acuerdo con el *Método A* a partir de 6-cloro-7-metoxi-9H-ß-carbolina-8-ilamina y ácido 1-(2-dimetilamino-etil)-5-oxo-pirrolidina-3-carboxílico

con un rendimiento de 60% siguiendo la purificación usando una columna semi-preparativa Chiralcel OD con 85/7,5/7,5 de hexano/EtOH/MeOH como eluyente.

^{1}H RMN (300 MHz, DMSO-d_6): d 2,44 (s, 6H), 2,75 (t, 2H), 2,88 (d, 2H), 3,56 (t, 2H), 3,66 (m, 1H), 3,85 (m, 2H), 3,90 (s, 3H), 8,03 (d, 1H), 8,21 (s, 1H), 8,28 (d, 1H), 8,79 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,42 min.

MS ($M+H^+$): 430.

INTERMEDIO 26: 6-cloro-7-fluoro-9H-ß-carbolin-8-ilamina

Una suspensión de 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina (500 mg, 1,88 mmol) en MeOH (25 ml) se desgasificó con argón. Se añadió paladio sobre carbono (al 20% p/p sobre C, 50 mg) y el recipiente de reacción se lavó abundantemente con hidrógeno. La suspensión se agitó en una atmósfera de hidrógeno durante 6 h, después se filtró a través de celite y se concentró a presión reducida para producir 6-cloro-7-fluoro-9H-ß-carbolin-8-ilamina (400 mg) en forma de un sólido pardo.

^{1}H RMN (300 MHz, DMSO-d6): d 11,48 (s a, 1); 8,99-8,98 (m, 1); 8,37-8,35 (m, 1); 8,11-8,09 (m, 1); 7,74-7,72 (m, 1); 5,65 (s a, 2).

condiciones convencionales de HCOOH.

DAD R_t = 1,00 min.

MS ($M+H^+$): 236.

EJEMPLO 15: N-(6-cloro-7-fluoro-9H-ß-carbolin-8-il)-2-metil-nicotinamida

Una solución de 6-cloro-7-fluoro-9H-ß-carbolin-8-ilamina (100 mg, 0,424 mmol) en piridina (2,5 ml) se agitó a TA. Se añadió ácido 2-metil-nicotínico (70 mg, 0,509 mmol) seguido de EDCI (130 mg, 0,678 mmol). La suspensión se agitó a 100°C durante a día. La piridina se retiró a presión reducida y el aceite oscuro resultante se trituró con $NaHCO_3$ acuoso saturado. El precipitado que se formó se filtró y se lavó con MeOH. El material se trató con HCl 2 M en Et_2O para

producir un sólido gris, la sal di-HCl de N-(6-cloro-7-fluoro-9H-ß-carbolin-8-il)-2-metil-nicotinamida (110 mg).

^{1}H RMN (300 MHz, DMSO-d6): d 13,15 (s, 1); 11,03 (s, 1); 9,33 (s, 1); 8,99-8,97 (m, 1); 8,92-8,89 (m, 1); 8,79-8,73 (m, 2); 8,50 (m, 1); 7,70 (m, 1); 2,80 (s, 3).

condiciones convencionales de HCOOH.

DAD R_t = 0,91 min.

MS ($M+H^+$): 355.

INTERMEDIO 27: 6-cloro-7-metilsulfanil-8-nitro-9H-ß-carbolina

Un matraz de fondo redondo de 250 ml con agitador magnético se cargó con 6-cloro-7-fluoro-8-nitro-ß-carbolina (*Intermedio 5*, 3,959 g, 14,9 mmol) y 100 ml de DMF anhidra. La mezcla naranja resultante se enfrió a 0ºC (baño de hielo y agua) y se añadió lentamente a la misma tiometóxido sódico (1,809 g, 25,8 mmol). La mezcla de reacción se agitó durante 1 h a 0ºC, se calentó a TA, y se añadió lentamente a una mezcla en agitación de 4:1 de H_2O/bicarbonato sódico acuoso saturado (500 ml). El sólido precipitado se recogió por filtración por succión y se secó al aire para producir 4,017 g de 6-cloro-7-metilsulfanil-8-nitro-9H-ß-carbolina en forma de un polvo naranja. El material bruto se usó directamente en las siguientes etapas.

^{1}H RMN (300 MHz, Metanol-d_4): d 8,91 (1H, d) 8,63 (1H, s) 8,42 (1H, d) 8,17 (1H, dd) 2,54 (3H, s).

LCMS (método de patrón de ácido fórmico) tiempo de retención = 1,43 min.

MS ($M+H^+$): 294.

INTERMEDIO 28: 6-cloro-7-metilsulfanil-9H-ß-carbolin-8-ilamina

Un matraz de fondo redondo de 500 ml con agitador magnético se cargó con 6-cloro-7-metilsulfanil-8-nitro-9H-ß-carbolina (4,011 g, 13,6 mmol) y 200 ml de etanol anhidro. A la mezcla resultante se le añadieron cloruro amónico acuoso (75 ml de una solución 0,33 M, 24,7 mmol), ácido clorhídrico acuoso (10 ml de una

solución 1 M, 10 mmol) y polvo de hierro (7,734 g, 138 mmol). La mezcla resultante se calentó a 60°C (baño de aceite) y se agitó vigorosamente durante 3,5 h. La reacción se enfrió a TA, se diluyó con EtOAc (75 ml) y se añadió carbono activado (aprox. 2,5 g). La mezcla resultante se agitó a TA durante 1,5 h más, se filtró a través de una capa de celite y el filtrado resultante se concentró (evaporador rotatorio, después bomba de vacío) para producir 5,153 g de un sólido de color amarillento-naranja. El sólido se redisolvió en MeOH (50-100 ml) y se añadió lentamente a bicarbonato sódico acuoso saturado (500 ml) con agitación. La mezcla se agitó a TA durante 45 min y el sólido resultante se recogió por filtración por succión y se secó al aire para producir 3,476 g de 6-cloro-7-metilsulfanil-9H-ß-carbolin-8-ilamina en forma de un sólido castaño que se usó sin purificación adicional en las siguientes etapas.

^{1}H RMN (300 MHz, Metanol-d_4): d 8,87 (1H, s) 8,35-8,24 (1H, m) 8,16-8,06 (1H, m) 7,67 (1H, s) 2,32 (3H, s).

LCMS (método de patrón de acetato amónico) tiempo de retención = 2,12 min.

MS ($M+H^+$): 264.

EJEMPLO 16: N-(6-cloro-7-metilsulfanil-9H-ß-carbolin-8-il)-2-metil-nicotinamida

Un matraz de fondo redondo de 250 ml con agitador magnético se cargó con 6-cloro-7-metilsulfanil-9H-ß-carbolin-8-ilamina (*Intermedio 28*, 2,336 g, 8,86 mmol) y ácido 2-metilnicotínico (3,219 g, 23,4 mmol) en 80 ml de piridina anhidra. A la mezcla de reacción resultante a TA se le añadió clorhidrato de 1-(3-dimetilaminopropil)-3-etilcarbodiimida sólido (7,080 g, 36,9 mmol) y la mezcla de reacción se calentó a 100°C (baño de aceite) durante 2 días. La mezcla resultante se enfrió a TA y se concentró (evaporador rotatorio) para producir un residuo pardo. El residuo se redisolvió en MeOH (50 ml), se añadió lentamente a una mezcla en agitación de 5:1 de H_2O/bicarbonato sódico acuoso saturado (600 ml) y se agitó a TA durante ~18 h. El sólido precipitado se recogió por filtración por

succión, se lavó con Et_2O (2 x 150 ml) y se secó al aire para producir 3,036 g de N-(6-cloro-7-metilsulfanil-9H-ß-carbolin-8-il)-2-metil-nicotinamida bruta en forma de un sólido pardo. El material bruto se purificó por HPLC (rendimientos = ~40–60%)

^{1}H RMN (300 MHz, DMSO-d6): d 11,72 (1H, s) 10,50 (1H, s) 8,97 (1H, s) 8,62 (1H, dd) 8,57 (1H, s) 8,41 (1H, d) 8,33-8,27 (1H, m) 8,21 (1H, d) 2,73 (3H, s) 2,41 (3H, s).

LCMS (método de patrón de acetato amónico) tiempo de retención = 1,89 min.

MS ($M+H^+$): 383.

INTERMEDIO 29: 6-cloro-7-etilsulfanil-8-nitro-9H-ß-carbolina

Un matraz de fondo redondo de 25 ml con un agitador magnético se cargó con 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina (102 mg, 0,38 mmol) en 5 ml de DMF anhidra. A la mezcla naranja resultante a TA se le añadió lentamente tioetóxido sódico (puro al 80%, 69,7 mg, 0,66 mmol) en forma de polvo. La mezcla de reacción se agitó a TA durante 45 minutos y después se añadió gota a gota a una mezcla de 5:1 de H_2O/bicarbonato sódico saturado (~30 ml). El sólido precipitado resultante se recogió por filtración por succión, se lavó con 1:1 de hexanos/éter dietílico (2 x 20 ml) y se secó al aire para producir 95,0 mg de 6-cloro-7-etilsulfanil-8-nitro-9H-ß-carbolina en forma de un sólido naranja (79%).

^{1}H RMN (300 MHz, CD_3OD, ppm) d 8,91 (1H, s) 8,63 (1H, s) 8,42 (1H, d) 8,18 (1H, d) 3,04 (2H, q) 1,20 (3H, t). Tiempo de retención (LC, método de patrón de ácido fórmico): 1,71 min.

MS ($M+H^+$): 308.

INTERMEDIO 30: 6-cloro-7-etilsulfanil-9H-ß-carbolin-8-ilamina

Un matraz de fondo redondo de 50 ml con agitador magnético se cargó con 6-cloro-7-etilsulfanil-8-nitro-9H-ß-carbolina (85,0 mg, 0,28 mmol) en 10 ml de etanol anhidro. A la mezcla naranja resultante a TA se le añadieron cloruro

amónico acuoso 0,33 M (2,0 ml, 0,66 mmol) y polvo de hierro (680 mg, 12,2 mmol). La mezcla de reacción se calentó a 60°C y se agitó vigorosamente durante 20 h. Luego, la mezcla se enfrió a TA, se diluyó con acetato de etilo (15 ml) y se añadió carbono activado (~180 mg). La mezcla resultante se filtró a través de una capa de celite y el filtrado se concentró para producir 77,8 mg de 6-cloro-7-etilsulfanil-9H-ß-carbolin-8-ilamina en forma de un sólido amarillo (>99%).

^{1}H RMN (300 MHz, CD_3OD, ppm) d 8,94 (1H, s) 8,33-8,29 (1H, m) 8,21-8,18 (1H, m) 7,73 (1H, s) 2,85 (2H, q) 1,21 (3H, t).

LCMS (método de patrón de acetato amónico) tiempo de retención = 2,13 min.

(M^+ = 278; M^- = 276).

EJEMPLO 17: N-(6-cloro-7-etilsulfanil-9H-ß-carbolin-8-il)-2-metil-nicotinamida

Un matraz de fondo redondo de 25 ml con agitador magnético se cargó con 6-cloro-7-etilsulfanil-9H-ß-carbolin-8-ilamina (37,2 mg, 0,13 mmol) y ácido 2-metilnicotínico (36,2 mg, 0,26 mmol) en 3 ml de piridina anhidra. A la mezcla naranja clara resultante a TA se le añadió clorhidrato de 1-(3-dimetilaminopropil)-3-etilcarbodiimida sólido (73,2 mg, 0,38 mmol) y la mezcla de reacción resultante se calentó a 80°C durante 5 días. Luego, la mezcla de reacción se enfrió a TA y se concentró para producir un jarabe viscoso pardo. el jarabe se disolvió en una cantidad mínima de MeOH (~2 ml), se añadió lentamente a una mezcla 5:1 de H_2O/bicarbonato sódico saturado (~20 ml) y se agitó a TA durante 2,5 h. El sólido precipitado resultante se recogió por filtración por succión, se lavó con 1:1 de hexanos/éter dietílico (2 x 20 ml) y se secó al aire para producir 21,0 mg de N-(6-cloro-7-etilsulfanil-9H-ß-carbolin-8-il)-2-metil-nicotinamida en forma de un sólido castaño (~38%). LCMS (método de patrón de acetato amónico) tiempo de retención = 2,33 min. (M^+ = 397; M^- = 395).

^{1}H RMN (300 MHz, CD_3OD, ppm) d 8,89 (1H, s) 8,63-8,58 (1H, m) 8,44-8,38 (2H, m) 8,36 (1H, d) 8,15 (1H, d) 7,52-7,44 (1H, m) 2,98 (2H, c) 2,84 (3H, s) 1,20 (3H, t).

INTERMEDIO 31: [2-(6-cloro-8-nitro-9H-ß-carbolin-7-ilsulfanil)-etil]-dimetil-amina

Un matraz de fondo redondo de 25 ml con agitador magnético se cargó con 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina (98 mg, 0,37 mmol) en 2 ml de DMF anhidra. Un segundo matraz de fondo redondo de 10 ml con agitador magnético se cargó con clorhidrato de 2-dimetilamino-etanotiol (100 mg, 0,70 mmol) en 2 ml de DMF anhidra. A la suspensión resultante se le añadió n-butillitio (0,43 ml de una solución 1,6 M en hexanos, 0,69 mmol) mediante una jeringa y la mezcla se agitó durante 5 min a TA. Luego, la solución del tioanión se añadió mediante una jeringa a 6-cloro-7-fluoro-8-nitro-9H-ß-carbolina, y la solución roja resultante se agitó a TA durante 30 min. La mezcla de reacción se añadió lentamente a una mezcla 5:1 de H_2O/bicarbonato sódico acuoso saturado (30 ml) y se dejó en reposo a TA durante varias horas. El sólido precipitado resultante se recogió por filtración por succión y se secó al aire para producir 109 mg de [2-(6-cloro-8-nitro-9H-ß-carbolin-7-ilsulfanil)-etil]-dimetil-amina en forma de un sólido naranja (83%).

LCMS (método de patrón de acetato amónico) tiempo de retención = 1,35 min.

(M^+ = 351; M^- = 349).

^{1}H RMN (300 MHz, CD_3OD, ppm) d 8,92 (1H, d, J = 1,0 Hz) 8,66 (1H, s) 8,43 (1H, d) 8,19 (1H, dd) 3,18-3,13 (2H, m) 2,57-2,52 (2H, m) 2,21 (6H, s).

INTERMEDIO 32: 6-cloro-7-(2-dimetilamino-etilsulfanil)-9H-ß-carbolin-8-ilamina

Un matraz de fondo redondo de 50 ml con un agitador magnético se cargó con [2-(6-cloro-8-nitro-9H-ß-carbolin-7-ilsulfanil)-etil]-dimetil-amina (106 mg, 0,30 mmol) en 8 ml de etanol anhidro. A la mezcla naranja resultante a TA se le añadieron cloruro amónico acuoso 0,33 M (1,95 ml, 0,64 mmol) y polvo de hierro

(540 mg, 9,67 mmol). La mezcla de reacción se calentó a 60°C y se agitó vigorosamente durante 20 h. Luego, la mezcla se enfrió a TA, se diluyó con acetato de etilo (20 ml) y se añadió carbono activado (aprox. 150 mg). La mezcla resultante se filtró a través de una capa de celite y el filtrado resultante se concentró para producir 103 mg de 6-cloro-7-(2-dimetilamino-etilsulfanil)-9H-ß-carbolin-8-ilamina en forma de un sólido amarillo. El producto bruto se usó directamente en la etapa de acoplamiento.

LCMS (método de patrón de acetato amónico) tiempo de retención = 1,34 min.

(M^+ = 321; M^- = 319).

^{1}H RMN (300 MHz, CD_3OD, ppm) d 8,87 (1H, s) 8,30 (1H, d) 8,06 (1H, d) 7,75 (1H, s) 3,23-3,13 (4H, m) 2,84 (6H, s).

EJEMPLO 18: N-[6-cloro-7-(2-dimetilamino-etilsulfanil)-9H-ß-carbolin-8-il]-2-metil-nicotinamida

Un matraz de fondo redondo de 25 ml con agitador magnético se cargó con 6-cloro-7-(2-dimetilamino-etilsulfanil)-9H-ß-carbolin-8-ilamina (45,2 mg, 0,14 mmol) y ácido 2-metilnicotínico (39,0 mg, 0,28 mmol) en 4,5 ml de piridina anhidra. A la mezcla naranja clara resultante a TA se le añadió clorhidrato de 1-(3-dimetilaminopropil)-3-etilcarbodiimida sólido (95,0 mg, 0,49 mmol). La mezcla de reacción resultante se calentó a 80°C durante 3 días. Luego, la reacción se enfrió a TA y se concentró para producir un jarabe viscoso pardo. el jarabe se disolvió en una cantidad mínima de MeOH (~2 ml), se añadió lentamente a una mezcla 5:1 de H_2O/bicarbonato sódico saturado (~25 ml) y se extrajo con acetato de etilo (3 x 30 ml). Las capas orgánicas combinadas se lavaron con salmuera (1 x 30 ml), se secaron sobre Na_2SO_4, se filtraron y se concentraron para producir un residuo pardo (79,6 mg). El residuo se redisolvió en MeOH (~5 ml) y se filtró a través de un lecho de algodón. Al filtrado resultante se le añadió HCl en 1,4-dioxano (1,0 ml, 4,0 mmol), la solución resultante se agitó a TA durante 3 h y se añadió gota a gota

a éter dietílico (30 ml) con agitación. El producto precipitado resultante se recogió por filtración por succión, se lavó con éter y se secó al aire para producir 36,3 mg de tris-clorhidrato de N-[6-cloro-7-(2-dimetilamino-etilsulfanil)-9H-ß-carbolin-8-il]-2-metil-nicotinamida en forma de un polvo amarillo.

^{1}H RMN (300 MHz, CD_3OD, ppm) d 8,95 (1H, s) 8,62 (1H, dd) 8,43 (1H, s) 8,38-8,34 (2H, m) 8,16 (1H, d) 7,50 (1H, dd) 3,09 (2H, t) 2,85 (3H, s) 2,30 (2H, t) 1,99 (6H, s).

LCMS (método de patrón de acetato amónico) tiempo de retención = 1,56 min.

(M^+ = 440; M^- = 438).

INTERMEDIO 33: 4-terc-butil éster del ácido morfolin-3(S),4-dicarboxílico

Una suspensión de ácido morfolin-3(S)-carboxílico (2,00 g, 15,3 mmol) en DMF (75 ml) se agitó a TA. Se añadieron trietilamina (7,47 ml, 53,6 mmol) y dicarbonato de di-terc-butilo (BOC_2O, 4,02 g, 18,4 mmol). La suspensión se agitó a TA durante una hora, tiempo durante el cual la reacción formó una solución amarilla transparente. La solución se concentró a un volumen reducido (~25 ml) y se diluyó con agua (15 ml) y HCl 1 N (15 ml). La mezcla se vertió en un embudo de decantación, se diluyó de nuevo con agua (100 ml) y salmuera (100 ml) y se extrajo con Et_2O (3 x 100 ml). La capa orgánica se lavó con salmuera, se secó, se filtró y se concentró para producir un sólido blanco. El sólido, que contenía exceso de BOC_2O, se disolvió en Et_2O (500 ml) y se extrajo con NaOH 1 N (3 x 100 ml). La capa acuosa se acidificó con HCl 6 N aproximadamente a un pH de 2 y después se extrajo rápidamente con Et_2O (3 x 100 ml). La capa de Et_2O se secó, se filtró y se concentró para producir un sólido blanco(3,07 g).

^{1}H RMN (300 MHz, DMSO-d6): d 12,95 (s a, 1); 4,34-4,30 (m, 1); 4,18-4,10 (m, 1); 3,83-3,74 (m, 1); 3,59-3,51 (m, 2); 3,39-3,32 (m, 1); 3,21-2,95 (m, 1); 1,41-1,36 (m, 9).

Condiciones convencionales de NH_4OAc.

ELSD R_t = 1,08 min.

M-H = 230.

EJEMPLO 19: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-metil-morfolin-3(S)-carboxílico

Una suspensión de ácido morfolin-3(S)-carboxílico (3,00 g, 22,9 mmol) en EtOH (115 ml) se agitó a TA. Se añadió una solución de CH_2O acuoso (3,42 ml, 45,8 mmol, 37% p/p en H_2O), seguido de $Pd(OH)_2$ (600 mg, 20% p/p sobre carbono). El matraz se cargó con hidrógeno (1 atm) y la suspensión gris se agitó durante 24 h a TA en una atmósfera de hidrógeno. El matraz se purgó con nitrógeno y la suspensión negra se diluyó con MeOH, se filtró a través de papel de filtro y se concentró a un volumen reducido. La solución gris pálida se filtró a través de un filtro de jeringa de 0,45 µm para retirar el $Pd(OH)_2$ residual y se concentró para producir un aceite incoloro transparente. El aceite se puso a alto vacío durante 24 h y se aisló una espuma sólida blanca. La espuma se disolvió en piridina (200 ml) y se añadió 6-cloro-9H-ß-carbolin-8-ilamina (3,74 g, 17,2 mmol), seguido de EDCI (5,87 g, 30,6 mmol). La solución naranja pálida transparente se agitó a TA durante 24 h. La solución se diluyó con H_2O (300 ml) se vertió en un embudo de decantación que contenía EtOAc (300 ml). La mezcla se agitó y las capas se separaron. La capa acuosa se extrajo con EtOAc (3 x 150 ml) y las capas orgánicas combinadas se lavaron con H_2O y salmuera. La capa orgánica se secó, se filtró y se concentró para producir un aceite pardo que se puso a alto vacío. La espuma parda resultante se trituró con MeOH y el precipitado que se formó se filtró y se lavó con MeOH. El sólido amarillo pálido resultante se purificó por HPLC quiral para producir un sólido blanco (3,23 g).

^{1}H RMN (300 MHz, DMSO-d6): d 11,36 (s, 1); 10,02 (s, 1); 9,04 (s, 1); 8,38 (d, 1); 8,22-8,21 (m, 1); 8,15 (d, 1); 7,91-7,90 (m, 1); 4,00 (dd, 1); 3,85-3,81 (m, 1); 3,69-3,58 (m, 2); 2,99-2,95 (dd, 1); 2,89-2,85 (m, 1); 2,32 (s, 3); 2,32-2,24 (m, 1).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,89 min.

M+H = 345.

HPLC preparativa quiral: 10% v/v de EtOH/Hexanos.

columna Chiralcel OD.

R_t = 11,5 - 14 min.

Pureza enantiomérica del producto =99% de ee.

MÉTODO C: PROCEDIMIENTO PARA ANÁLOGOS SUSTITUIDOS DE 4-MORFOLINA

Como se ha indicado para el *Intermedio 34*, *Intermedio 35* y Ejemplo 20:

INTERMEDIO 34: éster terc-butílico del ácido 3(S)-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-morfolin-4-carboxílico

Una solución de 4-terc-butil éster del ácido morfolin-3(S),4-dicarboxílico (2,83 g, 12,7 mmol) en piridina (106 ml) se agitó a TA. Se añadió 6-cloro-9H-ß-carbolin-8-ilamina (2,30 g, 10,6 mmol), seguido de EDCI (4,06 g, 21,2 mmol). La solución naranja a parda transparente se agitó a TA durante 14 h. La solución se diluyó con H_2O (120 ml) se vertió en un embudo de decantación que contenía EtOAc (200 ml), H_2O (100 ml) y salmuera (100 ml). La mezcla se agitó y las capas se separaron. La capa acuosa se extrajo con EtOAc (2 x 50 ml) y las capas orgánicas combinadas se lavaron con salmuera. La capa orgánica se secó, se filtró y se concentró a un volumen reducido, y después se añadió gota a gota a una solución en agitación de 1:1 de Et_2O/hexanos (500 ml). El precipitado que se formó se filtró y se lavó con 1:1 de Et_2O/Hexanos. El filtrado se concentró a un volumen reducido y se recogió un segundo cultivo del precipitado. El producto sólido se puso a alto vacío durante 2 h para producir terc-butil éster del ácido 3(S)-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-morfolin-4-carboxílico en forma de un sólido de amarillo pálido a pardo pálido (4,36 g).

^{1}H RMN (300 MHz, DMSO-d6): d 11,30 (s, 1); 10,13 (s, 1); 9,06 (s, 1); 8,40-8,38 (m, 1); 8,19-8,16 (m, 2); 7,98 (s, 1); 4,67-4,47 (m, 2); 3,96-3,60 (m, 2); 3,64-3,39 (m, 3); 1,42 (s, 9).

Condiciones convencionales de NH_4OAc.

DAD R_t = 2,31 min.

M+H = 431.

INTERMEDIO 35: terc-butil éster del ácido 2(R)-[3(S)-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-morfolin-4-ilmetil]-pirrolidina-1-carboxílico

Una solución de terc-butil éster del ácido 3(S)-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-morfolin-4-carboxílico (1,00 g, 2,32 mmol) en CH_2Cl_2 (6 ml) se agitó a TA. Se añadió ácido trifluoroacético (6 ml) y la solución se agitó a TA durante 45 min y después se concentró hasta un residuo. El residuo se concentró una vez más en CH_2Cl_2 para producir un sólido amarillo-pardo que se disolvió en THF (13 ml) en una atmósfera de argón. En algunas ocasiones se requirió un calentamiento suave para garantizar la completa disolución. Se añadió una solución de N-(terc-butoxicarbonil)-D-prolinal (693 mg, 3,48 mmol) en THF (2 ml), seguido de triacetoxiborohidruro sódico (738 mg, 3,48 mmol). La solución se agitó a TA durante 30 min y después se inactivó mediante la adición de NaOH acuoso 1 N (30 ml). La mezcla se vertió en un embudo de decantación que contenía EtOAc (100 ml), H_2O (100 ml) y salmuera (100 ml). La mezcla se agitó y las capas se separaron. La capa acuosa se extrajo con EtOAc (2 x 50 ml) y las capas orgánicas combinadas se lavaron con salmuera. La capa orgánica se secó, se filtró y se concentró para producir un sólido pardo claro. La cromatografía en columna (MeOH al 2%-4%/CH_2Cl_2) produjo terc-butil éster del ácido 2(R)-[3(S)-(6-Cloro-9H-ß-carbolin-8-ilcarbamoil)-morfolin-4-ilmetil]-pirrolidina-1-carboxílico en forma de un sólido blanco (915 mg).

^{1}H RMN (300 MHz, DMSO-d6): d 11,30 (s, 1); 9,88 (s, 1); 9,04 (s, 1); 8,39-8,37 (m, 1); 8,20-8,15 (m, 2); 7,95 (s, 1); 3,99-3,82 (m, 3); 3,69-3,63 (m, 2); 3,44-

3,32 (m, 1); 3,27-3,11 (m, 3); 2,92-2,80 (m, 1); 2,44-2,32 (m, 1); 1,99-1,67 (m, 5); 1,33 (s, 9).

condiciones convencionales de HCOOH.

DAD R_t = 1,39 min.

M+H = 514.

HPLC quiral.

Se comprobó la pureza enantiomérica de la muestra. Las muestras fueron =97% de ee.

columna Chiralpak AD.

EtOH al 15% v/v/Hexanos que contenía Et_2NH al 0,1%.

EJEMPLO 20: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-pirrolidin-2(R)-ilmetil-morfolin-3(S)-carboxílico, sal HCl)

A una solución de terc-butil éster del ácido 2(R)-[3(S)-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-morfolin-4-ilmetil]-pirrolidina-1-carboxílico (850 mg, 1,65 mmol) en MeOH (16 ml) se le añadió HCl acuoso concentrado (13 ml). La solución se agitó a TA durante 30-45 min, tiempo durante el cual se formó un precipitado amarillo. La mezcla de reacción se concentró para producir (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-pirrolidin-2(R)-ilmetil-morfolin-3(S)-carboxílico, sal HCl) en forma de un sólido amarillo pálido (755 mg).

^{1}H RMN (300 MHz, DMSO-d6): d 13,30 (s, 1); 11,56 (s a, 1); 9,63 (s a, 1); 9,46 (s, 1); 8,87-8,85 (m, 1); 8,66-8,57 (m, 2); 8,25 (s, 1); 4,38-4,26 (m, 1); 4,24-4,08 (m, 1); 4,04-3,86 (m, 2); 3,86-3,68 (m, 2); 3,57-3,39 (m, 1); 3,39-3,02 (m, 4); 2,99-2,76 (m, 1); 2,10-1,84 (m, 3); 1,75-1,56 (m, 1).

condiciones convencionales de HCOOH.

DAD R_t = 0,81 min.

M+H = 414.

INTERMEDIO 36: (6-cloro-9H-carbolin-8-il) amida del ácido cis-2-(terc-butoxicarbonilamino)-ciclopentanocarboxílico

Una solución de ácido cis-2-(terc-butoxicarbonilamino)-ciclopentanocarboxílico (550 mg, 2,4 mmol) en piridina (10 ml) se agitó a TA. Se añadió 6-cloro-9H-ß-carbolin-8-ilamina (436 mg, 2,0 mmol), seguido de EDCI (615 mg, 3,2 mmol) y la solución naranja se agitó a TA durante 1,5 h. La solución se diluyó con H_2O (20 ml) y se vertió en un embudo de decantación que contenía H_2O (50 ml) y EtOAc (100 ml). La mezcla se agitó y las capas se separaron. La capa acuosa se extrajo con EtOAc (100 ml). Las capas orgánicas combinadas se lavaron con salmuera, se secaron sobre $MgSO_4$, se filtraron y se concentraron hasta sólidos oleosos naranjas que después se trituraron con MeOH al 5% en Et_2O (20 ml) y se capturaron por filtración para producir (6-cloro-9H-carbolin-8-il)amida del ácido cis-2-(terc-butoxicarbonilamino)-ciclopentanocarboxílico en forma de un sólido amarillo claro (740 mg).

^{1}H RMN (300 MHz, DMSO-d6): d 11,17 (s, 1); 9,89 (s, 1); 9,04 (s, 1); 8,36 (d, 1); 8,18-8,08 (m, 2); 7,95 (s, 1); 6,92 (d, 1); 4,32-4,22 (m, 1); 3,16-3,09 (m, 1); 2,13-2,01 (m, 1); 1,96-1,75 (m, 3); 1,74-1,59 (m, 1); 1,58-1,42 (m, 1); 1,07 (s, 9).

Condiciones convencionales de NH_4OAc.

DAD R_t = 2,52 min.

M+H = 429.

EJEMPLO 21: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido cis-2-amino-ciclopentanocarboxílico

Una solución de (6-cloro-9H-ß-carbolin-8-il)amida del ácido 2-(terc-butoxicarbonilamino)-ciclopentanocarboxílico (736 mg, 1,72 mmol) en ácido trifluoroacético (5 ml) se agitó a TA durante 20 min y después se concentró hasta un aceite naranja. El aceite se disolvió en MeOH (5 ml) y se neutralizó con una solución acuosa saturada de bicarbonato sódico (25 ml). La mezcla resultante se diluyó de nuevo con H_2O (25 ml) y EtOAc (100 ml). La capa acuosa se retiró y se

extrajo con EtOAc (100 ml). Las fases orgánicas se combinaron, se lavaron con salmuera, se secaron sobre $MgSO_4$, se filtraron y se concentraron hasta sólidos amarillos (507 mg). Estos sólidos se disolvieron en MeOH (5 ml) y se añadió una solución de HCl en dioxano (4 M, 1,5 ml). La solución amarilla brillante se agitó durante 30 min y después se concentró para producir (6-cloro-9H-ß-carbolin-8-il)-amida del ácido cis-2-amino-ciclopentanocarboxílico en forma de un polvo amarillo (600 mg).

^{1}H RMN (300 MHz, DMSO-d6): d 9,29 (s, 1); 8,75 (d, 1); 8,53 (d, 1); 8,37 (s, 1); 8,02 (s, 1); 4,05-3,95 (m, 1); 3,42-3,34 (m, 1); 2,46-1,80 (m, 6).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,65 min.

M+H = 329.

EJEMPLO 22: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-(2-amino-etil)-morfolin-3-carboxílico, sal HCl

Se siguió el *Método C* usando ácido morfolin-3-carboxílico racémico alquilado reductoramente con 2-aminoacetaldehído.

^{1}H RMN (300 MHz, MeOH-d4): d 9,37 (s, 1); 8,76 (d, 1); 8,55 (d, 1); 8,44 (d, 1); 8,06 (d, 1); 4,68-4,55 (m, 2); 4,17-3,99 (m, 3); 3,84-3,73 (m, 2); 3,57-3,39 (m, 4).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,69 min.

M+H = 374.

EJEMPLO 23: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-(2(S)-amino-propil)-morfolin-3(S)-carboxílico, sal HCl (primer diastereómeros de elución)

Se siguió el *Método C* usando ácido morfolin-3-carboxílico racémico alquilado reductoramente con el aldehído de alanina apropiado. Los

diastereómeros se separaron por cromatografía en columna antes de la etapa de desprotección.

^{1}H RMN (300 MHz, DMSO-d6): d 9,32 (s, 1); 8,76 (d); 8,55 (d, 1); 8,41 (s, 1); 8,08 (s, 1); 4,38 (d, 1); 4,32-4,21 (m, 1); 4,16-4,09 (m, 1); 4,04-3,95 (m, 2); 3,79-3,57 (m, 2); 3,47-3,40 (m, 1); 3,22-3,05 (m, 2); 1,44 (d, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,38 min.

M+H = 388.

EJEMPLO 24: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-(2(S)-amino-propil)-morfolin-3(R)-carboxílico, sal HCl (segundo diastereómero de elución)

Se siguió el *Método C* usando ácido morfolin-3-carboxílico racémico alquilado reductoramente con el aldehído de alanina apropiado. Los diastereómeros se separaron por cromatografía en columna antes de la etapa de desprotección.

^{1}H RMN (300 MHz, DMSO-d6): d 9,34 (s, 1); 8,77 (d, 1); 8,55 (d, 1); 8,42 (s, 1); 8,06 (s, 1); 4,42 (d, 1); 4,30-4,12 (m, 1); 4,07-3,92 (m, 3); 3,89-3,74 (m, 1); 3,65-3,49 (m, 1); 3,25-2,90 (m, 3); 1,36 (d, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,57 min.

M+H = 388.

EJEMPLO 25: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 2-amino-ciclohexanocarboxílico

Una solución de ácido cis-2-(terc-butoxicarbonilamino)-ciclohexano-carboxílico (255 mg, 1,05 mmol) en piridina (10 ml) se agitó a TA. Se añadió 6-cloro-9H-ß-carbolin-8-ilamina (218 mg, 1,00 mmol) seguido de EDCI (315 mg, 1,64 mmol) y la solución naranja ligeramente turbia se agitó a TA durante 16 h. La solución se diluyó con H_2O (20 ml) y se vertió en un embudo de decantación que

contenía H_2O (50 ml) y EtOAc (50 ml). La mezcla se agitó y las capas se separaron. La capa acuosa se extrajo con EtOAc (50 ml) y las capas orgánicas combinadas se lavaron con salmuera. La capa orgánica se secó, se filtró y se concentró para producir un aceite amarillo que se puso a alto vacío durante 4 h. El vidrio amarillo-pardo resultante se suspendió en CH_2Cl_2 (10 ml) a TA. Se añadió ácido trifluoroacético (5 ml) y la suspensión se disolvió instantáneamente para formar una solución naranja transparente. La solución se agitó a TA durante 45 min y después se concentró hasta un residuo pardo. El residuo se destiló azeotrópicamente en tolueno (3 x 10 ml) para producir un sólido amarillo. Se preparó una solución de Na_2CO_3 acuoso diluido mediante la adición de un pequeño volumen de Na_2CO_3 acuoso al 10% a H_2O (50 ml) hasta que la solución acuosa alcanzó un pH de 10. El sólido amarillo se disolvió en una cantidad mínima de MeOH y se añadió gota a gota a la solución acuosa con agitación. El precipitado que se formó se filtró, se lavó con H_2O y se puso a alto vacío para producir (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 2-amino-ciclohexanocarboxílico en forma de un sólido amarillo pálido (147 mg).

^{1}H RMN (300 MHz, DMSO-d6): d 9,00 (s, 1); 8,37-8,34 (m, 1); 8,16-8,13 (m, 2); 7,83 (m, 1); 5,66-5,00 (s a, 2); 3,42-3,40 (m, 1); 2,70-2,62 (m, 1); 2,02-1,90 (m, 1); 1,70-1,54 (m, 5); 1,42-1,29 (m, 2).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,46 min.

M+H = 343.

EJEMPLO 26: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-(2(R)-amino-propil)-morfolin-3(S)-carboxílico, sal HCl

Se siguió el *Método C* usando ácido morfolin-3-carboxílico racémico alquilado reductoramente con el aldehído de alanina apropiado. Los diastereómeros se separaron por cromatografía en columna antes de la etapa de desprotección.

^{1}H RMN (300 MHz, DMSO-d6): d 9,35 (s, 1); 8,77 (m, 1); 8,55 (m, 1); 8,43 (s, 1); 8,01 (s, 1); 4,45 (d, 1); 4,26 (m, 1); 4,09-3,91 (m, 3); 3,79 (m, 1); 3,63 (m, 1); 3,28-2,99 (m, 3); 1,37 (d, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,39 min.

M+H = 388.

EJEMPLO 27: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-(2(R)-amino-3-fenil-propil)-morfolin-3(S)-carboxílico, sal HCl

Se siguió el *Método C* usando ácido morfolin-3-carboxílico racémico alquilado reductoramente con el aldehído de alanina apropiado. Los diastereómeros se separaron por cromatografía en columna antes de la etapa de desprotección.

^{1}H RMN (300 MHz, DMSO-d6): d 9,34 (s, 1); 8,77 (d, 1); 8,55 (d, 1); 8,42 (s, 1); 8,04 (s, 1); 7,44-7,23 (m, 5); 4,39 (d, 1); 4,65-4,07 (m, 1); 4,40-3,82 (m, 4); 3,50-3,25 (m, 1); 3,30-3,14 (m, 1); 3,11-2,88 (m, 3); 2,85-2,69 (m, 1).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,90 min.

M+H = 464.

MÉTODO D: CONDICIONES CROMATOGRÁFICAS

LCMS

Tipo de columna: columnas Phenomenex Luna C18(2), 5 µm, tamaño 50 x 4,6 mm

Tiempo de ejecución: ejecución de 5,00 minutos

Condiciones de NH_4OAc:

Disolvente A:

NH_4OAc 10 mM

H_2O al 99%

MeCN al 1%

Disolvente B:

NH_4OAc 10 mM

H2O al 5%

MeCN al 95%

Gradiente convencional:

condiciones iniciales - A al 95%, B al 5%

gradiente de 3,5 minutos de B al 5%-100%

mantenido durante 3,5-4,3 minutos a B al 100%

condiciones iniciales durante 4,3-5

Gradiente polar:

condiciones iniciales - A al 70%, B al 30%

gradiente durante 3,5 minutos de B al 70%-100%

mantenido durante 3,5-4,3 a B al 100%

condiciones iniciales durante 4,3-5

Gradiente no polar:

condiciones iniciales - A al 100%

gradiente durante 3,5 minutos de B al 0%-50%

mantenido durante 3,5-4,3 minutos a B al 100%

condiciones iniciales durante 4,3-5

Condiciones de HCOOH:

Disolvente C:

HCOOH al 0,1%

H_2O al 99%

MeCN al 1%

Disolvente D:

HCOOH al 0,1%

H_2O al 5%

- MeCN al 95%

Gradiente convencional:

condiciones iniciales - C al 95%, D al 5%

gradiente durante 3,5 minutos de D al 5%-100%

mantenido durante 3,5-4,3 a D al 100%

condiciones iniciales durante 4,3-5 minutos

Gradiente polar:

condiciones iniciales - C al 70%, D al 30%

gradiente durante 3,5 minuto de D al 70%-100%

mantenido durante 3,5-4,3 a D al 100%

condiciones iniciales durante 4,3-5 minutos

Gradiente no polar:

condiciones iniciales - C al 100%

gradiente durante 3,5 minutos de D al 0%-50%

mantenido durante 3,5-4,3 a D al 100%

condiciones iniciales durante 4,3-5 minutos

INTERMEDIO 37: 6-cloro-2,3,4,9-tetrahidro-1H-ß-carbolina, sal HCl

Se disolvió clorhidrato de 5-clorotriptamina (5 g, 20 mmol, 1 equiv.) en 40 ml de tampón NaOAc 3 M (pH = 4,8) y 40 ml de agua. Se añadió en una porción ácido glioxálico (1,84 g, 20 mmol, 1 equiv.) y la solución se agitó a TA durante una noche. La suspensión pegajosa resultante se filtró y los sólidos verdes claros se suspendieron en 100 ml de HCl 6 N y se calentaron a 125°C en un condensador de reflujo durante 1 hora con adiciones intermitentes de HCl conc. (2 ml cada 15 min). Después de enfriar a TA, se añadieron 4,38 g (90%) de 6-cloro-2,3,4,9-tetrahidro-1H-ß-carbolina, sal HCl en forma de un sólido azul-gris que se aisló por filtración.

^{1}H RMN (300 MHz, DMSO-d6): d 11,33 (a, 2H), 9,62 (a, 2H), 7,53 (d, 1), 7,39 (d, 1), 7,09 (dd, 1), 4,33 (a, 2H), 2,92 (t, 2)

Condiciones convencionales de ácido fórmico.

DAD RT = 1,56 min.

M+H = 207.

INTERMEDIO 38: (6-cloro-1,3,4,9-tetrahidro-ß-carbolin-2-il)-fenil-metanona

Se suspendió 6-cloro-2,3,4,9-tetrahidro-1H-ß-carbolina, sal HCl (10,2 g, 42 mmol, 1 equiv.) en 100 ml de piridina seca en atmósfera de N_2 y se enfrió a 0°C en un baño de agua enfriada con hielo. A la solución enfriada se le añadió gota a gota cloruro de benzoílo (7,3 ml, 63 mmol, 1,5 equiv.) después de que la reacción se retirase del baño de hielo y se dejó en agitación durante una noche a temperatura ambiente. La reacción se interrumpió mediante la adición de agua hasta que se atascó con sólidos. Estos sólidos se recogieron por filtración, se lavaron con una solución acuosa saturada de bicarbonato sódico, se suspendieron de nuevo en agua, se sonicaron y se filtraron de nuevo para dar 1,27 g (94%) de (6-cloro-1,3,4,9-tetrahidro-ß-carbolin-2-il)-fenil-metanona en forma de sólidos naranjas cristalinos.

^{1}H RMN (300 MHz, DMSO-d6): d 10,65-10,59 (a, 1H), 7,0-7,5 (m, 9H), 4,60-4,83 (a, 2H), 3,62-3,99 (a, 2H), 2,75 (a, 2H).

Condiciones convencionales de ácido fórmico.

DAD RT = 2,68 min.

M+H = 311.

INTERMEDIO 39: 2-benzoil-6-cloro-1,2,3,9-tetrahidro-ß-carbolin-4-ona

Se mezclaron (6-cloro-1,3,4,9-tetrahidro-ß-carbolin-2-il)-fenil-metanona (1,76 g, 5,66 mmol, 1 equiv.) y DDQ (2,31 g, 10,2 mmol, 1,8 equiv.) en forma de sólidos y se enfriaron a -78°C. Se enfriaron 15 ml de una solución 9:1 de THF/H_2O a -78°C y la suspensión resultante se añadió a los sólidos enfriados seguido de 15 ml más de THF (también enfriado a -78°C). La solución azul oscura se agitó a -78°C durante 2 horas y después se calentó gradualmente a temperatura ambiente

y se agitó durante dos horas más. La reacción se interrumpió mediante la adición de NaOH 1 N y se extrajo con 3 x 150 ml de EtOAc. Las capas orgánicas combinadas se lavaron con 2 x 100 ml de HCl 1 N y 1 x 100 ml de salmuera, se secaron sobre $MgSO_4$, se filtraron y se concentraron para producir 1,38 g (75%) de 2-benzoil-6-cloro-1,2,3,9-tetrahidro-ß-carbolin-4-ona en forma de un sólidos oleosos naranjas.

^{1}H RMN (300 MHz, DMSO-d6): d 12,11-12,48 (a, 1H), 7,29-7,88 (m, 8H), 4,93-5,18 (a, 2H), 4,60-4,46 (a, 2H).

Masa exacta: 324,07.

Condiciones convencionales de ácido fórmico

DAD RT = 2,15 min.

M+H = 325.

INTERMEDIO 40: 4-amino-6-cloro β-carbolina

Se disolvió 2-benzoil-6-cloro-1,2,3,9-tetrahidro-ß-carbolin-4-ona bruta (4 g) en 30 ml de hidrazina anhidra y se agitó a reflujo (baño de aceite a 130°C) en atmósfera de N_2 durante 6 horas, después de lo cual la mezcla de reacción se dejó enfriar a temperatura ambiente y se dejó en reposo durante una noche. Los sólidos amarillos precipitados se retiraron por filtración y se lavaron con agua, 2 x 5 ml, para producir 785 mg (30%) de 4-amino-6-cloro-β-carbolina en forma de un sólido blanquecino. Se añadió más agua a los filtrados combinados hasta que no se produjo más precipitación. Estos sólidos también se retiraron por filtración para dar 1,056 g (39%) de 4-amino-6-cloro-β-carbolina en forma de sólidos amarillos, (rendimiento total de 69%).

^{1}H RMN (300 MHz, DMSO-d6): d 11,48 (s, 1H), 8,44 (s, 1H), 8,13 (s, 1H), 7,77 (s, 1H), 7,42-7,52 (m, 2H), 5,86 (s, 2H).

Condiciones convencionales de ácido fórmico.

DAD RT = 1,68 min.

M+H = 218.

INTERMEDIO 41: N-(6-cloro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida

Se disolvió 4-amino-6-cloro-β-carbolina (1,05 g, 4,82 mmol, 1 equiv.) en 4 ml de piridina anhidra y 20 ml de THF y se enfrió a 0°C en atmósfera de N_2. A la solución enfriada se le añadió gota a gota ácido trifluoroanhídrido (3,4 ml, 24 mmol, 5 equiv.). Después de que se completara la adición, la reacción se retiró del baño de hielo y se agitó a temperatura ambiente durante ~1,5 horas. La reacción se interrumpió mediante la adición lenta de agua (10 ml) y se extrajo con 2 x 150 ml de EtOAc, se lavó con 2 x 100 ml de bicarbonato sódico acuoso saturado y con 1 x 100 ml de salmuera, se secó sobre $MgSO_4$, se filtró y se concentró hasta sólidos naranjas. Estos sólidos se trituraron en 10-15 ml de Et_2O y se recogieron por filtración para dar 1,23 g (81%) de N-(6-cloro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida en forma de un sólido amarillo.

^{1}H RMN (300 MHz, DMSO-d6): d 12,11 (s, 1H), 11,89 (s, 1H), 8,92 (s, 1H), 8,33 (s, 1H), 7,82 (s, 1H), 7,60-7,70 (m, 2H).

Condiciones convencionales de ácido fórmico.

DAD RT = 2,12 min.

M+H = 314.

INTERMEDIO 42: N-(6-cloro-8-nitro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida

Se disolvió N-(6-cloro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida (125 mg, 0,4 mmol, 1 equiv.) en 2 ml de TFA y se añadió en una porción $NaNO_2$ (541 mg, 7,84 mmol, 2 equiv.). La solución se agitó a temperatura ambiente durante 4 h. Los volátiles se retiraron con evaporador rotatorio y los sólidos oleosos naranjas resultantes se suspendieron en agua, se neutralizaron con una solución acuosa saturada de bicarbonato sódico y se filtraron para dar 132 mg (92%) de N-(6-cloro-8-nitro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida.

^{1}H RMN (300 MHz, DMSO-d6): d 12,87 (s, 1H), 12,03 (s, 1H), 9,11 (s, 1H), 8,56 (s, 1H), 8,53 (s, 1H), 8,26 (s, 1H).

Condiciones convencionales de ácido fórmico.

DAD RT = 2,27 min.

M+H = 359.

INTERMEDIO 43: N-(8-amino-6-cloro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida

La N-(6-cloro-8-nitro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida bruta (130 mg, 0,36 mmol) se disolvió en 7 ml de MeOH y el recipiente de reacción se purgó al vacío con 3 x N_2. Se añadió rápidamente platino (20 mg, al 20% en peso sobre carbono activado) y el recipiente de reacción se purgó de nuevo al vacío con 3 x N_2, seguido de 3 ciclos más de purgado al vacío con H_2. La reacción se dejó en agitación en atmósfera de H_2 a 1 atm durante una noche. Después de que se completara, el recipiente de reacción se purgó de H_2 y se filtró sobre celite. El celite se lavó varias veces con metanol hasta que los filtrados fueron transparentes. Los filtrados combinados se concentraron para dar N-(8-amino-6-cloro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida en forma de un sólido amarillo (112 mg, 95%).

^{1}H RMN (300 MHz, DMSO-d6): d 11,83 (s, 1H), 8,98 (s, 1H), 8,30 (s, 1H), 7,10 (s, 1H), 6,82 (s, 1H), 5,87 (a, 2H).

Condiciones convencionales de ácido fórmico.

DAD RT = 1,95 min.

M+H = 329.

INTERMEDIO 44: N-[6-cloro-4-(2,2,2-trifluoro-acetilamino)-9H-ß-carbolin-8-il]-2-metil-nicotinamida

Se disolvieron N-(8-amino-6-cloro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida (90 mg, 0,274 mmol, 1 equiv.) y ácido 2-metilnicotínico (45 mg, 0,329

mmol, 1,2 equiv.) en 1,5 ml de piridina anhidra en atmósfera de N_2. Se añadió en una porción EDCI (84 mg, 0,438 mmol, 1,6 equiv.) y la mezcla de reacción se agitó a temperatura ambiente durante 2 horas. La reacción se interrumpió con agua y los sólidos oscuros resultantes se recogieron por filtración. Estos sólidos se trituraron en una solución 3:1 de metanol-DMSO para dar N-[6-cloro-4-(2,2,2-trifluoro-acetilamino)-9H-ß-carbolin-8-il]-2-metil-nicotinamida en forma de sólidos amarillos claros (41 mg, 3%).

^{1}H RMN (300 MHz, DMSO-d6): d 11,94 (a, 1H), 11,78 (s, 1H), 10,61 (s, 1), 9,00 (s, 1), 8,64 (d, 1), 8,38 (s, 1H), 8,13 (d, 1), 8,01 (s, 1H), 7,73 (s, 1H), 7,45 (m, 1H), 2,68 (s, 3H).

Condiciones convencionales de ácido fórmico.

DAD RT = 1,98 min.

M+H = 448.

INTERMEDIO 45: N-[6-cloro-4-(2,2,2-trifluoro-acetilamino)-9H-ß-carbolin-8-il]-nicotinamida

Se disolvieron N-(8-amino-6-cloro-9H-ß-carbolin-4-il)-2,2,2-trifluoro-acetamida (90 mg, 0,274 mmol, 1 equiv.) y ácido 2-metilnicotínico (40 mg, 0,329 mmol, 1,2 equiv.) en 1,5 ml de piridina anhidra en atmósfera de N_2. Se añadió en una porción EDCI (84 mg, 0,438 mmol, 1,6 equiv.) y la mezcla de reacción se agitó a temperatura ambiente 2 horas. La reacción se interrumpió y se trató siguiendo el protocolo anterior para obtener 38 mg (32%) de N-[6-cloro-4-(2,2,2-trifluoro-acetilamino)-9H-ß-carbolin-8-il]-nicotinamida.

^{1}H RMN (300 MHz, DMSO-d6): d 11,89 (a, 1H), 10,73 (s, 1), 9,28 (s, 1), 8,97 (s, 1), 8,84 (d, 1), 8,43 (d, 1), 8,37 (s, 1), 7,85 (s, 1), 7,76 (s, 1), 7,67 (m, 1).

Condiciones convencionales de ácido fórmico.

DAD RT = 1,94 min.

M+H = 434.

EJEMPLO 28: N-(4-amino-6-cloro-9H-ß-carbolin-8-il)-2-metil-nicotinamida

Se suspendió N-[6-cloro-4-(2,2,2-trifluoro-acetilamino)-9H-ß-carbolin-8-il]-nicotinamida (41 mg, 0,092 mmol, 1 equiv.) en 5 ml de MeOH y se añadieron 2 ml de una solución acuosa de K_2CO_3 (127 mg, 0,92 mmol, 10 equiv.). La solución transparente resultante se calentó a 60°C durante 16 h y después se dejó enfriar a TA. Se añadió más agua, produciendo sólidos finos que se recogieron por filtración, se lavaron una vez con 10 ml de MeOH al 5% en Et_2O y se secaron a alto vacío para dar 18 mg de N-(4-amino-6-cloro-9H-ß-carbolin-8-il)-2-metil-nicotinamida en forma de sólidos amarillos en polvo (rendimiento de 56%).

^{1}H RMN (300 MHz, DMSO-d6): d 11,18 (s, 1H), 10,41 (s, 1H), 8,62 (d, J = 3,6, 1H),), 8,32 (s, 1H), 8,20 (s, 1H), 8,11 (d, J = 7,5, 1H), 7,91 (s, 1H), 7,79 (s, 1H), 7,43 (m, 1H), 5,91 (a, 2H), 2,66 (s, 3H).

Condiciones convencionales de ácido fórmico.

DAD RT = 1,63 min.

M+H = 352.

EJEMPLO 29: N-(4-amino-6-cloro-9H-ß-carbolin-8-il)-nicotinamida

Se suspendió N-[6-cloro-4-(2,2,2-trifluoro-acetilamino)-9H-ß-carbolin-8-il]-nicotinamida (38 mg, 0,088 mmol, 1 equiv.) en 5 ml de MeOH y se añadieron 2 ml de una solución acuosa de K_2CO_3 (121 mg, 0,92 mmol, 10 equiv.). La solución transparente resultante se calentó a 60°C durante 11 h. Después de enfriar a TA, precipitaron sólidos finos que se recogieron por filtración y se lavaron con 10 ml de agua para dar 2,78 mg (10%) de N-(4-amino-6-cloro-9H-ß-carbolin-8-il)-nicotinamida.

^{1}H RMN (300 MHz, DMSO-d6): d 11,32 (s, 1H), 10,56 (s, 1H), 9,26 (s, 1H), 8,82 (d, 1), 8,42 (d, 1), 8,36 (s, 1H), 8,18 (s, 1H), 7,79 (s, 1H), 7,70 (s, 1H), 7,64 (m, 1H), 5,91 (a, 2H).

Condiciones convencionales de ácido fórmico.

DAD RT = 1,55 min.

M+H = 338.

INTERMEDIO 46: éster terc-butílico del ácido 3-cianometil-indol-1-carboxílico

Una solución de 3-indoleacetonitrilo (10 g, 64 mmol) en DMF (160 ml) se agitó a TA. Se añadieron K_2CO_3 (13,3 g, 96 mmol) y dicarbonato de di-terc-butilo (15,35 g, 70 mmol) y la mezcla de reacción se agitó a TA durante 12 h. A la mezcla de reacción se le añadió H_2O (100 ml) y el precipitado resultante se recogió por filtración. Los sólidos se disolvieron en metanol caliente (20 ml) y la solución se dejó enfriar lentamente, produciendo sólidos naranjas claros que se aislaron por filtración para dar éster terc-butílico del ácido 3-cianometil-indol-1-carboxílico (9,2 g).

^{1}H RMN (300 MHz, DMSO-d6): d 8,08 (d, 1); 7,70-7,66 (m, 2); 7,42-7,29 (m, 2); 4,12 (s, 2); 1,63 (3, 9).

Condiciones convencionales de NH_4OAc.

DAD R_t = 3,31 min.

M+H = 257.

INTERMEDIO 47: éster terc-butílico del ácido 3-(ciano-metil-metil)-indol-1-carboxílico

Una solución agitada de éster terc-butílico del ácido 3-cianometil-indol-1-carboxílico (2,15 g, 8,39 mmol) en THF (40 ml) se enfrió a -78°C en una atmósfera de argón. Se añadió bis(trimetilsilil)amida sódica (1 M en THF, 10 ml, 10 mmol) y la solución fría se agitó durante 30 minutos. Se añadió yodometano (627 µl, 10 mmol) y la mezcla de reacción se agitó durante 1,5 h mientras se calentaba gradualmente a 0°C. Se añadió H_2O (100 ml) y la solución se llevó a TA y se diluyó con EtOAc (250 ml). La capa acuosa se retiró y se extrajo con EtOAc (250 ml). Las capas orgánicas combinadas se lavaron con HCl acuoso (1 N, 3 x 50 ml), seguido de salmuera, después se secaron sobre $MgSO_4$, se filtraron y se

concentraron hasta un aceite naranja. La purificación por cromatografía en columna (hexanos:acetato de etilo) dio éster terc-butílico del ácido 3-(ciano-metil-metil)-indol-1-carboxílico (1,8 g).

^{1}H RMN (300 MHz, DMSO-d6): d 8,09 (d, 1); 7,73 (d, 1); 7,69 (s, 1); 7,74-7,29 (m, 2); 4,56 (c, 1); 1,66 (d, J =.7,2, 3); 1,63 (s, 9).

INTERMEDIO 48: éster terc-butílico del ácido 3-(2-amino-1-metil-metil)-indol-1-carboxílico

Una solución de éster terc-butílico del ácido 3-(ciano-metil-metil)-indol-1-carboxílico (850 mg, 3,14 mol) en metanol (15 ml) se agitó a TA. Se añadió catalizador de níquel Raney (suspensión al 50% en peso en H_2O, 1 ml) y el recipiente de reacción se tapó y se purgó 3 veces al vacío con argón, seguido de forma similar por hidrógeno. La mezcla se agitó durante 22 h en 1 atm. de hidrógeno, se purgó al vacío con argón y se filtró a través de celite. El filtrado se concentró hasta un aceite (670 mg) y se determinó por LCMS que estaba compuesta principalmente del compuesto deseado éster terc-butílico del ácido 3-(2-amino-1-metil-metil)-indol-1-carboxílico.

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,78 min.

M+H = 275.

INTERMEDIO 49: Ácido 4-metil-2,3,4,9-tetrahidro-1H-ß-carbolina-1-carboxílico

Se disolvió éster terc-butílico del ácido 3-(2-amino-1-metil-metil)-indol-1-carboxílico bruto (670 mg, aprox. 2,44 mmol) en ácido trifluoroacético (2 ml), se agitó durante 30 min a TA y después se concentró hasta un sólido oleoso a presión reducida. El aceite resultante se disolvió en una solución 3 M de NaOAc:tampón AcOH (pH = 4,8, 12 ml) y H_2O (6 ml) a TA. Se añadió ácido glioxálico (225 mg, 2,44 mmol) y la mezcla de reacción se agitó durante 4 h a TA y después se concentró a sequedad. Se determinó que los sólidos resultantes se

componían principalmente del compuesto deseado ácido 4-metil-2,3,4,9-tetrahidro-1H-ß-carbolina-1-carboxílico por LCMS y se usaron posteriormente sin purificación.

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,24 min.

M+H = 231.

INTERMEDIO 50: 4-metil-2,3,4,9-tetrahidro-1H-ß-carbolina

Se suspendió ácido 4-metil-2,3,4,9-tetrahidro-1H-ß-carbolina-1-carboxílico bruto (aprox.. 2,44 mmol) en H_2O (5 ml) y HCl (12 N, 5 ml) y la suspensión se calentó a 120°C durante 1 h y después se dejó enfriar a TA. Los sólidos naranjas-pardos oscuros se retiraron por filtración y después se disolvieron en metanol (5 ml). Se añadió una solución saturada de bicarbonato sódico (20 ml), produciendo una suspensión amarilla pegajosa. Esta mezcla de reacción se filtró para producir sólidos amarillos compuestos principalmente por 4-metil-2,3,4,9-tetrahidro-1H-ß-carbolina (386 mg) según se determinó por LCMS.

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,23 min.

M+H = 187.

INTERMEDIO 51: 4-Metil-9H-ß-carbolina

Se suspendió 4-metil-2,3,4,9-tetrahidro-1H-ß-carbolina (214 mg, 1,17 mmol) en xilenos (10 ml). Se añadió catalizador de Pd (al 10% en peso sobre carbono, 21 mg) y la mezcla de reacción se agitó a 160°C durante 24 horas y después se enfrió a TA y se filtró a través de celite. El filtrado se concentró a sequedad para dar 4-metil-9H-ß-carbolina (210 mg).

^1H RMN (300 MHz, DMSO-d6): d 11,73 (s a, 1); 8,78 (s a, 1); 8,19 (d, 1); 8,13 (s, 1); 7,62 (s a, 1); 7,53 (t, 1); 7,25 (t, 1); 2,78 (s, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 2,24 min.

M+H = 183.

INTERMEDIO 52: 6-Cloro-4-metil-9H-ß-carbolina

Se disolvió 4-metil-9H-ß-carbolina (97 mg, 0,532 mmol) en HCl (1 N, 4 ml) y se agitó a TA. Se añadió NCS (85 mg, 0,637 mmol) y la mezcla de reacción se agitó durante 5 h. Se añadió una solución saturada de bicarbonato sódico (20 ml) y la mezcla de reacción se extrajo dos veces con EtOAc (100 ml). Las capas orgánicas combinadas se lavaron con salmuera, se secaron sobre $MgSO_4$, se filtraron y se concentraron para dar 6-cloro-4-metil-9H-ß-carbolina (108 mg) en forma de un aceite.

^{1}H RMN (300 MHz, DMSO-d6): d 8,67 (s, 1); 8,19 (s, 1); 8,11 (s, 1); 7,57-7,54 (m, 2); 2,82 (s, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 2,48 min.

M+H = 217.

INTERMEDIO 53: 6-cloro-4-metil-8-nitro-9H-ß-carbolina

Una solución de 6-cloro-4-metil-9H-ß-carbolina (100 mg, 0,462 mmol) en ácido trifluoroacético (10 ml) se agitó a TA. Se añadió $NaNO_3$ (106 mg, 1,25 mmol) y la mezcla de reacción se agitó durante 30 min y después se concentró para dar un residuo naranja. El residuo se disolvió en MeOH (5 ml) y se neutralizó mediante la adición de una solución saturada de bicarbonato sódico (20 ml), provocando la formación de sólidos amarillos que se recogieron por filtración y se lavaron con H_2O (10 ml) y Et_2O (2 x 10 ml) para dar 6-cloro-4-metil-8-nitro-9H-ß-carbolina (82 mg).

^{1}H RMN (300 MHz, DMSO-d6): d 8,90 (s, 1); 8,65 (s, 1); 8,55 (s, 1); 8,29 (s, 1); 2,89 (s, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 3,00 min.

M+H = 262.

INTERMEDIO 54: 6-cloro-4-metil-9H-ß-carbolin-8-ilamina

Una solución de 6-cloro-4-metil-8-nitro-9H-ß-carbolina (80 mg, 0,31 mmol) en MeOH (10 ml) se agitó a TA. Se añadió catalizador de platino (al 10% en peso sobre carbono, 24 mg) y el recipiente de reacción se tapó y se purgó 3 veces al vacío con argón, seguido de forma similar de hidrógeno. La mezcla de reacción se agitó durante 1,5 h a 1 atm de hidrógeno, después se purgó al vacío con argón, se diluyó con DCM (10 ml) y se filtró a través de un filtro de jeringa de 0,2 µM. El filtrado se concentró para producir 6-cloro-4-metil-9H-ß-carbolin-8-ilamina (67 mg) en forma de un aceite pardo claro.

^{1}H RMN (300 MHz, DMSO-d6): d 9,04 (s, 1); 8,29 (s, 1); 7,74 (d, 1); 7,10 (d, 1); 2,98 (s, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,89 min.

M+H = 231.

EJEMPLO 30: N-(6-cloro-4-metil-9H-ß-carbolin-8-il)-nicotinamida

Una solución de 6-cloro-4-metil-9H-ß-carbolin-8-ilamina (43 mg, 0,19 mmol) en piridina (4 ml) se agitó a TA en una atmósfera de argón. Se añadió clorhidrato de cloruro de nicotinoílo (40 mg, 0,22 mmol) y la mezcla de reacción se agitó durante 12 h. La solución se diluyó con H_2O (5 ml) y se vertió en un embudo de decantación que contenía H_2O (5 ml) y EtOAc (25 ml). La mezcla de reacción se agitó y las capas se separaron. La capa acuosa se extrajo con EtOAc (2 x 25 ml). Las capas orgánicas combinadas se lavaron con una solución saturada de bicarbonato sódico (15 ml), seguido de salmuera, después se secaron sobre

$MgSO_4$, se filtraron y se concentraron para producir N-(6-cloro-4-metil-9H-ß-carbolin-8-il)-nicotinamida (5,2 mg) en forma de un aceite viscoso naranja.

^1H RMN (300 MHz, DMSO-d6): d 11,67 (s, 1); 10,70 (s, 1); 9,27 (s, 1); 8,83 (s, 2); 8,45 (s, 1); 8,20-8,12 (m, 2); 7,83 (s, 1); 7,66 (s, 1); 2,80 (s, 3).

Condiciones convencionales de NH_4OAc.

DAD R_f = 1,92 min.

M+H = 337.

INTERMEDIO 55: 4-terc-butil éster del ácido 1,1-dioxo-17^6-tiomorfolin-3,4-dicarboxílico

Se disolvió 4-terc-butil éster del ácido tiomorfolin-3,4-dicarboxílico (120 mg, 0,485 mmol) en Et_2O (8 ml). A la solución se le añadió mCPBA (172 mg, 0,994 mmol), seguido de una segunda porción de mCPBA (84 mg, 0,485 mmol). El precipitado que se formó se filtró, se lavó con Et_2O y se secó para producir un sólido blanco de 4-terc-butil éster del ácido 1,1-dioxo-17^6-tiomorfolin-3,4-dicarboxílico (74 mg).

EJEMPLO 31: (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 1,1-dioxo-17^6-tiomorfolin-3-carboxílico

Una suspensión de 6-cloro-7-fluoro-9H-ß-carbolin-8-ilamina (43 mg, 0,198 mmol), 4-terc-butil éster del ácido 1,1-dioxo-17^6-tiomorfolin-3,4-dicarboxílico (72 mg, 0,257 mmol) y EDCI (76 mg, 0,396 mmol) en piridina (2 ml) se calentó a 70°C. Después de 1 h, el disolvente se retiró a presión reducida y el aceite oscuro resultante se disolvió en MeOH (1 ml). La solución de MeOH se añadió gota a gota a una solución en agitación de $NaHCO_3$ acuoso y se formó un precipitado amarillo. El sólido se filtró, se secó y se disolvió en HCl 2 M en Et_2O. Después de agitar durante una noche, el sólido amarillo resultante se filtró y se secó para producir un sólido amarillo, la sal di-HCl de (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 1,1-dioxo-17^6-tiomorfolin-3-carboxílico (78 mg).

^{1}H RMN (300 MHz, MeOH-d4): d 9,26 (s, 1); 8,74 (d, 1); 8,54 (d, 1); 8,37 (d, 1); 7,94 (d, 1); 5,01 (dd, 1); 4,15-3,75 (m, 4); 3,64-3,58 (m, 2).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,57 min.

M+H = 379.

INTERMEDIO 56: 4-terc-butil éster del ácido 6,6-Dimetil-morfolin-3,4-dicarboxílico

A una suspensión de ácido 6,6-dimetil-morfolin-3-carboxílico (5,56 g, 34,9 mmol) en dioxano (58 ml) se le añadió carbonato potásico acuoso (1 M, 58 ml). A la solución incolora transparente resultante se le añadió dicarbonato de di-terc-butilo (9,14 g, 41,9 mmol). La solución se agitó a temperatura ambiente durante una noche. La mezcla de reacción se diluyó con agua (200 ml) y se confirmó que el pH de la solución era de aproximadamente 7. La mezcla de reacción se vertió en un embudo de decantación y se extrajo con Et_2O (2 x 100 ml) para retirar el exceso de dicarbonato de di-terc-butilo. La capa acuosa se acidificó mediante la adición de HCl 6 N acuoso con agitación hasta que se alcanzó un pH de 3. La mezcla se extrajo rápidamente con Et_2O (2 x 200 ml) y las capas orgánicas se combinaron, se secaron sobre sulfato de magnesio, se filtraron y se concentraron para producir un aceite incoloro transparente. El aceite se disolvió en Et_2O (50 ml), se trituró con hexanos (150 ml) y se concentró para producir un sólido blanco. El producto sólido se puso sobre la bomba de alto vacío durante varias horas, después de lo cual se obtuvieron 8,85 g de 4-terc-butil éster del ácido 6,6-dimetil-morfolin-3,4-dicarboxílico (rendimiento de 97%).

^{1}H RMN (300 MHz, DMSO-d6): d 12,95 (s, 1); 4,35 ("dd", 1); 3,98-3,83 (m, 2); 3,48 ("dd", 1); 2,81 ("dd", 1); 1,39 ("dd", 9); 1,15 (s, 3); 1,08 ("dd", 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 0,98 min.

M-H = 258.

INTERMEDIO 57: éster terc-butílico del ácido (S)-5-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-carboxílico

El compuesto deseado se preparó de acuerdo con el *Método C* a partir de 6-cloro-9H-ß-carbolina-8-ilamina y 4-terc-butil éster del ácido 6,6-dimetil-morfolin-3,4-dicarboxílico con un rendimiento de 87%.

^{1}H RMN (DMSO-d6, 300 MHz) d 11,33 (s, 1); 10,14 (s, 1); 9,05 (s, 1); 8,38 (d, 1); 8,21 (d, 1); 8,16 (d, 1); 7,94 (s, 1); 4,70-4,56 (m, 1); 4,25-4,14 (m, 1); 4,07 (dd, 1); 3,64-3,56 (m, 1); 3,30-3,14 (m, 1); 1,41 ("dd", 9); 1,21 (s, 3); 1,15 (s, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,84 min.

M+H = 459.

HPLC quiral: =95% de ee.

Columna Chiralpak AD.

15% v/v de EtOH/Hexanos que contenía Et_2NH al 0,1%.

INTERMEDIO 58: sal trifluoroacetato de (6-cloro-9H-beta-carbolin-8-il)-amida del ácido [(S)-4-((S)-2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico

El compuesto deseado se preparó usando el mismo procedimiento que para el Intermedio 35 partiendo de éster terc-butílico del ácido (S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-carboxílico y usando Boc-alaninal en la etapa de alquilación reductora con un rendimiento de 60%.

^{1}H RMN (300 MHz, D_2O): d 1,16 (d, 3H), 1,25 (s, 3H), 1,28 (s, 3H), 2,41 (d, 1H), 2,59 (dd, 1H), 2,89 (dd, 1H), 2,95 (d, 1H), 3,35-3,50 (m, 2H), 3,95-4,15 (m, 2H), 7,59 (d, 1H), 7,97 (d, 1H), 8,11 (d, 1H), 8,30 (d, 1H), 8,40 (d, 1H), 8,94 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,57 min.

MS ($M+H^+$): 416,2.

MÉTODO E: Procedimiento de acoplamiento usando sal trifluoroacetato de (6-cloro-9H-beta-carbolin-8-il)-amida del ácido [(S)-4-((S)-2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico. Se recogieron sal trifluoroacetato de (6-cloro-9H-beta-carbolin-8-il)-amida del ácido [(S)-4-((S)-2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico (1,0 mmol), TBTU (1,2 mmol), el ácido (1,25 mmol) para acoplarse y Et_3N (4-6 mmol, pH básico) en acetonitrilo (10 ml). La mezcla resultante se agitó a temperatura ambiente durante 4-15 h. Después, la mezcla de reacción se repartió entre EtOAc y una solución acuosa al 10% de Na_2CO_3. La fase acuosa separada se extrajo de nuevo con EtOAc. Los extractos combinados se lavaron sucesivamente con una solución acuosa al 10% de Na_2CO_3 y salmuera, se secaron sobre Na_2SO_4 y se concentraron completamente. El residuo se purificó sobre sílice (MeOH al 2-7%/CH_2Cl_2) para dar el producto correspondiente.

EJEMPLO 39: (6-Cloro-9*H*-□-carbolin-8-il)-amida del ácido 6,6-Dimetil-4-[2-(2,2,2-trifluoro-acetilamino)-propil]-morfolin-3-carboxílico:

Se suspendió (6-cloro-9*H*-□-carbolin-8-il)-amida del ácido 4-(2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico (sal 3 CF_3COOH) (1,5 g) en diclorometano (80 ml) junto con 5 equivalentes de trietilamina. Se añadió ácido trifluoroanhídrido acético (56 µl, 2 equivalentes) y la mezcla se agitó a temperatura ambiente durante una hora. El disolvente se retiró por evaporación rotatoria. El producto se purificó por cromatografía ultrarrápida sobre gel de sílice (metanol al 5%/diclorometano, Rf del producto 0,3) para producir 1 g.

^{1}H RMN (300 MHz, en relación al pico de $CDCl_3$ a 7,3 ppm) d 10 (s, 1H), 9,7 (d, 1H) , 8,7 (s, 1H), 8,6 (s, 1H), 8,2 (d, 1H), 7,6 (s, 2H), 6,6 (s, 1H), 4,3 (m, 1H), 3,9 (m, 1H), 3,8 (t, 1H), 3,2 (m, 1H), 2,7-2,9 (m, 2H), 2,5 (m, 1H), 2,2 (d, 1 H), 1,4 (d, 3H), 1,3 (s, 3H), 1,2 (s, 3H).

LCMS (método de patrón de acetato amónico) tiempo de retención = 1,84 min.

($M^+ = 512$; $M^- = 510$).

EJEMPLO 40: (6-Cloro-9H-b-carbolin-8-il)-amida del ácido 4-((S)-2-acetilamino-propil)-6,6-dimetil-morfolin-3-(S)-carboxílico

El compuesto deseado se preparó de acuerdo con el ejemplo anterior a partir de (6-cloro-9*H*-□-carbolin-8-il)-amida del ácido 4-(2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico (sal 3 CF_3COOH) y anhídrido acético.

^{1}H RMN (300 MHz, metil-d_3 alcohol-d): d 1,15 (d, 3H), 1,23 (s, 3H), 1,39 (s, 3H), 1,98 (s, 3H), 2,24 (d, 1H), 2,38 (m, 1H), 2,68 (m, 1H), 2,92 (d, 1H), 3,24 (m, 1H), 3,98 (m, 2H), 4,22 (m, H), 7,78 (d, 1H), 7,98 (m, 2H), 8,27 (d, 1H), 8,84 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,54 min.

MS ($M+H^+$): 458.

EJEMPLO 41: (6-Cloro-9H-b-carbolin-8-il)-amida del ácido 4-((S)-2-metanosulfonilamino-propil)-6,6-dimetil-morfolin-3-(S)-carboxílico

El compuesto deseado se preparó de acuerdo con el Método E.

^{1}H RMN (300 MHz, metil-d_3 alcohol-d): d 1,28 (s, 3H), 1,29 (d, 3H), 1,43 (s, 3H), 2,28 (d, 1H), 2,57 (m, 1H), 2,66 (m, 1H), 2,98 (s, 3H), 3,03 (d, 1H), 3,34 (m, 1H), 3,66 (m, 1H), 4,05 (m, 2H), 7,67 (d, 1H), 8,10 (m, 2H), 8,32 (d, 1H), 8,89 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,54 min.

MS ($M+H^+$): 494.

EJEMPLO 42: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 4-[2-[(-4,6-dimetil-pirimidin-5-carbonil)-amino]-propil]-6,6-dimetil-morfolin-3-carboxílico

El compuesto deseado se preparó usando [4-(2-amino-propil)-6,6-dimetil-morfolin-3-ilmetil]-(6-cloro-9*H*-beta-carbolin-8-il)-amina y ácido 4,6-dimetil-pirimidin-5-carboxílico siguiendo el Método E con un rendimiento de 51%.

^{1}H RMN (300 MHz, DMSO): d 11,27 (1H, s), 10,02 (1H, s), 9,0 (1H, s), 8,86 (1H, s), 8,5 (1H, d), 8,3 (1H, d), 8,22 (2H, m), 7,88 (1H, s), 4,1 (1H, m), 3,9 (2H, m), 2,99 (2H, m), 2,36 (6H, s), 2,1 (2H, m), 1,3 (3H, s), 1,24 (6H, m).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,50 min.

MS (M+H+): 551

EJEMPLO 43: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-6,6-dimetil-4-{(S)-2-[(2-metil-piridin-3-carbonil)amino]propil}-morfolin-3-carboxílico.

El compuesto deseado se preparó de acuerdo con el Método E a partir de sal trifluoroacetato de (6-cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-4-((S)-2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico y ácido 2-metil-nicotínico con un rendimiento de 75%.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,21 (s, 3H), 1,22 (d, 3H), 1,36 (s, 3H), 2,10 (d, 1H), 2,42 (m, 1H), 2,60 (m, 1H), 2,99 (d, 1H), 3,20 (m, 1H), 3,92 (m, 2H), 4,22 (m, 1H), 7,22 (dd, 1H), 7,65 (d, 1H), 7,90 (s, 1H), 8,16 (d, 1H), 8,23 (s, 1H), 8,31 (d, 1H),8,38 (d, 1H), 8,45 (d, 1H), 9,02 (s, 1H),10,04 (s, 1H), 11,26 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,16 min.

MS ($M+H^+$): 535,5.

EJEMPLO 44: (6-Cloro-9H-b-carbolin-8-il)-amida del ácido 6,6-dimetil-4-{2-[(tetrahidro-piran-4-carbonil)-amino]-propil}-morfolin-3-carboxílico:

Se suspendió (6-cloro-9H-b-carbolin-8-il)-amida del ácido 4-(2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico (sal 3 CF_3COOH) (300 mg) en cloruro de metileno (12 ml) junto con 3 equivalentes de trietilamina. Se añadió cloruro de morfolin-4-carbonilo (70 mg, 1,3 equivalentes) y la mezcla se agitó a temperatura ambiente durante una noche. El disolvente se retiró por evaporación rotatoria. El producto se separó por TLC preparativa sobre placas de sílice (10/90 de metanol/acetato de etilo como eluyente, Rf del producto 0,4) Rendimiento: 83 mg.

^{1}H RMN (300 MHz, en relación al pico de CD_3OD a 3,3 ppm) d 8,8 (s, 1H), 8,27 (d, 1H), 7,9-7,99 (m, 3H), 3,95-4,15 (m, 2H), 3,85-3,95 (m, 2H), 3,5-3,6 (m,

5H), 3,3-3,45 (m, 2H), 3,15-3,3 (m, 2H), 2,85-2,95 (d, 1H), 2,6-2,72 (m, 1H), 2,3-2,43 (m, 1H), 2,2-2,28 (d, 1H), 2,0 (s, 2H), 1,35-1,45 (d, 3H), 1,05-1,25 (m, 6H).

LCMS (método de patrón de acetato amónico) tiempo de retención = 2,39 min.(M+ = 529; M- = 527).

EJEMPLO 45: (6-Cloro-9H-b-carbolin-8-il)-amida del ácido 4-((S)-2-[(1-acetil-pirrolidina-2-(S)-carbonil)-amino]-propil)-6,6-dimetil-morfolin-3-(S)-carboxílico

El compuesto deseado se preparó de acuerdo con el Método E.

^{1}H RMN (300 MHz, metil-d_3 alcohol-d): d 1,21 (d, 3H), 1,28 (s, 3H), 1,39 (s, 3H), 1,95 (m, 3H), 2,05 (s, 3H), 2,17 (m, 1H), 2,28 (d, 1H), 2,51 (m, 1H), 2,74 (m, 1H), 3,05 (d, 1H), 3,30 (m, 1H), 3,55 (m, 1H), 3,61 (m, 1H), 4,05 (m, 2H), 4,18 (m, 1H), 4,34 (m 1H), 7,78 (d, 1H), 8,10 (m, 2H), 8,33 (d, 1H), 8,90 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,07 min.

MS ($M+H^+$): 555.

EJEMPLO 46: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 6,6-dimetil-4-[-2-[(5-metil-isoxazol-3-carbonil)-amino]-propil]-morfolin-3-carboxílico

El compuesto deseado se preparó usando [4-(2-amino-propil)-6,6-dimetil-morfolin-3-ilmetil]-(6-cloro-9*H*-beta-carbolin-8-il)-amina y cloruro de 5-metilisoxazol-carbonilo siguiendo el Método E con un rendimiento de 61%.

^{1}H RMN (300 MHz, DMSO): d 11,2 (1H, s), 9,98 (1H, s), 9,0 (1H, s), 8,7 (1H, d), 8,6 (1H, d), 8,2 (2H, m), 7,9 (1H, s). 6,47 (1H, s), 3,87 (2H, m), 3,17 (2H, m), 2,9 (1H, d), 2,7 (1H, m), 2,3 (4H), 2,1 (1H, d), 1,29 (3H, s), 1,15 (6H, m).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,81 min.

MS (M+H+): 526

EJEMPLO 47: (6-Cloro-9*H*-□-carbolin-8-il)-amida del ácido 6,6-dimetil-4-[2-(3-metil-ureido)-propil]-morfolin-3-carboxílico:

Se suspendió (6-cloro-9*H*-□-carbolin-8-il)-amida del ácido 4-(2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico (sal 3 HCl) (300 mg) en diclorometano (10 ml) y se añadieron simultáneamente trietilamina (4 equivalentes) e isocianato de metilo (2 equivalentes). Después de una hora a temperatura ambiente, el disolvente se retiró por evaporación rotatoria. El producto se purificó por cromatografía ultrarrápida sobre gel de sílice (metanol al 5%/diclorometano, Rf del producto = 0,3) para producir 200 mg.

^{1}H RMN (300 MHz, en relación al pico de $CDCl_3$ a 7,3 ppm) d 12,3 (s, 1H), 10,2 (s, 1H), 8,9 (s, 1H), 8,6 (s, 1H), 8,4 (d, 1H), 7,9 (d, 1H), 7,8 (s, 1H), 5,4 (s, 1H), 5,2 (d, 1H), 4,2 (s, 1H), 3,8 (m, 2H), 3,2 (m, 1H), 2,6-3 (m, 7H), 2,3 (d, 1H), 2,2 (t, 1H), 1,4 (s, 3H), 1,2 (s, 3H), 1,1 (d, 3H)

LCMS (método de patrón de acetato amónico) tiempo de retención = 1,62 min. (M^+ = 473; M^- = 471).

EJEMPLO 49: Éster metílico del ácido {(S)-2-[(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-1-metil-etil}-carbámico.

A una solución de sal clorhidrato de (6-cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-4-((S)-2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico (3,45 g, 6,59 mmol) en 68 ml de piridina seca se le añadió en tres porciones durante 1,5 h una solución 3 M en DCM de cloroformiato de metilo (9,2 ml, 27,6 mmol, 4,2 equiv.). Después de 2 h, se añadieron 10 ml de agua y la mezcla se concentró a sequedad. El residuo se repartió en 150 ml de EtOAc y 100 ml de una solución acuosa 0,5 M de K_2CO_3. La fase acuosa separada se extrajo con 50 ml de EtOAc. Los extractos orgánicos combinados se lavaron sucesivamente con agua (2 x 50 ml) y salmuera (50 ml), se secaron sobre Na_2SO_4 y se concentraron a sequedad. El residuo se purificó sobre sílice (MeOH al 5%/CH_2Cl_2) para dar 2,48 g (aceite pegajoso, rendimiento de 77%) del producto deseado.

^{1}H RMN (300 MHz, $CDCl_3$): d 1,15 (d, 3H), 1,28 (s, 3H), 1,42 (s, 3H), 2,33 (dd, 1H), 2,42 (d, 1H), 2,78 (dd, 1H), 2,86 (d, 1H), 3,32 (dd, 1H), 3,86 (s, 3H), 3,92

(t, 1H), 4,01 (dd, 1H), 4,18 (m, 1H), 4,78 (d, 1H), 7,95 (d, 1H), 7,97 (s, 1H), 8,29 (s, 1H), 8,50 (d, 1H), 8,98 (s, 1H), 9,88 (s, 1H), 10,94 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,70 min.

MS ($M+H^+$): 474,1

EJEMPLO 50: (6-Cloro-9*H*-beta-carbolin-8-il)-amida del ácido 4-[2-[(2,4-dimetil-piridin-3-carbonil)-amino]-propil]-6,6-dimetil-morfolin-3-carboxílico

El compuesto deseado se preparó a partir de [4-(2-amino-propil)-6,6-dimetil-morfolin-3-ilmetil]-(6-cloro-9*H*-beta-carbolin-8-il)-amina y ácido 2,4-dimetil-nicotínico siguiendo el Método E con un rendimiento de 50%.

^{1}H RMN (300 MHz, DMSO): d 11,27 (1H, s), 10 (1H, s), 8,9 (1H, s), 8,37 (2H, d), 8,24 (3H, m), 7,8 (1H, s), 7,04 (1H, d), 3,91 (2H, m), 3,1 (2H, m), 2,36 (4H, m), 2,1 (3H, m), 2,05 (1H, d), 1,3 (3H, s), 1,2 (6H, m).

Tiempo de retención (LC, método: patrón de acetato amónico) : 1,53 min.

MS (M+H+): 550

EJEMPLO 51: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 6,6-dimetil-4-[2-[(pirazina-2-carbonil)-amino]-propil]-morfolin-3-carboxílico

El compuesto deseado se preparó a partir de [4-(2-Amino-propil)-6,6-dimetil-morfolin-3-ilmetil]-(6-cloro-9*H*-beta-carbolin-8-il)-amina y ácido 2-pirazina-carboxílico siguiendo el Método E con un rendimiento de 62%.

^{1}H RMN (300 MHz, DMSO): d 12,9 (1H, s), 10,89 (1H, s), 9,42 (1H, s), 9,0 (1H, s), 8,8 (1H, d), 8,6 (1H, d), 8,4 (1H, s), 8,2 (1H, m), 4,5 (1H, m), 4,1 (2H, m), 3,1 (1H, m), 2,1 (4H, m), 1,23 (9H, m).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,69 min.

MS (M+): 522,2

EJEMPLO 52: 4-({2-[5-(6-Cloro-9H-ß-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-1-metil-etil}-amida)-3-metilamida del ácido del ácido piridin-3,4-dicarboxílico

A una solución de [4-(2-amino-propil)-6,6-dimetil-morfolin-3-ilmetil]-(6-cloro-9H-beta-carbolin-8-il)-amina (100 mg, 0,132 mmol) en 0,6 ml de acetonitrilo seco se le añadieron anhídrido 3,4-piridinadicarboxílico (21 mg, 0,15 mmol) y trietilamina (102 ml, 0,8 mmol). La mezcla de reacción se agitó a temperatura ambiente durante 1 h. Después, el disolvente se retiró a presión reducida y el residuo se recogió en piridina (0,6 ml). A la mezcla resultante se le añadieron una solución 2 M de metilamina en THF (0,2 ml, 0,4 mmol) y EDCI (40 mg, 0,21 mmol). La mezcla de reacción se agitó durante 4 h, el disolvente se retiró a presión reducida y el residuo se repartió en EtOAc y K_2CO_3 acuoso 1 M. La fase acuosa separada se extrajo dos veces con EtOAc. Las fases orgánicas combinadas se lavaron sucesivamente con agua y salmuera, se secaron sobre $MgSO_4$ y se concentraron completamente. El residuo se purificó sobre gel de sílice (MeOH al 10%-CH_2Cl_2) para dar el compuesto del título en forma de un sólido blanco con un rendimiento de 36%.

^{1}H RMN (300 MHz, DMSO): □ 9,24 (1H, s), 8,17 (1H,s), 7,81 (2H, m), 7,5 (3H, m), 7,3 (1H, s), 7,0 (1H, s) 6,5 (1H, d), 3,08 (2H, d), 3,35 (1H, m), 2,4 (3H, m), 1,86 (4H, m), 0,5 (3H, s), 0,37 (6H, m).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,46 min.

MS (M+H+): 579

EJEMPLO 53: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 6,6-dimetil-4-{(4-metil-pirimidin-5-carbonil)-amino)-propil}-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el Método E a partir de [4-(2-amino-propil)-6,6-dimetil-morfolin-3-ilmetil]-(6-cloro-9H-beta-carbolin-8-il)-amina y ácido 4-metil-pirimidin-5-carboxílico con un rendimiento de 55%.

^{1}H RMN (300 MHz, DMSO): □ 11,26 (1H, s), 10,04 (1H, s) 9,04 (2H, m), 8,66 (1H, d) 8,3 (2H, m) 8,22-8,17 (2H, m), 7,9 (1H, s), 4,2 (1H, m), 3,93 (2H, m), 3,2 (1H, m), 2,98 (2H, m), 2,6 (3H, m), 2,1 (2H, m), 1,36 (3H, s), 1,23 (6H, m).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,55 min.

MS (M+H+): 537

EJEMPLO 54: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-6,6-dimetil-4-[(S)-2-[(4-metil-piridin-3-carbonil)-amino]-propil]-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el Método E a partir de sal trifluoroacetato de (6-cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-4-((S)-2-amino-propil)-6,6-dimetil-morfolin-3-carboxílico y ácido 4-metil-nicotínico con un rendimiento de 79%.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,21 (s, 3H), 1,22 (d, 3H), 1,36 (s, 3H), 2,10 (d, 1H), 2,40 (m, 1H), 2,62 (m, 1H), 2,99 (d, 1H), 3,22 (m, 1H), 3,94 (m, 2H), 4,23 (m, 1H), 7,26 (d, 1H), 7,90 (s, 1H), 8,16 (d, 1H), 8,23 (s, 1H), 8,34-8,46 (m, 3H), 9,02 (s, 1H),10,04 (s, 1H), 11,27 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,22 min.

MS (M+H$^+$): 535,5.

EJEMPLO 47: (6-Cloro-9H-ß-carbolin-8-il)-amida del ácido 4-[2-(2-amino-2-metil-propionil)]-6,6-dimetil-morfolin-3-carboxílico

Una solución de éster terc-butílico del ácido {2-[5-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-1-metil-etil}-carbámico (70,2 mg, 0,14 mmol) en TFA (2 ml) se agitó a temperatura ambiente. Después de 15 min, la reacción se concentró y el producto bruto se destiló azeotrópicamente con CH_2Cl_2 (2 x 5 ml). Una mezcla del intermedio bruto, TBTU (54,0 mg, 0,17 mmol), trietilamina (0,2 ml, 1,43 mmol) y ácido 2-terc-butoxicarbonilamino-2-metil-propiónico (45,0 mg, 0,22 mmol) en MeCN (1 ml) se agitó a temperatura ambiente durante 18 h. La solución se diluyó con H_2O (20 ml) y se vertió en un embudo de decantación que contenía

EtOAc (50 ml) y salmuera (50 ml). La mezcla se agitó y las capas se separaron. La capa acuosa se extrajo con EtOAc (2 x 50 ml). Las capas orgánicas combinadas se secaron, se filtraron y se concentraron. El producto bruto se purificó por cromatografía ultrarrápida para producir un sólido amarillo (51,0 mg, 62%) que se mostró por RMN y LCMS que era (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-[2-(2-amino-2-metil-propionil]-6,6-dimetil-morfolin-3-carboxílico.

^{1}H RMN (300 MHz, DMSO-d6): d 12,94 (s a, 1); 11,36 (s a, 1); 10,16 (s, 1); 9,06 (s, 1); 8,39 (d, 1); 8,22 (d, 1); 8,19 (d, 1); 7,95 (s, 1); 4,77-4,52 (m, 1); 4,28-4,13 (m, 1); 4,13-4,00 (m, 1); 3,68-3,52 (m, 1); 3,22-3,12 (m, 1); 1,44 (s, 3); 1,41-1,38 (m, 6); 1,28-1,24 (m, 2); 1,22 (s, 3); 1,25 (s, 3); 1,11-1,07 (m, 1).

Condiciones convencionales de NH_4OAc.

DAD R_f = 1,31 min.

M+H = 501.

EJEMPLO 55: (6-Cloro-9H-beta-carbolin-8-il)amida del ácido 6,6-dimetil-4-(1,2,3,4-tetrahidroisoquinolin-3-ilmetil)-morfolin-3-carboxílico

El compuesto deseado se fabricó siguiendo el procedimiento indicado en el Método C usando ácido 6,6-dimetil-morfolin-3-carboxílico y (s)-tetrahidroisoquinolin-aldehído.

^{1}H RMN (300 MHz, D_2O): d 9,1 (1H, s), 8,68 (1H, d), 8,52 (1H, d), 8,41 (1H, d), 7,68 (1H, d), 7,27 (1H, d), 7,06 (1H, m), 6,97 (1H, d), 6,84 (1H, m), 4,31 (2H, m), 4,09 (2H, m), 3,68 (1H, m), 3,56 (1H, t), 3,2 (2H, m), 3,06 (2H, m), 2,7 (2H, m) 1,48 (3H, s), 1,32 (3H, s)

Tiempo de retención (LC, método: patrón de acetato amónico) : 2,43 min.

MS (M+H+): 505

INTERMEDIO 59: (6-Cloro-9H-ß-carbolin-8-il)-amida del ácido 6,6-dimetil-morfolin-3-carboxílico, sal HCl

A una solución parda transparente de éster terc-butílico del ácido 5-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-carboxílico (10,4 g, 22,7 mmol) en metanol (41 ml) se le añadió HCl en dioxano (4 M, 91 ml). La reacción se agitó durante 30 minutos a temperatura ambiente, tiempo durante el cual comenzó a formarse un precipitado pardo pálido. La mezcla se vertió en un volumen de 250 ml de Et_2O en agitación vigorosa. La suspensión resultante se agitó a temperatura ambiente durante 15 minutos y después se filtró para producir un sólido naranja pálido. El sólido se puso sobre la bomba de alto vacío durante una noche, después de lo cual se obtuvieron 9,71 g de (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 6,6-dimetil-morfolin-3-carboxílico (rendimiento de 99%).

^{1}H RMN (DMSO-d6, 300 MHz) d 13,47 (s, 1); 11,77 (s, 1); 9,42 (s, 1); 8,86 (d, 1); 8,66 (d, 1); 8,58 (d, 1); 8,25 (d, 1); 4,41-4,37 (m, 2); 4,05 (dd, 1); 3,32-3,28 (m, 1); 3,04-3,00 (m, 1); 1,34 (s, 3); 1,30 (s, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,48 min.

M+H = 359.

EJEMPLO 56: (6-Cloro-9H-ß-carbolin-8-il)-amida del ácido 4-(2-amino-butil)-6,6-dimetil-morfolin-3-carboxílico (*también INTERMEDIO 60*)

Se siguió el *Método C*, usando éster terc-butílico del ácido 5-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-carboxílico y el aldehído apropiado, éster terc-butílico del ácido (1-formil-propil)-carbámico.

^{1}H RMN (DMSO-d6, 300 MHz) d 9,06 (s, 1); 8,37 (d, 1); 8,19 (d, 1); 8,15 (d, 1); 7,85 (d, 1); 6,75 (s a, 2); 3,96-3,85 (m, 2); 3,17-3,13 (m, 1); 2,89 (d, 1); 2,78-2,74 (m, 1); 2,67-2,59 (m, 1); 2,26-2,20 (m, 1); 2,14 (d, 1); 1,58-1,50 (m, 1); 1,32 (s, 3); 1,32-1,23 (m, 1); 1,18 (s, 3); 0,87 (t, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,27 min.

M+H = 430.

EJEMPLO 57: (6-Cloro-9H-ß-carbolin-8-il)-amida del ácido 6,6-dimetil-4-{2-[(2-metil-piridin-3-carbonil)-amino]-butil}-morfolin-3-carboxílico, sal HCl

A una solución de (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-(2-amino-butil)-6,6-dimetil-morfolin-3-carboxílico (100 mg, 0,233 mmol) en piridina (4 ml) se le añadieron ácido 2-metil-nicotínico (38,4 mg, 0,280 mmol) y EDCI (71,5 mg, 0,373 mmol). La solución se agitó durante una noche a temperatura ambiente y después se diluyó con agua (5 ml). La mezcla se vertió en un embudo de decantación y se diluyó de nuevo con agua (20 ml). La mezcla se extrajo con EtOAc (2 x 20 ml) y después las capas orgánicas combinadas se lavaron con salmuera. La capa orgánica se secó sobre sulfato de magnesio, se filtró y se concentró para producir un aceite amarillo-pardo que se purificó por cromatografía en columna. El sólido amarillo resultante se disolvió en metanol (2 ml) y se añadió HCl en Et_2O (2 M, 2 ml). La mezcla se agitó durante 5 minutos y después se concentró para producir 103 mg de (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 6,6-dimetil-4-{2-[(2-metil-piridin-3-carbonil)-amino]-butil}-morfolin-3-carboxílico (rendimiento de 71%).

^{1}H RMN (DMSO-d6, 300 MHz) d 13,31 (s a, 1); 11,41 (s a, 1); 11,14 (s a, 1); 9,44 (s, 1); 8,85 (d, 1); 8,73-8,64 (m, 3); 8,52 (s, 1); 8,32 (s, 1); 7,72 (s, 1); 4,64-3,55 (m, 6); 3,24-3,06 (m, 1); 2,95-2,81 (m, 1); 2,71 (s, 3); 1,89-1,74 (m, 1); 1,55-1,41 (m, 1); 1,32 (s, 3); 1,23 (s, 3); 0,93 (t, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,66 min.

M+H = 549.

INTERMEDIO 61: (6-Cloro-9H-ß-carbolin-8-il)-amida del ácido 4-(2-amino-3-metil-butil)-6,6-dimetil-morfolin-3-carboxílico

Se siguió el *Método C*, usando éster terc-butílico del ácido 5-(6-cloro-9H-ß-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-carboxílico y el aldehído apropiado, éster terc-butílico del ácido (1-formil-2-metil-propil)-carbámico.

^{1}H RMN (DMSO-d6, 300 MHz) d 9,05 (s, 1); 8,37 (d, 1); 8,21 (d, 1); 8,16 (dd, 1); 7,90 (d, 1); 6,71 (s a, 2); 3,93-3,86 (m, 2); 3,18-3,14 (m, 1); 2,91 (d, 1); 2,70-2,65 (m, 2); 2,21 (dd, 1); 2,15 (d, 1); 1,74-1,66 (m, 1); 1,31 (s, 3); 1,19 (s, 3); 0,85 (d, 3); 0,80 (d, 3).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,27 min.

M+H = 444.

EJEMPLO 58: (6-Cloro-9H-ß-carbolin-8-il)-amida del ácido 6,6-dimetil-4-[3-metil-2-[(2-metil-piridin-3-carbonil)-amino]-butil]-morfolin-3-carboxílico

A una solución de (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 4-(2-amino-3-metil-butil)-6,6-dimetil-morfolin-3-carboxílico (1,47 g, 3,31 mmol) en piridina (35 ml) se le añadieron ácido 2-metil-nicotínico (544 mg, 3,97 mmol) y EDCI (1,02 g, 5,30 mmol). La solución se agitó durante 6,5 horas a temperatura ambiente y después se diluyó con agua (100 ml). La mezcla se vertió en un embudo de decantación y se diluyó de nuevo con agua (50 ml) y EtOAc (150 ml). Las capas se agitaron y se separaron. La capa acuosa se extrajo con EtOAc (3 x 50 ml) y después las capas orgánicas combinadas se lavaron con salmuera. La capa orgánica se secó sobre sulfato de magnesio, se filtró y se concentró para producir un residuo semi-sólido naranja que se purificó por cromatografía en columna. El sólido amarillo resultante se puso sobre la bomba de alto vacío durante una noche, después de lo cual se obtuvieron 1,43 g de (6-cloro-9H-ß-carbolin-8-il)-amida del ácido 6,6-dimetil-4-[3-metil-2-[(2-metil-piridin-3-carbonil)-amino]-butil]-morfolin-3-carboxílico (rendimiento de 77%).

^{1}H RMN (DMSO-d6, 300 MHz) d 11,32 (s, 1); 10,08 (s, 1); 9,02 (s, 1); 8,46 (dd, 1); 8,38 (d, 1); 8,21-8,14 (m, 3); 7,97 (d, 1); 7,64 (dd, 1); 7,23 (dd, 1); 4,23-

4,14 (m, 1); 3,99-3,87 (m, 2); 3,22-3,19 (m, 1): 3,02 (d, 1); 2,85 (dd, 1); 2,52 (s, 3); 2,30 (dd, 1); 2,11 (d, 1); 2,05-1,95 (m, 1); 1,32 (s, 3); 1,21 (s, 3); 0,93 (d, 3); 0,86 (d, 3).

Condiciones convencionales de NH_4OAc.

DAD R_f = 1,67 min.

M+H = 563.

EJEMPLO 59: (6-Cloro-9H-b-carbolin-8-il)-amida del ácido 6,6-dimetil-4-[3-metil-2-(S)-[(tetrahidro-furan-3-carbonil)-amino]-butil]-morfolin-3-(S)-carboxílico

El compuesto deseado se preparó siguiendo el Método E a partir del Intermedio 61 y el ácido apropiado.

^1H RMN (300 MHz, metil-d_3 alcohol-d): d 0,87 (m, 6H), 1,25 (d, 3H), 1,37 (d, 3H), 1,81 (m, 1H), 2,10-2,47 (m, 4H), 2,93 (m, 2H), 3,10 (m, 1H), 3,26 (m, 1H), 3,80 (m, 1H), 3,86-4,07 (m, 6H), 7,86 (d, 1H), 8,10 (m, 2H), 8,32 (d, 1H), 8,89 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,73 min. MS ($M+H^+$): 542.

INTERMEDIO 62: Éster terc-butílico del ácido {(S)-2-[(S)-5-(6-cloro-4-metil-9H-□-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-1-metil-etil}-carbámico

Una solución de ácido (S)-4-((S)-2-terc-butoxicarbonilamino-propil)-6,6-dimetil-morfolin-3-carboxílico (3,316 g, 10,5 mmol) (preparado alquilando reductoramente ácido (S)-6,6-dimetil-morfolin-3-carboxílico con N-(terc-butoxicarbonil)-L-alanal) en piridina anhidra (75 ml) se agitó a temperatura ambiente. Se añadió 6-cloro-4-metil-9H-ß-carbolin-8-ilamina (1,869 g, 8,09 mmol), seguido de EDCI (2,894 g, 15,1 mmol). La reacción se agitó a temperatura ambiente durante 14-18 horas en una atmósfera de argón. La reacción se concentró parcialmente, se diluyó con H_2O (20 ml) y se transfirió a un embudo de decantación. La mezcla se diluyó con salmuera (50 ml) y se extrajo con EtOAc (3 x

100 ml). Las capas orgánicas combinadas se lavaron con salmuera, se secaron, se filtraron y se concentraron para producir un residuo oscuro. La cromatografía en columna (MeOH al 0-8%/CH_2Cl_2) produjo éster terc-butílico del ácido {(S)-2-[(S)-5-(6-cloro-4-metil-9H-□-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-1-metil-etil}-carbámico en forma de un sólido castaño claro (2,688 g).

^{1}H RMN (300 MHz, DMSO-d6): d 11,25 (s, 1) 9,94 (s, 1) 8,88 (s, 1) 8,18 (s, 1) 8,02 (s, 1) 7,92 (s, 1) 6,73 (d, 1) 3,95-3,85 (m, 2) 3,66 (s a, 1) 3,16-3,08 (m, 1) 2,88 (d, 1) 2,76 (s, 3) 2,51-2,40 (m, 1) 2,23 (dd, 1) 1,99 (d, 1) 1,34 (s a, 12) 1,17 (s, 3) 1,08 (d, 3).

Condiciones convencionales de NH_4OAc.

ELSD R_f = 2,07 min.

M+H = 530.

EJEMPLO 60: (6-Cloro-4-metil-9H-□-carbolin-8-il)-amida del ácido (S)-6,6-dimetil-4-{(S)-2-[(2-metil-piridin-3-carbonil)-amino]-propil}-morfolin-3-carboxílico

Una solución de éster terc-butílico del ácido {(S)-2-[(S)-5-(6-cloro-4-metil-9H-□-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-1-metil-etil}-carbámico (2,688 g, 5,08 mmol) en EtOH (60 ml) se agitó a temperatura ambiente. Se añadió HCl concentrado (10 ml) y la reacción se agitó durante 14 horas a temperatura ambiente en una atmósfera de argón. La reacción se concentró para producir un sólido amarillo (2,84 g). El sólido se disolvió en piridina anhidra (40 ml) y se agitó a temperatura ambiente en una atmósfera de argón. Se añadieron trietilamina (2,20 ml, 15,7 mmol) y EDCI (1,39 g, 7,28 mmol). La mezcla de reacción se agitó a temperatura ambiente durante 10 minutos y se añadió ácido 2-metil-nicotínico (0,868 g, 6,33 mmol). La reacción se agitó a temperatura ambiente durante 14-18 horas y se diluyó con H_2O (40 ml). La mezcla se vertió en un embudo de decantación que contenía H_2O (40 ml), salmuera (40 ml) y EtOAc (40 ml). La mezcla se agitó y las capas se separaron. La capa acuosa se extrajo con EtOAc (2 x 40 ml) y las capas orgánicas combinadas se lavaron con salmuera. La capa

orgánica se secó, se filtró y se concentró. El residuo resultante se disolvió en EtOAc (10-20 ml) y se añadió gota a gota a una solución en agitación de 4:1 de hexanos/Et_2O (300 ml). El precipitado que se formó se recogió por filtración y se secó al aire para producir (6-cloro-4-metil-9H-□-carbolin-8-il)-amida del ácido (S)-6,6-Dimetil-4-{(S)-2-[(2-metil-piridin-3-carbonil)-amino]-propil}-morfolin-3-carboxílico en forma de un sólido castaño (3,151 g).

^{1}H RMN (300 MHz, DMSO-d6): d 11,28 (s, 1) 9,98 (s, 1) 8,81 (s, 1) 8,45-8,39 (m, 1) 8,26 (d, 1) 8,13 (s, 1) 7,97 (s, 1) 7,89 (s, 1) 7,64-7,56 (m, 1) 7,24-7,12 (m, 1) 4,25-4,10 (m, 1) 3,91-3,82 (m, 2) 3,20-3,10 (m, 1) 2,94 (d, 1) 2,71 (s, 3) 2,61-2,49 (m, 1) 2,37 (s, 3) 2,06-2,02 (m, 2) 1,30 (s, 3) 1,16 (d, 3) 1,15 (s, 3).

Condiciones convencionales de NH_4OAc.

ELSD R_t = 1,57 min. M+H = 549.

INTERMEDIO 63: 3,5-Difluoro-4-tributilstanil-piridina

Se añadió *n*-butillitio (1,0 equiv., 76 mmol, 47,6 ml, 1,6 M en hexanos) mediante un embudo de goteo a una solución de diisopropilamina (1,05 equiv., 80 mmol, 11,2 ml) en THF (300 ml) a -78°C en atmósfera de nitrógeno (N_2). La solución se agitó durante 30 min a -78°C y después se añadió gota a gota una solución de 3,5-difluoropiridina (1,05 equiv., 80 mmol, 9,2 g) en THF (20 ml) mediante una jeringa. Se observó la formación de un precipitado beige. La reacción se agitó a -78°C durante 90 min y después se añadió gota a gota cloruro de tributilestaño (1,0 equiv., 76 mmol, 20,7 ml) mediante una jeringa y la solución resultante se dejó calentar a TA durante 2 h. Se añadió agua (5 ml) y después se retiraron aproximadamente 250 ml de THF en un evaporador rotatorio. El material resultante se diluyó con éter dietílico (350 ml) y se lavó sucesivamente con agua (2 x 200 ml) y una solución saturada de cloruro sódico (1 x 150 ml), se secó sobre sulfato de magnesio, se filtró y se concentró al vacío para producir la 3,5-difluoro-4-tributilstanil-piridina en forma de un aceite incoloro (27,5 g, 88%). Este material se usó bruto sin purificación adicional.

Tiempo de retención (LC, método: patrón de acetato amónico): 3,35 min. MS ($M+H^+$): 406.

INTERMEDIO 64: 4-Cloro-2-(3,5-difluoro-piridin-4-il)-fenilamina

Acoplamiento de Stille: Una solución en dimetilformamida (256 ml) del Intermedio 63 bruto (1,1 equiv., 70 mmol, 27,5 g) y 2-yodo-4-cloro-fenilamina (1,0 equiv., 64 mmol, 16,2 g) se desgasificó con N_2 durante 15 min. Se añadieron diclorobis(trifenilfosfina)paladio (II) (0,05 equiv., 3,2 mmol, 2,2 g) y yoduro de cobre (I) (0,1 equiv., 6,4 mmol, 1,2 g) y la suspensión calentó a reflujo durante 15 h en atmósfera de N_2. La mezcla se enfrió a TA, se filtró a través de un lecho corto de celite® y la dimetilformamida se retiró en un evaporador rotatorio. El material bruto se disolvió en acetonitrilo (300 ml), se lavó con hexanos (2 x 200 ml) y después se concentró al vacío. Después, el material se disolvió en acetato de etilo (400 ml) y se lavó sucesivamente con agua (2 x 200 ml), con una solución saturada de bicarbonato sódico (1 x 200 ml) y una solución saturada de cloruro sódico (200 ml), se secó sobre sulfato de magnesio, se filtró y se concentró al vacío. El sólido resultante se trituró con éter dietílico (50 ml) para retirar el color oscuro, después se disolvió en el volumen mínimo de metanol, se filtró para retirar una impureza insoluble y se concentró al vacío para producir la 4-cloro-2-(3,5-difluoro-piridin-4-il)-fenilamina en forma de un sólido castaño (12,3 g, ~80%,) que se usó en la siguiente etapa sin purificación adicional.

^{1}H RMN (300 MHz, dmso-d_6): d 5,28 (s, 2H), 6,77 (d, 1H), 7,08 (d, 1H), 7,19 (dd, 1H) y 8,58 (s, 2H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,70 min. MS ($M+H^+$): no se observó.

INTERMEDIO 65: 6-Cloro-4-fluoro-9H-□-carbolina

Se añadió bis(trimetilsilil)amida sódica (3,0 equiv., 130 mmol, 130 ml, 1,0 M en THF) mediante un embudo de goteo a una solución del Intermedio 64 bruto (1,0

equiv., 43 mmol, 10,4 g) en THF a TA en atmósfera de N_2. Después de agitar durante 15 h, el exceso de base se inactivó mediante la adición cuidadosa de una solución saturada de cloruro amónico (100 ml) y la mayor parte del THF se retiró en un evaporador rotatorio. La suspensión resultante se extrajo con acetato de etilo (400 ml y después 2 x 200 ml) y después los extractos combinados se lavaron sucesivamente con una solución saturada de bicarbonato sódico (300 ml) y con una solución saturada de cloruro sódico saturado (300 ml), se secaron sobre sulfato sódico y se filtraron. Se añadió gel de sílice y la suspensión se concentró en un evaporador rotatorio. El material se purificó usando un sistema de purificación Biotage Flash 75 (columna corta) eluyendo con 96:4 de diclorometano/metanol para producir la 6-cloro-4-fluoro-9H-□-carbolina en forma de un sólido blanquecino (7,8 g, 82%).

^{1}H RMN (300 MHz, dmso-d_6): d 7,71-7,61 (m, 3H), 8,11 (d, 1H) y 12,16 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,70 min. MS ($M+H^+$): 221.

INTERMEDIO 66: 6-cloro-4-fluoro-9H-□-carbolin-8-ilamina

Se añadió en porciones nitrato sódico (1,5 equiv., 53 mmol, 4,5 g) a una solución del Intermedio 65 (1,0 equiv., 35 mmol, 7,8 g) en ácido trifluoroacético (200 ml) y la mezcla resultante se calentó a 70ºC durante 3 h. Después de enfriar a TA, el ácido trifluoroacético se retiró en un evaporador rotatorio para producir un sólido bruto que se suspendió en un pequeño volumen de metanol y se añadió gota a gota a una mezcla agitada vigorosamente de una solución saturada de bicarbonato sódico (500 ml). La suspensión resultante se agitó durante 15 min y después los sólidos precipitados se recogieron por filtración por succión, se lavaron con agua (300 ml) y después se secaron al vacío para producir 6-cloro-4-fluoro-8-nitro-9H-□-carbolina (aproximadamente 9,5 g) que se usó en la siguiente etapa sin purificación adicional.

Tiempo de retención (LC, método: patrón de acetato amónico): 1,79 min. MS ($M+H^+$): 266

Se añadió platino sulfatado (~0,1 equiv., 1 g) a una suspensión de 6-cloro-4-fluoro-8-nitro-9H-□-carbolina (1,0 equiv., 35 mmol, 9,3 g) y formiato amónico (3,0 equiv., 105 mmol, 6,6 g) en etanol (175 ml) y la mezcla resultante se calentó a 75°C durante 4 h. Después de enfriar a TA, la mezcla se filtró a través de un lecho corto de Celite®, lavando con cantidades copiosas de metanol y después el filtrado se concentró al vacío para producir un sólido beige. El sólido se suspendió en el volumen mínimo de metanol y se añadió gota a gota a una mezcla agitada vigorosamente de una solución saturada de bicarbonato sódico y una solución saturada de cloruro sódico. Después de agitar durante 15 min, los sólidos precipitados se recogieron por filtración por succión, se lavaron con agua (200 ml) y se secaron al vacío para producir 6-cloro-4-fluoro-9H-□-carbolin-8-ilamina (5,8 g, 70% en 2 etapas) en forma de un polvo beige.

^{1}H RMN (300 MHz, dmso-d_6): d 5,76 (s, 2H), 6,81 (d, 1H), 7,29 (d, 1H), 8,24 (d, 1H), 8,82 (d, 1H), y 11,71 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,59 min. MS ($M+H^+$): 236.

EJEMPLO 61: (6-Cloro-4-fluoro-9H-b-carbolin-8-il)-amida del ácido 4-(2-acetilamino-propil)-6,6-dimetil-morfolin-3-carboxílico

El compuesto deseado se preparó a partir del Intermedio 66 y anhídrido acético.

^{1}H RMN (300 MHz, dmso-d_6): d 11,70 (s, 1H), 10,17 (s, 1H), 8,92 (s, 1H), 8,34 (d, 1H), 7,95 (s, 2H), 7,81 (d, 1H), 4,05-3,95 (m, 1H), 3,92-3,83 (m, 2H), 3,18-3,12 (m, 1H), 2,87 (d, 1H), 2,55-2,47 (m, 1H), 2,40-2,31 (m, 1H), 2,03 (d, 1H), 1,78 (s, 3H), 1,32 (s, 3H), 1,63 (s, 3H) y 1,08 (d, 3H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,73 min. MS ($M+H^+$): 474.

EJEMPLO 62: (6-Cloro-4-fluoro-9H-□-carbolin-8-il)-amida del ácido ·6,6-dimetil-4-[2-[(2-metil-piridin-3-carbonil)-amino]-propil]-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el Método E a partir del Intermedio 66 y ácido metilnicotínico.

^{1}H RMN (300 MHz, dmso-d_6): d 11,63 (s, 1H), d 10,10 (s, 1H), d 8,90 (s, 1H), d 8,44 (d, 1H), d 8,34 (s, 1H), d 8,29 (d, 1H), 7,94 (s, 2H), 7,65 (d, 1H), 7,21 (dd, 1H), 4,25-4,15 (m, 1H), 3,97-3,88 (m, 2H), 3,24-3,15 (m, 1H), 2,99 (d, 1H), 2,59 (t, 1H), 2,49 (s, 3H), 2,40 (dd, 1H), 2,09 (d, 1H), 1,35 (s, 3H), 1,21 (d, 3H) y 1,20 (s, 3H). Tiempo de retención (LC, método: patrón de acetato amónico): 1,67 min. MS ($M+H^+$): 553.

Ejemplo 63: Ácido [(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético

A una suspensión de (6-cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-6,6-dimetil-morfolin-3-carboxílico (2,6 g, 6,0 mmol) en 60 ml de metanol se le añadieron 1,7 ml de trietilamina (2,0 equiv.), cianoborohidruro sódico (575 mg, 9,1 mmol) y ácido glioxílico (780 mg, 8,5 mmol). La mezcla de reacción se agitó a temperatura ambiente durante 1,5 h. Se añadió agua (5 ml) y la mezcla se concentró hasta una suspensión amarilla pegajosa. Después, se añadió más agua (30 ml) y la suspensión resultante se agitó a temperatura ambiente durante 10 min y se filtró. El sólido amarillo recogido se lavó con agua y se secó a alto vacío para dar 1,80 g (71%) del producto deseado.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,17 (s, 3H), 1,30 (s, 3H), 2,81 (d, 1H), 3,34 (d, 1H), 3,46 (d, 1H), 3,56 (dd, 1H), 3,84-3,90 (m, 2H), 7,92 (s, 1H), 8,15 (d, 1H), 8,21 (s, 1H), 8,36 (d, 1H), 9,01 (s, 1H), 10,28 (s, 1H),11,40 (s, 1H) .

Tiempo de retención (LC, método: patrón de acetato amónico): 1,26 min.

MS ($M+H^+$): 417,1.

MÉTODO F: Procedimiento de acoplamiento para amidas inversas a partir de *ácido [(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético*

Se recogieron ácido [(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético (1,0 mmol), EDCI (1,6 mmol) y la amina (1,2 mmol) para acoplarse en un matraz de fondo redondo y se suspendieron en piridina (5 ml). La mezcla resultante se agitó durante una noche. Después, la piridina se retiró a presión reducida y el residuo se repartió en EtOAc y una solución acuosa al 5% de Na_2CO_3. La fase acuosa separada se extrajo de nuevo con EtOAc. Los extractos combinados se lavaron sucesivamente con agua y salmuera, se secaron sobre Na_2SO_4 y se concentraron completamente. El residuo se purificó sobre sílice para dar el producto deseado.

EJEMPLO 64: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-6,6-dimetil-4-(2-oxo-2-pirrolidin-1-il-etil)-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el Método F a partir de ácido [(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y pirrolidina con un rendimiento de 82%.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,21 (s, 3H), 1,29 (s, 3H), 1,75-1,92 (m, 4H), 2,46 (d, 1H), 2,77 (d, 1H), 3,35-3,68 (m, 7H), 3,94 (m, 2H), 8,08 (s, 1H), 8,19 (d, 1H), 8,23 (s, 1H), 8,41 (d, 1H), 9,05 (s, 1H), 10,71 (s, 1H),11,51 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,75 min.

MS ($M+H^+$): 470,3.

EJEMPLO 65: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-6,6-dimetil-4-(2-oxo-2-piperidin-1-il-etil)morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el Método F a partir de ácido [(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y piperidina con un rendimiento de 90%.

^{1}H RMN (300 MHz, DMSO-d6): d 1,17 (s, 3H), 1,29 (s, 3H), 1,35-1,60 (m, 6H), 2,25 (d, 1H), 2,71 (d, 1H), 3,15 (d, 1H), 3,26 (dd, 1H), 3,37 (dd, 1H), 3,45-3,65 (m, 2H), 3,65 (d, 1H), 3,70 (m, 1H), 3,89 (m, 2H) 7,95 (d, 1H), 8,15 (d, 1H), 8,20 (d, 1H), 8,37 (d, 1H), 9,01 (s, 1H), 10,43 (s, 1H),11,32 (s, 1H)

Tiempo de retención (LC, método: patrón de acetato amónico): 1,86 min.

MS ($M+H^+$): 484,3.

EJEMPLO 66: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-6,6-dimetil-4-(2-morfolin-4-il-2-oxo-etil)-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el Método F a partir de ácido [(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y morfolina con un rendimiento de 86%.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,17 (s, 3H), 1,30 (s, 3H), 2,19 (d, 1H), 2,71 (d, 1H), 3,03 (d, 1H), 3,16 (d, 1H), 3,22 (dd, 1H), 3,45-3,72 (m, 7H), 3,80-3,98 (m, 3H), 7,94 (d, 1H), 8,15 (d, 1H), 8,21 (d, 1H), 8,37 (d, 1H), 9,03 (s, 1H), 10,35 (s, 1H),11,28 (s, 1H) .

Tiempo de retención (LC, método: patrón de acetato amónico): 1,56 min.

MS ($M+H^+$): 486,3.

EJEMPLO 67: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-4-{[(2-hidroxi-etil)-metil-carbamoil]-metil}-6,6-dimetil-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el Método F a partir de ácido [(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y 2-metilamino-etanol con un rendimiento de 55%.

^{1}H RMN (300 MHz, DMSO-d_6): d 1,21 (s, 3H), 1,30 (s, 3H), 2,39 (m, 1H), 2,75 (d a,1H), 2,96 (s, 1,5H), 3,17 (s, 1,5H), 3,50-3,65 (m, 2,5H), 3,70-3,85 (m, 1,5H), 3,93 (m, 2H), 4,69 (m, 0,5H), 4,94(m, 0,5H), 8,05 (d, 1H), 8,18-8,25 (m, 2H), 8,42 (d, 1H), 9,06 (s, 1H), 10,68 (s, 1H),11,38 (s, 1H) .

Tiempo de retención (LC, método: patrón de acetato amónico): 1,47 min.

MS ($M+H^+$): 474.

EJEMPLO 68: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-6,6-dimetil-4-(piridin-3-ilcarbamoilmetil)-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el Método F a partir de ácido [(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y 3-aminopiridina. El producto se aisló en forma de la sal clorhidrato con un rendimiento de 55%.

^1H RMN (300 MHz, D_2O): d 1,34 (s, 3H), 1,54 (s, 3H), 2,60 (d, 1H), 3,4 (d, 1H), 3,50 (d, 1H), 3,70 (t, 1H), 3,81 (d, 1H) 4,23 (d, 2H), 7,69 (d, 1H), 7,98 (d, 1H), 8,01 (d, 1H), 8,17(d, 1H), 8,42 (d, 1H), 8,48-8,55 (m, 3H), 9,09 (s, 1H), 9,30 (d, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,56 min.

MS ($M+H^+$): 493,2.

EJEMPLO 69: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido (S)-6,6-dimetil-4-{[(piridin-4-ilmetil)-carbamoil]-metil}-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con el Método F a partir de ácido [(S)-5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y 4-(aminometil)piridina. El producto se aisló en forma de la sal clorhidrato con un rendimiento de 53%.

^1H RMN (300 MHz, D_2O): d 1,34 (s, 3H), 1,49 (s, 3H), 2,64 (d, 1H), 3,03 (d, 1H), 3,48 (d, 1H), 3,72 (t, 1H), 3,74 (d, 1H), 4,15-4,25 (m, 2H), 7,66 (d, 1H), 7,88 (s, 1H), 7,91 (s, 1H), 8,21(d, 1H), 8,44 (d, 1H), 8,53 (d, 1H), 8,56 (s, 1H), 8,58 (s, 1H), 9,08 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,47 min.

MS ($M+H^+$): 507,3.

EJEMPLO 70: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 4-[2-(4-hidroximetil-piperidin-1-il)-2-oxo-etil]-6,6-dimetil-morfolin-3-carboxílico

Se disolvieron ácido [5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético (200 mg, 0,48 mmol) y piperidin-4-il-metanol (111 mg, 0,96 mmol) en piridina (4 ml). La solución amarilla resultante se agitó a temperatura ambiente durante 10 min y después se añadió en una única porción EDC (184 mg, 0,96 mmol). La mezcla de reacción se dejó en agitación durante una noche (16 h). Se añadió agua (4 ml) y la mezcla se concentró a presión reducida. El residuo resultante se repartió entre acetato de etilo (50 ml) y carbonato potásico acuoso 1 M. La capa acuosa se extrajo de nuevo con acetato de etilo (3 x 50 ml) y los extractos combinados se lavaron con agua y salmuera, se secaron sobre sulfato sódico, se filtraron y se concentraron. El residuo bruto se purificó por cromatografía sobre gel de sílice (gradiente de cloruro de metileno y metanol) para producir (6-cloro-9H-beta-carbolin-8-il)-amida del ácido 4-[2-(4-hidroximetil-piperidin-1-il)-2-oxo-etil]-6,6-dimetil-morfolin-3-carboxílico pura en forma de una espuma amarilla (152 mg, 62%). La sal bis-HCl se preparó mediante la adición de 2 equivalentes de HCl conc. a una solución etanólica de la base libre. La concentración, seguido de trituración con éter, produjo la sal en forma de un polvo amarillo de fluido libre.

^{1}H RMN (base libre, 300 MHz, $CDCl_3$) □: 11,03 (d, 1 H), 10,51 (d, 1 H), 8,97-8,80 (m, 1 H), 8,47-8,27 (m, 2 H), 7,94-7,76 (m, 2 H), 4,84 (d, 1 H), 4,16-3,77 (m, 3 H), 3,68-3,26 (m, 5 H), 3,08 (ddd, 1 H), 2,89-2,64 (m, 2 H), 2,50-2,37(m, 2 H), 1,98-1,70 (m, 3 H), 1,38 (s, 3 H), 1,25 (s, 3 H), 1,19-0,97 (m, 1).

MS (Condiciones convencionales de NH_4OAc, ES+) e/z = 514 $(M+H)^+$

DAD R_t = 1,51 min

EJEMPLO 71: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 4-[2-(4-hidroxi-piperidin-1-il)-2-oxo-etil]-6,6-dimetil-morfolin-3-carboxílico

El compuesto deseado se sintetizó usando ácido [5-(6-cloro-9*H*-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y 4-hidroxipiperazina siguiendo el Método F con un rendimiento de 31%.

^{1}H RMN (300 MHz, D_2O): d 8,85 (1H,s), 8,19 (2H, s), 7,82 (1H, s), 7,44 (1H, s), 4,15 (2H, m), 3,9-3,75 (4H, m), 3,6 (2H, m), 3,56 (2H, m), 3,12 (1H, m), 2,9 (3H, m), 2,8 (1H, m), 1,7 (2H, m) 1,34 (3H, s), 1,19 (3H, s)

Tiempo de retención (LC, método: patrón de acetato amónico): 1,5 min

MS (M+H+): 501

EJEMPLO 72: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 4-dietilcarbamoilmetil-6,6-dimetil-morfolin-3-carboxílico

El compuesto deseado se fabricó usando ácido [5-(6-cloro-9*H*-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y dietilamina siguiendo el Método F con un rendimiento de 60%.

^{1}H RMN (300 MHz, D_2O): d 8,87 (1H, s), 8,26 (2H, m), 7,8 (1H, s), 7,52 (1H, s), 4,1 (3H, m), 3,8 (2H, m), 3,22 (2H, m), 3,0 (1H, d), 2,7 (1H, d), 1,3 (3H, s), 1,19 (3H, s), 0,09 (6H, m).

Tiempo de retención (LC, método: patrón de acetato amónico) : 1,96 min

MS (M+H+): 472

EJEMPLO 73: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 6,6-dimetil-4-[-2-(4-metil-piperazin-1-il)-2-oxo-etil]-morfolin-3-carboxílico

El compuesto deseado se preparó usando ácido [5-(6-cloro-9*H*-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y 1-metil-piperazina siguiendo el Método F con un rendimiento de 12%.

^{1}H RMN (DMSO) d 11,28 (1H, s), 10,35 (1H, s), 9,01 (1H,s), 8,37 (1H, s), 8,21-8,15 (2H, m), 7,9 (1H, s), 3,89 (2H, m), 3,7 (5H, m), 3,1 (2H, m), 2,85 (2H, m), 2,49 (3H, s), 2,4 (3H, m), 1,2 (3H, s), 1,16 (3H, s)

Tiempo de retención (LC, método: patrón de acetato amónico): 1,34 min.

MS (M+H+): 500

EJEMPLO 74: (6-Cloro-9H-b-carbolin-8-il)-amida del ácido 4-[2-(2,6-dimetil-morfolin-4-il)-2-oxo-etil]-6,6-dimetil-morfolin-3-carboxílico

El compuesto deseado se preparó usando ácido [5-(6-cloro-9*H*-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético y 2,6-dimetil-morfolina siguiendo el Método F. La purificación cromatográfica dio el producto deseado con un rendimiento de 70-80%.

^{1}H RMN (300 MHz, $CDCl_3$): d 9,31 (s, 1H), 8,75 (d, 1H), 8,55 (d, 1H), 8,37 (d, 1H), 8,14 (m, 1H), 4,73 (m, 2H), 4,96 (m, 1H), 4,37 (m, 2H), 3,69 (m, 2H), 3,57 (m, 4H), 2,82 (t, 1H), 2,47 (t, 1H), 1,50 (d, 3H), 1,42 (d, 3H), 1,18 (m, 6H)

Tiempo de retención (LC, método: patrón de ácido fórmico): 1,14 min (Serie de diodos).

MS ($M+H^+$): 514, ($M-H^+$): 512

EJEMPLO 75: Éster metílico del ácido 1-{2-[5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acetil}-piperidina-4-carboxílico

Se disolvieron ácido [5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético (200 mg, 0,48 mmol) y isonipecotato de metilo (137 mg, 130 µl, 0,96 mmol) en piridina (4 ml) y se agitaron a temperatura ambiente 10 min. Se añadió EDC y la mezcla de reacción se dejó en agitación a temperatura ambiente durante una noche (16 h). Se añadió más isonipecotato de metilo (137 µl, 0,96 mmol) y la mezcla se agitó durante 24 h más. Se añadió agua (4 ml) y la mezcla se concentró. El residuo bruto se repartió entre acetato de etilo (75 ml) y carbonato potásico acuoso 1 M (50 ml). La fase acuosa se extrajo de nuevo con más acetato de etilo (75 + 50 ml). Los extractos combinados se lavaron con agua y salmuera, se secaron sobre sulfato sódico, se filtraron, se concentraron y se purificaron por cromatografía sobre gel de sílice (gradiente de cloruro de metileno/metanol) para

producir la base libre en forma de una espuma amarilla (150 mg, 57%). La sal bis-HCl se preparó mediante la adición de 2 equivalentes de HCl conc. a una solución etanólica de la base libre. La concentración, seguido de trituración con éter, produjo la sal en forma de un polvo amarillo fluido.

^{1}H RMN (base libre, 300 MHz, $CDCl_3$) □ 11,04 (d, 1 H), 10,48 (d, 1 H), 8,95 (s, 1 H), 8,48-8,31 (m, 2 H), 7,96-7,79 (m, 2 H), 4,48 (dd, 1 H), 4,13-3,92 (m, 2 H), 3,88-2,97 (m, 9 H), 2,84-2,35 (m, 3 H), 2,09-1,90 (m, 2 H), 1,85-1,52 (m, 2 H), 1,38 (s, 3 H), 1,23 (s, 3 H).

MS (Condiciones convencionales de NH_4OAc, ES+) e/z = 542 $(M+H)^+$

DAD R_t = 1,72 min

EJEMPLO 76: Sal *bis*-clorhidrato de (6-cloro-9*H*-beta-carbolin-8-il)-amida del ácido (*S*)-4-[2-(3,3-dimetil-morfolin-4-il)-2-oxo-etil]-6,6-dimetil-morfolin-3-carboxílico

Se disolvió 2,2-dimetilmorfolina (3,0 g, 26,1 mmol) (preparada de acuerdo con el procedimiento de Cottle, D.L., et al. *J. Org. Chem.* 1946, *11*, 286-291) en diclorometano (60 ml). Se añadió trietilamina (3,6 ml, 2,66 g, 26,1 mmol) y la reacción se enfrió a -10°C. Se añadió gota a gota cloruro de bromoacetilo (2,2 ml, 4,08 g, 26,1 mmol) y la solución se calentó lentamente a temperatura ambiente. La reacción se concentró a sequedad al vacío, se disolvió de nuevo en acetato de etilo y se pasó a través de un lecho de gel de sílice. El eluyente se concentró hasta un aceite amarillo (3,45 g, 56%) que se llevó a la siguiente etapa.

Tiempo de retención (LC, método: patrón de acetato amónico): 1,20 min.

MS $(M+H^+)$: 237.

El Intermedio 59 (60 mg, 0,14 mmol) se suspendió en diclorometano (3 ml). Se añadió una solución de carbonato potásico 1 M (0,5 ml). La capa orgánica se separó, se secó sobre $MgSO_4$ y se concentró a sequedad. La base libre se disolvió en DMF (1 ml) y se agitó a temperatura ambiente. Se añadió gota a gota 2-bromo-1-(3,3-dimetil-morfolin-4-il)-etanona (30 mg, 0,13 mmol) disuelto en DMF (1 ml). La reacción se agitó durante 3 horas a temperatura ambiente y se

concentró a sequedad al vacío. La cromatografía ultrarrápida en columna (93:7 de diclorometano:metanol) produjo un aceite amarillo que se disolvió en HCl 4 N/dioxano (2 ml). La concentración al vacío produjo el compuesto del título en forma de un sólido amarillo (22 mg, 29%).

^{1}H RMN (300 MHz, MeOH-d_4): d 1,35 (s, 3H), 1,41 (s, 3H), 1,43 (s, 6H), 2,98 (m, 1H), 3,45 (m, 4H), 3,56 (m, 1H), 3,76 (m, 3H), 4,30 (m, 4H), 8,04 (s, 1H), 8,39 (s, 1H), 8,52 (d, 1H), 8,73 (d, 1H), 9,24 (s, 1H)

Tiempo de retención (LC, método: patrón de acetato amónico): 1,16 min.

MS ($M+H^+$): 514

EJEMPLO 77: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 4-[(*trans*-4-hidroxi-ciclohexilcarbamoil)-metil]-6,6-dimetil-morfolin-3-carboxílico

Se disolvieron ácido [5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético (200 mg, 0,48 mmol), clorhidrato de *trans*-4-aminociclohexanol (145 mg, 0,96 mmol) y diisopropiletilamina (124 mg, 167 µl, 0,96 mmol) en piridina (4 ml) y se agitaron a temperatura ambiente durante 10 min. Se añadió EDC (184 mg) y la reacción se agitó a temperatura ambiente durante una noche (16 h). Se añadió agua (2 ml) y la mezcla resultante se concentró a presión reducida. El residuo resultante se diluyó con 50 ml de carbonato potásico acuoso 1 M y se extrajo con acetato de etilo (75 + 2 x 50 ml). Los extractos combinados se lavaron con agua y salmuera, se secaron sobre sulfato sódico, se filtraron y se concentraron. El residuo bruto se purificó por cromatografía sobre gel de sílice (gradiente de metanol/cloruro de metileno) para producir (6-cloro-9H-beta-carbolin-8-il)-amida del ácido 4-[(*trans*-4-hidroxi-ciclohexilcarbamoil)-metil]-6,6-dimetil-morfolin-3-carboxílico puro (148 mg, 59%) en forma de una espuma amarilla. La sal bis-HCl se preparó mediante la adición de 2 equivalentes de HCl conc. a una solución etanólica de la base libre. La

concentración, seguido de trituración con éter, produjo la sal en forma de un polvo amarillo de flujo libre.

^{1}H RMN (base libre, 300 MHz, $CDCl_3$) □ 11,71 (s a, 1 H), 10,21 (s, 1 H), 9,06 (s, 1 H), 8,45-8,27 (m, 2 H), 8,08-7,84 (m, 2 H), 6,36 (s a, 1 H), 4,13-3,81 (m, 3 H), 3,72-3,56 (m, 1H), 3,51-3,37 (m, 2 H), 3,03 (d, 1 H), 2,79-2,26 (m, 5 H), 2,16-1,94 (m, 4 H), 1,54-1,30 (m, 5 H), 1,22 (s, 3 H).

MS (Condiciones convencionales de NH_4OAc, ES+) e/z = 514 $(M+H)^+$

DAD R_t = 1,39 min

EJEMPLO 78: Sal *bis*-clorhidrato de (6-cloro-9*H*-beta-carbolin-8-il)-amida del ácido (*S*)-4-[2-((*2R,5R*)-2,5-dimetil-pirrolidin-1-il)-2-oxo-etil]-6,6-dimetil-morfolin-3-carboxílico

Se disolvió clorhidrato de (2R,5R)-2,5-dimetil-pirrolidina (270 mg, 2,0 mmol) (preparado usando el procedimiento de Masamune, S., et al. *J. Org. Chem.* 1989, *54*, 1756) en diclorometano (3 ml) y se enfrió a 0°C. Se añadió trietilamina (405 mg, 0,56 ml, 4,0 mmol). Se añadió gota a gota cloruro de cloroacetilo (226 mg, 0,16 ml, 2,0 mmol) disuelto en diclorometano (1 ml). La mezcla se calentó a temperatura ambiente y se agitó durante 30 minutos más. La reacción se diluyó con diclorometano (5 ml), se extrajo con HCl 1 N y salmuera y después se secó sobre $MgSO_4$. La capa orgánica se concentró hasta un aceite pardo. Peso: 242 mg (82%).

Tiempo de retención (LC, método: patrón de acetato amónico): 1,24 min.

MS ($M+H^+$): 176,5, El material se llevó a la siguiente etapa sin purificación adicional.

El Intermedio 59 (495 mg, 1,15 mmol) se disolvió en una mezcla de acetonitrilo (8 ml) y agua (2 ml). Se añadió carbonato potásico (477 mg, 3,45 mmol) y la reacción se calentó a 40°C. Se añadió gota a gota 2-cloro-1-((2R,5R)-2,5-dimetil-pirrolidin-1-il)-etanona (242 mg, 1,38 mmol) (preparada como se ha descrito anteriormente) disuelta en acetonitrilo (1 ml). La reacción se calentó a

80°C y se agitó durante una noche. La reacción se enfrió a 40°C. Se añadió en una porción yoduro sódico (207 mg, 1,38 mmol) disuelto en acetona (1 ml). La reacción se agitó durante una noche a 40°C. La reacción se concentró al vacío y se diluyó con acetato de etilo (40 ml). La capa orgánica se extrajo dos veces con agua y salmuera y después se secó sobre $MgSO_4$. La capa orgánica se filtró y se concentró al vacío hasta una espuma naranja. La cromatografía ultrarrápida en columna (95:5 de diclorometano:metanol) produjo un sólido amarillo que se disolvió en HCl 4 N/dioxano (1 ml) y se concentró a sequedad. La trituración con éter produjo el compuesto del título en forma de un sólido amarillo (18 mg, 3%).

^{1}H RMN (300 MHz, MeOH-d_4): d 1,23 (dd, 6H), 1,38 (s, 3H), 1,48 (s, 3H), 1,65 (m, 2H), 2,23 (m, 2H), 3,18 (m, 1H), 3,50 (m, 1H), 3,66 (m, 1H), 4,18 (m, 2H), 4,35 (m, 4H), 8,15 (s, 1H), 8,42 (s, 1H), 8,55 (d, 1H), 8,78 (d, 1H), 9,29 (s, 1H).

Tiempo de retención (LC, método: patrón de acetato amónico): 2,02 min.

MS ($M+H^+$): 498.

EJEMPLO 79: (6-Cloro-9H-b-carbolin-8-il)-amida del ácido 4-{2-[4-(1-hidroxi-1-metil-etil)-piperidin-1-il]-2-oxo-etil}-6,6-dimetil-morfolin-3-carboxílico.

Una solución de éster metílico del ácido 1-{2-[5-(6-cloro-9H-b-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acetil}-piperidina-4-carboxílico (62 mg, 0,11 mmol) en una mezcla de éter anhidro y tolueno (1 ml: 1 ml) se agitó a 0°C en atmósfera de N_2. A esta solución se le añadió lentamente bromuro de metilmagnesio (3,0 M en éter, 306 µl, 0,917 mmol). La mezcla de reacción se agitó a temperatura ambiente durante una noche y después se inactivó mediante la adición de bicarbonato sódico acuoso saturado. La mezcla resultante se diluyó de nuevo con agua (10 ml) y acetato de etilo (30 ml). La capa acuosa se retiró y se extrajo con acetato de etilo (30 x 2 ml). Las fases orgánicas se combinaron, se lavaron con salmuera, se secaron sobre sulfato de magnesio, se filtraron y se

concentraron para producir un sólido amarillo (85 mg). El residuo se purificó por HPLC, para producir el producto puro (13 mg, 20%).

^{1}H RMN (300 MHz, $HCDCl_3$): d 10,94 (d, 1 H), 10,47 (d, 1 H), 8,94 (s, 1 H), 8,41 (d, 1 H), 8,32 (d, 1 H), 7,89-7,81 (m, 2H), 4,91 (d, 1 H), 4,04-3,91 (m, 2 H); 3,64-3,57 (m, 1 H), 3,43 (s, 1 H), 3,37-3,31 (m, 1 H), 3,15-2,90 (m, 1 H), 2,77-2,58 (m, 2 H), 2,47-2,41 (m, 1 H), 2,00-1,86 (m, 2 H), 1,53-1,03 (m, 17 H).

Condiciones convencionales de NH_4OAc.

DAD R_t = 1,85 min

M+H = 542

EJEMPLO 80: (6-Cloro-9H-beta-carbolin-8-il)-amida del ácido 4-[2-(3,3-dimetil-4-oxo-piperidin-1-il)-2-oxo-etil]-6,6-dimetil-morfolin-3-carboxílico

Se disolvió éster terc-butílico del ácido 4-oxo-piperidina-1-carboxílico (5 g, 25 mmol) en tetrahidrofurano (100 ml) y la solución resultante se enfrió a 0°C. A la solución enfriada se le añadió en una única porción hidruro sódico (al 60% en aceite mineral, 2,10 g, 53 mmol) y la mezcla turbia resultante se dejó en agitación durante 10 min. Después, se añadió yoduro de metilo y la mezcla se dejó calentar a temperatura ambiente durante varias horas. La agitación se continuó durante una noche (12 h). La mezcla naranja clara se concentró a presión reducida. El residuo se repartió entre éter y agua. La fase acuosa se extrajo de nuevo con más éter. Los extractos combinados se lavaron con agua y salmuera, se secaron sobre sulfato sódico, se filtraron y se concentraron hasta un sólido amarillo pálido. El sólido se trituró con acetato de etilo al 4% en hexanos (50 ml) para producir éster terc-butílico del ácido 3,3-dimetil-4-oxo-piperidina-1-carboxílico en forma de un sólido de color crema (1,8 g, 32%).

^{1}H RMN ($CDCl_3$, 300 MHz) □ 3,73 (t, 2 H), 3,43 (s a, 2 H), 2,49 (t, 2 H), 1,49 (s, 9 H), 1,13 (s, 6 H).

El éster terc-butílico del ácido 3,3-dimetil-4-oxo-piperidina-1-carboxílico preparado de esta manera (450 mg, 1,97 mmol) se disolvió en cloruro de metileno

(10 ml). Se añadió ácido trifluoroacético (305 µl) y la solución resultante se agitó a temperatura ambiente 2 h. Se añadió más ácido trifluoroacético (300 µl) y la reacción se agitó a temperatura ambiente durante 3 días. La solución amarilla pálida se concentró para producir un residuo oleoso que se trituró con éter. Los sólidos se recogieron por filtración por succión y se secaron al vacío. Se aisló 3,3-dimetil-piperidin-4-ona y se usó en forma de su sal del ácido trifluoroacético (381 mg, 80%).

^{1}H RMN (d_6-DMSO, 300 MHz) □ 3,44-3,33 (m, 4 H), 2,63-2,57 (m, 2 H), 1,11 (s, 6 H).

Se disolvieron ácido [5-(6-cloro-9H-beta-carbolin-8-ilcarbamoil)-2,2-dimetil-morfolin-4-il]-acético (100 mg, 0,24 mmol), sal del ácido 3,3-dimetil-piperidin-4-ona trifluoroacético (116 mg, 0,48 mmol) y diisopropiletilamina (62 mg, 85 µl) en piridina (3 ml) y se agitaron durante 10 min. Se añadió EDC (92 mg, 0,48 mmol) y la mezcla se agitó a temperatura ambiente 4 días. Se añadió agua (3 ml) y la reacción inactivada se concentró. El residuo se repartió entre acetato de etilo (50 ml) y carbonato sódico acuoso 1 M (50 ml). La fase acuosa se extrajo con más acetato de etilo (50 ml) y los extractos se combinaron. Después, los extractos se lavaron con agua y salmuera, se secaron sobre sulfato sódico, se filtraron, se secaron y se concentraron a presión reducida. El residuo resultante se purificó por cromatografía sobre gel de sílice (gradiente de cloroformo, acetato de etilo, metanol), produciendo (6-cloro-9H-beta-carbolin-8-il)-amida del ácido 4-[2-(3,3-dimetil-4-oxo-piperidin-1-il)-2-oxo-etil]-6,6-dimetil-morfolin-3-carboxílico en forma de una espuma amarilla (91 mg, 73%). La sal bis-HCl se preparó mediante la adición de 2 equivalentes de HCl conc. a una solución etanólica de la base libre. La concentración, seguido de trituración con éter, produjo la sal en forma de un polvo amarillo de flujo libre.

^{1}H RMN ($CDCl_3$, 300 MHz) □ 10,67 (s, 1 H), 10,37 (d, 1 H), 8,93 (s, 1 H), 8,41 (d, 1 H), 8,15 (dd, 1 H), 7,82 (d, 1 H), 7,71 (d, 1 H), 4,07-3,92 (m, 3 H), 3,83-

3,89 (m, 6 H), 2,79-2,68 (m, 1 H), 2,62-2,40 (m, 3 H), 1,41-1,36 (m, 3 H), 1,27-1,22 (m, 3 H), 1,18-1,13 (m, 3 H), 1,07-0,99 (m, 3 H).

MS (Condiciones convencionales de NH_4OAc, ES+) e/z = 526 $(M+H)^+$

DAD R_t = 1,79 min

EJEMPLO 81: (6-Cloro-4-metil-9H-b-carbolin-8-il)-amida del ácido 6,6-dimetil-4-(2-oxo-2-pirrolidin-1-il-etil)-morfolin-3-carboxílico

El compuesto deseado se preparó de acuerdo con los Métodos C, E y F a partir de 6-cloro-4-metil-9H-ß-carbolin-8-ilamina (Intermedio 54) y pirrolidina.

^1H RMN (300 MHz, MeOD-d4) d 9,17 (s, 1) 8,39 (s, 1) 8,26 (d, 1) 8,17 (d, 1) 4,74-4,52 (m, 3) 4,47-4,30 (m, 1) 3,80-3,52 (m, 3) 3,48-3,34 (m, 4) 3,01 (s, 3) 1,76-1,55 (m, 4) 1,50 (s, 3) 1,43 (s, 3).

Condiciones convencionales de NH_4OAc ELSD R_t = 2,01 min.

M+H = 498

Ensayo Biológico

Los compuestos de esta invención son inhibidores eficaces de IκB quinasa (IKK), y por lo tanto, son útiles para tratar afecciones provocadas o agravadas por la actividad de esta quinasa. Las actividades inhibidora de IκB quinasa *in vitro* e *in vivo* de los compuestos de fórmula I pueden determinarse por diversos procedimientos conocidos en la técnica. Las potentes afinidades por IκB quinasa mostradas por los compuestos de la invención pueden medirse como un valor de IC_{50} (en nM), que es la concentración (en nM) de compuesto necesaria para proporcionar una inhibición del 50% de IκB quinasa.

Lo siguiente son ejemplos de ensayos que pueden ser útiles para evaluar y seleccionar un compuesto que module IKK.

Ensayo para medir la inhibición de la enzima IκB quinasa

Un ensayo *in vitro* para detectar y medir la actividad de inhibición frente al complejo I?B quinasa por agentes farmacológicos candidatos puede emplear un polipéptido que abarque la Ser^{32} y Ser^{36} de I?B (SwissProt Nº de Acceso 25963, Swiss Institute of Bioinformatics, Geneva, Suiza) y un agente para la detección del producto fosforilado, por ejemplo, un anticuerpo específico que se une sólo a la forma fosforilada del polipéptido, siendo monoclonal o policlonal (por ejemplo, anticuerpos anti-fosfo-serina^{32} I?B disponibles en el mercado). En el ejemplo para detectar el producto fosforilado por una anticuerpo anti-fosfoserina^{32} I?B, una vez está formado el complejo anticuerpo-fofo-polipéptido, el complejo puede detectarse por una diversidad de métodos analíticos (por ejemplo, radiactividad, luminiscencia, fluorescencia, o absorbancia óptica). Para el uso del método DELFIA (Inmunoensayo de Fluorescencia de Lantánidos con Potenciamiento de Disociación) (fluorometría resuelta en el tiempo, Perkin Elmer Life and Analytical Sciences Inc., Boston, MA), el complejo puede inmovilizarse en un placa de unión a biotina (por ejemplo, placa recubierta con Neutravidina) y detectarse con un anticuerpo secundario conjugado a Europio, o en una placa de unión a anticuerpo (por ejemplo, placa recubierta con Proteína-A) y detectarse con proteína de unión a biotina conjugada a Europio (por ejemplo, Estreptavidina-Europio). El nivel de actividad puede correlacionarse con una curva patrón usando fosfopéptidos sintéticos que corresponden a polipéptido sustrato. Cómo preparar materiales para y dirigir este ensayo se describe con más detalle a continuación.

<u>Aislamiento del complejo I?B quinasa</u>

Se preparó un complejo I?B-a quinasa diluyendo primero 10 ml de la fracción S100 de extractos de células HeLa S3 (Lee *et al.* (1997) Cell 88:213-222) con 40 ml de HEPES 50 mM pH 7,5, Después, se añadió sulfato de amonio al 40% y se incubó en hielo durante 30 minutos. El sedimento precipitado resultante se redisolvió con 5 ml de tampón SEC (HEPES 50 mM pH 7,5, DTT 1 mM, EDTA 0,5 mM, 2-glicerofosfato 10 mM), se aclaró por centrifugación a 20.000 x g durante 15 min, y se filtró a través de una unidad de filtro de 0,22 µm. La muestra se cargó

en una columna de FPLC de filtración en gel SUPEROSE-6 (Amersham Biosciences AB, Uppsala, Suecia) equilibrada con un tampón SEC aplicado a un caudal de 2 ml/min a 4°C. Las fracciones que abarcan el marcador de peso molecular de 670-kDa se combinaron para la activación. Después se activó una combinación que contenía quinasa por incubación con MEKK1? 100 nM (Lee *et al.* (1997) Cell 88:213-222), MgATP 250 µM, $MgCl_2$10 mM, DTT 5 mM, 2-glicerofosfato 10 mM, Microcistina-LR 2,5 µM, durante 45 minutos a 37°C. La enzima activada se almacenó a –80°C hasta su uso adicional.

Medida de la actividad I?B quinasa fosfo-transferasa

En cada pocillo de una placa de 96 pocillos, se preincubaron los compuestos de diversas concentraciones en 5 µl de DMSO al 20% durante 30 minutos a 25°C con 40 µl de enzima activada diluida 1:25 con tampón de ensayo (Hepes 50 mM pH 7,5, DTT 5 mM, $MgCl_2$10 mM, 2-glicerofosfato 10 mM, Microcistina-LR 2 µM, Albúmina de Suero Bovino al 0,1%). Se añadieron 5 µl de sustrato peptídico (biotina-$(CH_2)_6$-DRHDSGLD(fosfoS)MKD-$CONH_2$) a 200 µM + ATP 500 µM a cada pocillo y se incubaron durante 1 hora antes de interrumpir con 50 µL de Hepes 50 mM pH 7,5, BSA al 0,1%, EDTA 100 mM. Se transfirieron 5 µl de reacción de quinasa interrumpida a una placa de Proteína A (Pierce Biotechnology, Inc., Rockford, IL, EEUU) que contenía 90 µl de anticuerpo anti-fosfo IκB S32/S36 (Cell Signaling Technologies Beverly, MA, EEUU) a 2 µg/ml. Las muestras se incubaron durante 2 horas con agitación. Después de 3 lavados con PBS + Tween20 al 0,05%, se añadieron 90 µl de quelato de europio unido a estreptavidina (Perkin Elmer Life and Analytical Sciences, Boston, MA, EEUU) a 0,1 µg/ ml a cada pocillo y se incubaron durante 1 hora con agitación. Después de 3 lavados con PBS + Tween20 al 0,05%, se añadieron 100 µl of Solución de Ponclamiento DELFIA (Perkin Elmer Life and Analytical Sciences, Boston, MA, EEUU) a cada pocillo. Se leyó una señal de europio con una excitación de 330 nM y emisión de 615 nM en un lector de placa Wallac Victor (Perkin Elmer Life and

Analytical Sciences, Boston, MA). Como el ensayo demostró ser lineal previamente con respecto a la concentración de enzima y tiempo para la dilución de enzima ensayados, los niveles de señal de europio se usaron para determinar la actividad de inhibición de los agentes farmacológicos candidatos.

Los compuestos de la invención fueron inhibidores activos del complejo IKK. Se apreciará que los compuestos de esta invención pueden mostrar actividades inhibidoras de IκB quinasa de grados variados. Después de procedimientos de ensayo tales como los ensayo in vitro y los basados en células descritos en este documento, los valores de IC_{50} medios de inhibición de IκB quinasa para los compuestos de la invención estuvieron generalmente por debajo de aproximadamente 10 micromolar, preferiblemente, por debajo de aproximadamente 1,0 micromolar y más preferiblemente por debajo de aproximadamente 100 nanomolar. Los compuestos de la invención también son selectivos para inhibir IKK-2 a diferencia de IKK-1.

Ensayos Celulares

Líneas celulares de Mieloma Múltiple (MM) y asilamiento de células MM obtenidas de un paciente

Se obtuvieron células MM humanas RPMI 8226 y U266 de la American Type Culture Collection (Manassas, VA). Todas las líneas celulares MM se cultivaron en RPMI-1640 que contenía suero bovino fetal al 10% (FBS, Sigma-Aldrich Co., St. Louis, MO), L-glutamina 2 mM, 100 U/ml de penicilina (Pen) y 100 µg/ml de estreptomicina (Strep) (productos de cultivo celular de la marca GIBCO disponibles de Invitrogen Life Technologies, Carlsbad, CA). Las células MM obtenidas de un paciente se purificaron de aspirados de la médula ósea (BM) del paciente usando el sistema de separación ROSETTESEP (kit de enriquecimiento de células B) (StemCell Technologies, Vancouver, Canadá). La pureza de las células MM se confirmó por citometría de flujo usando anticuerpo anti-CD138 conjugado con PE (BD Biosciences, Bedford, MA).

Cultivos Celulares de Estroma de Médula Ósea

Se obtuvieron muestras de médula ósea (BM) de pacientes con MM. Las células mononucleadas (MNC) separadas por sedimentación por densidad de Ficoll-Hypaque se usaron para establecer cultivos de BM de larga duración como se ha descrito previamente (Uchiyama *et al.*, *Blood* 1993, 82:3712-3720). Las células se recogieron en Solución Salina Tamponada de Hank (HBSS) que contenía tripsina al 0,25% y EDTA al 0,02%, se lavaron, y se recogieron por centrifugación.

Proliferación Celular mediante medida de velocidad de síntesis de ADN

La proliferación se midió como se ha descrito previamente (Hideshima *et al.*, *Blood* 96:2943 (2000)). Las células MM (3×10^4 células/pocillo) se incubaron en placas de cultivo de 96 pocillos (Corning Life Sciences, Corning, NY) en presencia de medios o un inhibidor de IKK de esta invención durante 48 h a 37°C. La síntesis de ADN se midió por incorporación de [^{3}H]-timidina ([^{3}H]-TdR, New England Nuclear division of Perkin Elmer Life and Analytical Sciences, Boston, MA) en células en división. La células se sometieron a pulsos con [^{3}H]TdR (0,5 μCi/pocillo) durante las últimas 8 h de cultivos de 48 h. Todos los experimentos se realizaron por triplicado.

Ensayo de Viabilidad de Celular con MTT

El efecto inhibidor de los presentes compuestos en el crecimiento de MM se evaluó midiendo la reducción de amarillo de tatrazolio MTT (bromuro de 3-(4,5-dimetiltiazolil-2)-2,5-difeniltetrazolio) por células metabólicamente activas (J. Immunol. Methods 174: 311-320, 1994). Las células de cultivos de 48 h se sometieron a pulsos con 10 μl de 5 mg/ml de MTT a cada pocillo durante las últimas 4 h de los cultivos de 48 h, seguido de 100 μl de isopropanol que contenía HCl 0,04 N. La absorbancia se midió a 570 nm usando un espectrómetro (Molecular Devices Corp., Sunnyvale CA).

Activación de NF-κB mediante Ensayo de Cambio de Movilidad Electroforética

Los análisis de cambio de movilidad electroforética (EMSA) se realizaron como se ha descrito previamente (Hideshima *et al.*, *Oncogene* 2001, 20:4519). Brevemente, las células MM se preincubaron con un inhibidor de IKK de esta invención (10 μM durante 90 min) antes de estimulación con TNF-α (5 ng/ml) durante 10 a 20 min. Después las células se sedimentaron, se resuspendieron en 400 μl de tampón de lisis hipotónico (HEPES 20 mM, pH 7,9, KCl 10 mM, EDTA 1 mM, Triton X-100 al 0,2%, Na_3VO_4 1 mM, NaF 5 mM, PMSF 1 mM, 5 μg/ml de leupeptina, 5 μg/ml aprotinina), y se mantuvieron en hielo durante 20 min. Después de centrifugación (14000 g durante 5 min) a 4°C, el sedimento nuclear se extrajo con 100 μl de tampón de lisis hipertónico (HEPES 20 mM, pH 7,9, NaCl 400 mM, EDTA 1 mM, Na_3VO_4 1 mM, NaF 5 mM, PMSF 1 mM, 5 μg/ml de leupeptina, 5 μg/ml de aprotinina) en hielo durante 20 min. Después de centrifugación (14000 g durante 5 min) a 4°C, el sobrenadante se recogió como extracto nuclear. Se marcó en el extremo una sonda oligonucleotídica consenso de doble cadena de NF-κB (5'-GGGGACTTTCCC-3', Santa Cruz Biotechnology Inc., Santa Cruz CA) con [(^{32}P]ATP (50 μCi a 222 TBq/mM; New England Nuclear division of Perkin Elmer Life and Analytical Sciences, Boston, MA). Las reacciones de unión que contenían 1 ng de oligonucleótido y 5 μg de proteína nuclear se realizaron a temperatura ambiente durante 20 min en un volumen total de 10 μl de tampón de unión (Tris-HCl 10 mM, pH 7,5, NaCl 50 mM, $MgCl_2$ 1 mM, EDTA 0,5 mM, DTT 0,5 mM, glicerol al 4% (v/v), y 0,5 μg de poli (dI-dC) (Amersham Biosciences AB, Uppsala, Suecia). Para el análisis de supercambio, se añadió 1 μg de Ab anti-p65 de NF-κB 5 min antes de las mezclas de reacción, inmediatamente después de la adición de la sonda radiomarcada. Las muestras se cargaron en un gel de poliacrilamida al 4%, se transfirieron a papel Whatman (Whatman International, Maidstone, U.K.), y se visualizaron por autorradiografía.

Ensayo de Proliferación Celular de Linfoma Difuso de Células B Grandes

Se mantuvieron las líneas celulares DLBCL tipo ABC (LY3 y Ly10) y tipo GCB (Ly7 y Ly19) (Alizadeh et al (2000) Nature 403:503-511; Davis et al. (2001) J. Exp. Med. 194:1861-1874) en medio de crecimiento (GM, DMEM de Iscove + FBS al 10%) pasando las células dos veces por semana. Las células se privaron durante una noche en medio DMEM de Iscove + FBS al 0,5% durante una noche antes de sembrarlas en placas en el ensayo de proliferación. En el día del ensayo, se contaron las células y se controló la viabilidad usando tinción de Azul de Tripano. Para las células Ly3 y Ly10, se sembraron 5000 células en GM por pocillo en una placa de 96 pocillos. Las células Ly7 y Ly19 se sembraron a 10.000 células por pocillo. Los inhibidores de IKK primero se disolvieron en DMSO y después se diluyeron en GM para alcanzar las concentraciones finales de 80 μM-0,01 μM. Cada concentración se sembró en placas por triplicado. La viabilidad celular se determinó usando un ensayo de viabilidad celular WST-1 convencional (Roche Applied Science, Indianapolis, IN).

Ensayo de Liberación de Citoquinas por monocitos sanguíneos periféricos (PBMC) humanos

Se purificaron PBMC de la sangre completa de un donante normal por el método de gradiente de Ficoll. Después de un lavado con PBS, se resuspendieron PBMC en medio AIM-V. Se añadieron los inhibidores de IKK de esta invención diluidos de manera seriada en DMSO al 100% a 1 μl a la parte superior de una placa de 96 pocillos y se mezclaron con 180 μl de 4,5 X 10^5 PBMC en medios AIM-V por pocillo. Después de preincubar PBMC con inhibidor a 37°C durante 40 min, las células se estimularon con 20 μl de LPS (100 ng/ml) o anti-CD3 (0,25 μg/ml) y anti-CD28 (0,25 μg/ml) (Pharmingen division of BD Biosciences, Bedford, MA) a 37°C durante 5 horas. Los sobrenadantes se recogieron y se ensayaron para la liberación de IL-1β o TNF-α usando kit de ELISA disponibles en el mercado convencionales.

Ensayo de Liberación de Metaloproteasas de Matriz (MMP) de Condrocito Humano

La línea celular SW1353 de condrocito humano (ATCC, Manassas, VA) se cultivó conteniendo suero bovino fetal al 10% (Hyclone, Logan, UT), L-glutamina 2 mM (productos de cultivo celular de la marca GIBCO disponibles de Invitrogen Life Technologies, Carlsbad, CA) y Pen/Strep al 1% (GIBCO). Las células se sembraron en una placa de Poli-D-Lisina de 96 pocillos (BD BIOCOAT, Black/Clear bottom, BD Biosciences, Bedford, MA). Se añadieron inhibidores de IKK diluidos de manera seriada a 1 µl a cada pocillo de placas de 96 pocillos y se mezclaron con 180 µl de 4,5 X 10^5 condrocitos por pocillo. Después de pre-incubar las células con compuestos durante 1 h a 37°C, las células se estimularon con 20 µl de IL-1β (10 ng/ml, R&D Systems Inc.) a 37°C durante 24 h. Después se recogieron los sobrenadantes y se evaluaron para la producción de metaloproteinasas de matriz (MMP) usando kits de ELISA disponibles en el mercado.

Ensayo de Sinoviocito Tipo Fibroblasto Humano (HFLS)

Se proporcionó HFLS aislado de tejidos sinoviales RA obtenidos de cirugía de remplazamiento articular por Cell Applications Inc. (San Diego, CA). Los inhibidores de IKK de la invención se ensayaron para su capacidad de bloquear la liberación de IL-6 o IL-8 inducida por TNF-α o IL-1β de estas células usando kits de ELISA disponibles en el mercado. Las condiciones de cultivo celular y métodos de ensayo se describieron en Aupperle et al., *Journal of Immunology*, 163:427-433 (1999).

Ensayo de Mastocitos Obtenidos de Sangre de Cordón Umbilical Humano

Se obtuvo sangre de cordón umbilical humano de Cambrex (Walkersville, MD). Los mastocitos se diferenciaron y se cultivaron de un modo similar al descrito por Hsieh et al., *J. Exp. Med.*, 193:123-133 (2001). Los inhibidores de IKK de la

Invención se ensayaron para su capacidad de bloquear la liberación de TNFα inducida por IgE o LPS usando kits de ELISA disponibles en el mercado.

Ensayos de Diferenciación y Funcionales de Osteoclastos

Se obtuvieron precursores de osteoclastos humanos en forma crioconservada de Cambrex (Walkersville, MD). Las células se diferenciaron en cultivo en base a las instrucciones del fabricante. Los inhibidores de IKK de la invención se ensayaron para su capacidad de bloquear la diferenciación, resorción ósea y degradación colágeno como se ha descrito previamente (véase, Khapli, S. M., *Journal of Immunol*, 171:142-151 (2003); Karsdal, M. A., *J Biol Chem*, 278:44975-44987 (2003); y Takami, M., *Journal of Immunol*, 169:1516-1523 (2002)).

Modelos de Rata para Artritis Reumatoide

Se descubrió que ciertos compuestos de esta invención son activos en uno o más modelos de rata para artritis reumatoide. Dicho ensayo se conoce en la bibliografía e incluye un modelo de LPS de rata convencional como se describe en Conway et al., "Inhibition of Tumor Necrosis Factor-a (TNF-a) Production and Arthritis in the Rat by GW3333, a Dual Inhibitor of TNF—Converting Enzyme and Matrix Metalloproteinases", *J. Pharmacol. Exp. Ther.* 298(3), 900-908 (2001); un modelo de rata de artritis inducida por adyuvante como se describe en Pharmacological Methods in the Control of Inflammation (1989) páginas 363-380 "Rat Adjuvant Arthritis: A Model of Chronic Inflammation" Barry M. Weichman autor del capítulo del libro {Alan R. Liss Inc Publisher}; y un modelo de rata de artritis inducida por colágeno como se describe en Pharmacological Methods in the Control of Inflammation (1989) páginas 395-413 "Type II Collagen Induced Arthritis en the Rat" DE Trentham and RA Dynesuis-Trentham autores del capítulo del libro {Alan R. Liss Inc Publisher}. Véase, también, "Animal Models of Arthritis: Relevance to Human Disease" (1999) por A. Bendele, J. McComb, T. Gould, T.

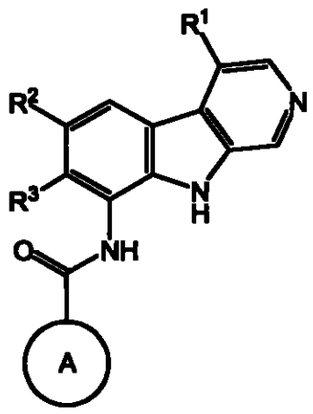
McAbee, G. Sennello, E. Chlipala y M. Guy. Toxicologic Pathology Vol 27 (1) 134-142.

En base a los resultados de uno o más modelos de rata tales como los descritos anteriormente, se descubrió que los compuestos de fórmula II-C son sorprendentemente superiores en comparación con otros compuestos donde el Anillo A es un anillo de piridina. También en los modelos de rata, se descubrió que los compuestos de fórmula III-A-a, especialmente los compuestos de fórmula III-A-aa, son sorprendentemente superiores en comparación con otros compuestos donde el Anillo A es un anillo de morfolina.

Aunque se han descrito varias realizaciones de esta invención, es evidente que los ejemplos básicos pueden alterarse para proporcionar otras realizaciones, que utilizan los compuestos y métodos de esta invención. Por lo tanto, se apreciará que el alcance de esta invención se definen por las reivindicaciones adjuntas en lugar de por las realizaciones específicas, que se han representado a modo de ejemplo.

REIVINDICACIONES

1. Un compuesto de fórmula I:



I

o a una sal farmacéuticamente aceptable del mismo, donde:

el Anillo A se selecciona entre el grupo compuesto por:

(a) un anillo piridinilo o pirimidinilo que está sustituido con (i) $-CH_2C(O)$-G y 0-1 R^{6a} o (ii) 1-2 R^{6a}, y

(b) un anillo morfolinilo, piperidinilo, piperazinilo, pirrolidinilo, piranilo, tetrahidrofuranoilo, ciclohexilo, ciclopentilo o tiomorfolinilo que está sustituido con (i) $-C(R^9)_3$, –W-G o –G, (ii) 0-4 R^{6b} y (iii) 0-1 grupos oxo sobre un carbono del anillo o 0-2 grupos oxo sobre un azufre del anillo;

cada R^{6a} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, alcoxi C_{1-6} o amino;

cada R^{6b} se selecciona independientemente entre un grupo alifático C_{1-3} o $-N(R^7)_2$, y dos R^{6b} sobre el mismo o un carbono adyacente se toman opcionalmente junto con el/los átomo(s) de carbono que intervienen para formar un anillo de 5-6 miembros que tiene 1-2 heteroátomos en el anillo seleccionados entre N, O o S;

W es –Q-, –Q-C(O)-, -$C(R^9)_2$-$C(R^9)(R^{12})$- o -$C(R^9)_2$-$[C(R^9)(R^{12})]_2$-;

Q es -$C(R^9)_2$- o -$C(R^9)_2C(R^9)_2$-;

G es –OH, –NR^4R^5, -$N(R^9)CONR^4R^5$, -$N(R^9)SO_2$(un grupo alifático C_{1-3}), -$N(R^9)COCF_3$, -$N(R^9)CO$(un grupo alifático C_{1-6}), -$N(R^9)CO$(heterociclilo), -$N(R^9)CO$(heteroarilo), -$N(R^9)CO$(arilo), un anillo heterociclilo de 3-7 miembros o un heteroarilo de 5-6 miembros, donde cada uno de los restos heteroarilo, arilo y heterociclilo de G está opcionalmente sustituido con 1-3 R^{10};

R^1 es hidrógeno, halo, un grupo alifático C_{1-3}, amino, ciano, (alquil C_{1-3})$_{1-2}$ amino, alcoxi C_{1-3}, -$CONH_2$, -$NHCOCF_3$ o -CH_2NH_2;

R^2 es hidrógeno, halo, un grupo alifático C_{1-3}, -CF_3;

R^3 es hidrógeno, halo, un grupo alifático C_{1-6}, haloalquilo C_{1-6}, alcoxi C_{1-6}, hidroxi, amino, ciano o (alquil C_{1-6})$_{1-2}$ amino;

R^4 es hidrógeno, heterocicilo de 3-7 miembros o un grupo alifático C_{1-6};

R^5 es hidrógeno, un grupo alifático C_{1-6} o un anillo heterocíclico de 3-7 miembros que tiene 1-2 heteroátomos en el anillo seleccionados entre N, O o S, donde R^5 está opcionalmente sustituido con halo, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$S(O)_2N(R^7)_2$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^8$ o -$N(R^7)C(O)N(R^7)_2$;

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};

cada R^9 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-3};

cada R^{10} se selecciona independientemente entre oxo, -R^{11}, -T-R^{11} o -V-T-R^{11};

cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$ o $-N(R^7)C(O)N(R^7)_2$;

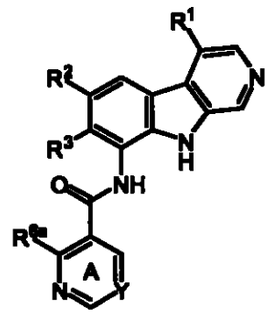
T es una cadena alquileno C_{1-4} lineal o ramificada;

V es –O-, $-N(R^7)-$, -S-, -S(O)-, $-S(O)_2-$, -C(O)- o $-CO_2-$; y

R^{12} es hidrógeno o una cadena lateral de aminoácidos.

2. El compuesto de la reivindicación 1, en el que el Anillo A es un anillo 3-piridinilo o 5-pirimidinilo sustituido con 1-2 grupos R^{6a}.

3. El compuesto de la reivindicación 2, que tiene la fórmula II-C:



II-C

donde:

Y es N o CH;

R^1 es hidrógeno, halo, un grupo alifático C_{1-3}, amino, ciano, (alquil C_{1-3})$_{1-2}$ amino, alcoxi C_{1-3}, $-CONH_2$, $-NHCOCF_3$ o $-CH_2NH_2$;

R^2 es hidrógeno, halo, un grupo alifático C_{1-3}, $-CF_3$;

R^3 es hidrógeno, halo, un grupo alifático C_{1-6}, haloalquilo C_{1-6}, alcoxi C_{1-6}, hidroxi, amino, ciano o (alquil C_{1-6})$_{1-2}$ amino; y

R^{6a} se selecciona entre un grupo alifático C_{1-6} o halo.

4. El compuesto de la reivindicación 3, en el que R^{6a} es metilo.

5. El compuesto de la reivindicación 4, en el que R^1 es hidrógeno, metilo, amino o fluoro; R^2 es hidrógeno o halo; y R^3 es hidrógeno, halo o alcoxi C_{1-4}.

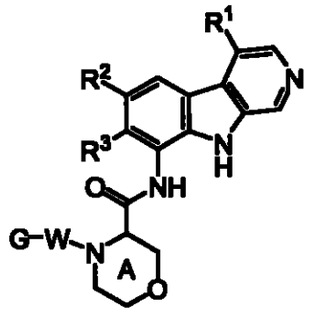
6. El compuesto de la reivindicación 5 en el que Y es CH.

7. El compuesto de la reivindicación 5 en el que Y es N.

8. El compuesto de la reivindicación 1 en el que el Anillo A se selecciona entre morfolinilo, piperidinilo, piperazinilo, pirrolidinilo, piranilo, tetrahidrofuranollo, ciclohexilo, ciclopentilo o tiomorfolinilo y donde el Anillo A está sustituido con (i) $-C(R^9)_3$ o -W-G, (ii) 0-4 R^{6b} y (iii) 0-1 grupos oxo sobre un carbono del anillo o 0-2 grupos oxo sobre un azufre del anillo.

9. El compuesto de la reivindicación 8 en el que el sustituyente –W-G o $–C(R^9)_3$ sobre el anillo A está en posición orto donde se une a la porción beta-carbolina.

10. Un compuesto de fórmula III-A:



III-A

o a una sal farmacéuticamente aceptable del mismo, donde:

El Anillo A está sustituido con 0-4 R^{6b};

cada R^{6b} se selecciona independientemente entre un grupo alifático C_{1-3} o $-N(R^7)_2$, y dos R^{6b} sobre el mismo o un carbono adyacente se toman opcionalmente junto con el/los átomo(s) de carbono que intervienen para formar un anillo de 5-6 miembros que tiene 1-2 heteroátomos en el anillo seleccionados entre N, O o S;

W es –Q-, –Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$ o $-C(R^9)_2-[C(R^9)(R^{12})]_{2}-$;

Q es $-C(R^9)_2-$ o $-C(R^9)_2C(R^9)_2-$;

G es –OH, $-NR^4R^5$, $-N(R^9)CONR^4R^5$, $-N(R^9)SO_2$(un grupo alifático C_{1-3}), $-N(R^9)COCF_3$, $-N(R^9)CO$(un grupo alifático C_{1-6}), $-N(R^9)CO$(heterociclilo), $-N(R^9)CO$(heteroarilo), $-N(R^9)CO$(arilo), un anillo heterocíclilo de 3-7 miembros o un heteroarilo de 5-6 miembros, donde cada uno de los restos heteroarilo, arilo y heterociclilo de G está opcionalmente sustituido con 1-3 R^{10};

R^1 es hidrógeno, halo, un grupo alifático C_{1-3}, amino, ciano, (alquil $C_{1-3})_{1-2}$ amino, alcoxi C_{1-3}, $-CONH_2$, $-NHCOCF_3$ o $-CH_2NH_2$;

R^2 es hidrógeno, halo, un grupo alifático C_{1-3}, $-CF_3$;

R^3 es hidrógeno, halo, un grupo alifático C_{1-6}, haloalquilo C_{1-6}, alcoxi C_{1-6}, hidroxi, amino, ciano o (alquil $C_{1-6})_{1-2}$ amino;

R^4 es hidrógeno, un grupo heterociclilo de 5-6 o un grupo alifático C_{1-6};

R^5 es hidrógeno, un grupo alifático C_{1-6} o un anillo heterocíclico de 5-6 miembros que tiene 1-2 heteroátomos en el anillo seleccionados entre N, O o S, donde R^5 está opcionalmente sustituido con halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$ o $-N(R^7)C(O)N(R^7)_2$;

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};

cada R^9 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-3};

cada R^{10} se selecciona independientemente entre oxo, $-R^{11}$, $-T-R^{11}$ o $-V-T-R^{11}$;

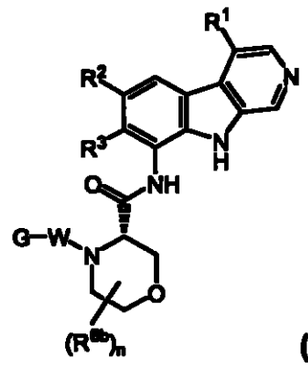
cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$ o $-N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada;

V es –O-, $-N(R^7)-$, -S-, -S(O)-, $-S(O)_2-$, -C(O)- o $-CO_2-$; y

R^{12} es hidrógeno o una cadena lateral de aminoácidos.

11. El compuesto de la reivindicación 10 que tiene la fórmula (S)-III-A:



(*S*)-III-A

donde n es 0-4 y R^1, R^2, R^3, W, G y R^{6b} son como se han definido en la reivindicación 10.

12. El compuesto de la reivindicación 11, en el que:

R^1 es hidrógeno, halo, metilo o amino;

R^2 es hidrógeno, metilo o halo;

R^3 es hidrógeno, halo, alcoxi o (un grupo alifático $C_{1-6})_2$ amino;

n es 0-2;

R^{6b} es un grupo alifático C_{1-3};
W es –Q-, –Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$ o $-C(R^9)_2-[C(R^9)(R^{12})]_{2-}$;
Q es $-C(R^9)_2-$ o $-C(R^9)_2C(R^9)_2-$;
G es $-NR^4R^5$, $-N(R^9)C(O)NR^4R^5$, $-N(R^9)SO_2$(un grupo alifático C_{1-3}), $-N(R^9)C(O)CF_3$, $-N(R^9)CO$(un grupo alifático C_{1-6}), y $-N(R^9)CO$(heterocicilo), $-N(R^9)CO$(heteroarilo), $-N(R^9)CO$(arilo), un anillo heterocicilo de 5-6 miembros o un heteroarilo de 5-6 miembros, donde cada uno de los restos heteroarilo, arilo y heterocicilo de G está opcionalmente sustituido con 1-3 R^{10};
R^4 es hidrógeno o un grupo alifático C_{1-6};
R^5 es hidrógeno o un grupo alifático C_{1-6} que está opcionalmente sustituido con halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$ o $-N(R^7)C(O)N(R^7)_2$;
cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterocicilo de 5-6 miembros;
cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};
R^9 es hidrógeno;
cada R^{10} se selecciona independientemente entre oxo, R^{11}, $T-R^{11}$ o $V-T-R^{11}$;
cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$ o $-N(R^7)C(O)N(R^7)_2$;
T es una cadena alquileno C_{1-4} lineal o ramificada;
V es –O-, $-N(R^7)-$, -S-, -S(O)-, $-S(O)_2-$, -C(O)- o $-CO_2-$; y
R^{12} es hidrógeno, un grupo alifático C_{1-6}, fenilo sustituido o no sustituido o bencilo sustituido o no sustituido.

13. El compuesto de la reivindicación 12, en el que:
R^1 es hidrógeno, metilo, flúor o amino;
R^2 es cloro;

R^3 es hidrógeno o alcoxi;

n es cero ó 2;

R^{6b} es metilo;

W es –Q-, –Q-C(O)- o -$C(R^9)_2$-$C(R^9)(R^{12})$-;

Q es -$C(R^9)_2$- o -$C(R^9)_2C(R^9)_2$-;

G es –NR^4R^5, -$N(R^9)C(O)NR^4R^5$, -$N(R^9)C(O)CF_3$, -$N(R^9)$CO(un grupo alifático C_{1-6}), y -$N(R^9)$CO(heterociclilo), -$N(R^9)$CO(heteroarilo), un anillo heterociclilo de 5-6 miembros o un heteroarilo de 5-6 miembros, donde cada uno de los restos heteroarilo y heterociclilo de G está opcionalmente sustituido con 1-3 R^{10};

R^4 es hidrógeno o un grupo alifático C_{1-6};

R^5 es hidrógeno o un grupo alifático C_{1-6};

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};

R^9 es hidrógeno;

cada R^{10} se selecciona independientemente entre oxo, R^{11}, T-R^{11} o V-T-R^{11};

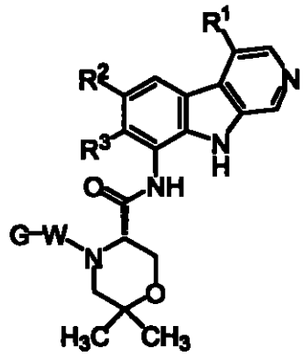
cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, -$S(O)_2N(R^7)_2$, -OR^7, -CN, -SR^8, -$S(O)_2R^8$, -$C(O)R^7$, -CO_2R^7, -$N(R^7)_2$, -$C(O)N(R^7)_2$, -$N(R^7)C(O)R^7$, -$N(R^7)CO_2R^7$ o -$N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada;

V es –O-, -$N(R^7)$-, -S-, -S(O)-, -$S(O)_2$-, -C(O)- o -CO_2-; y

R^{12} es hidrógeno, un grupo alifático C_{1-6}, fenilo o bencilo.

14. Un compuesto de fórmula (*S*)-III-A':



(*S*)-III-A'

o a una sal farmacéuticamente aceptable del mismo, donde:

R^1 es hidrógeno, metilo, flúor o amino;

R^2 es cloro;

R^3 es hidrógeno o alcoxi;

W es –Q-, –Q-C(O)- o -$C(R^9)_2$-$C(R^9)(R^{12})$-;

Q es -$C(R^9)_2$- o -$C(R^9)_2C(R^9)_2$-;

G es –NR^4R^5, -$N(R^9)C(O)NR^4R^5$, -$N(R^9)C(O)CF_3$, -$N(R^9)$CO(un grupo alifático C_{1-6}), y -$N(R^9)$CO(heterociclilo), -$N(R^9)$CO(heteroarilo), un anillo heterociclilo de 5-6 miembros o un heteroarilo de 5-6 miembros, donde cada uno de los restos heteroarilo y heterociclilo de G está opcionalmente sustituido con 1-3 R^{10};

R^4 es hidrógeno o un grupo alifático C_{1-6};

R^5 es hidrógeno o un grupo alifático C_{1-6};

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;

cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};

R^9 es hidrógeno;

cada R^{10} se selecciona independientemente entre oxo, R^{11}, T-R^{11} o V-T-R^{11};

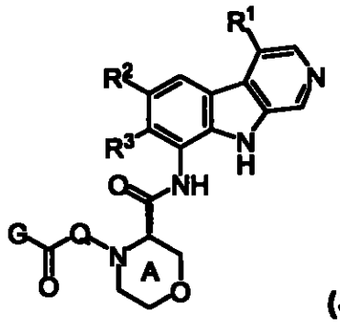
cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$ o $-N(R^7)C(O)N(R^7)_2$;

T es una cadena alquileno C_{1-4} lineal o ramificada;

V es –O-, $-N(R^7)-$, -S-, -S(O)-, $-S(O)_2-$, -C(O)- o $-CO_2-$; y

R^{12} es hidrógeno, un grupo alifático C_{1-6}, fenilo o bencilo.

El compuesto de la reivindicación 14 que tiene la fórmula (*S*)-III-A-a:



(*S*)-III-A-a

donde R^1 es hidrógeno, halo, metilo o amino;

R^2 es hidrógeno, metilo o halo;

R^3 es hidrógeno, halo, alcoxi o (un grupo alifático $C_{1-6})_2$ amino;

El Anillo A está sustituido con 0-2 R^{6b};

R^{6b} es un grupo alifático C_{1-3};

Q es $-C(R^9)_2-$ o $-C(R^9)_2C(R^9)_2-$;

G es $-NR^4R^5$ o un anillo heterociclilo de 5-6 miembros sustituido o no sustituido;

R^4 es hidrógeno o un grupo alifático C_{1-6};

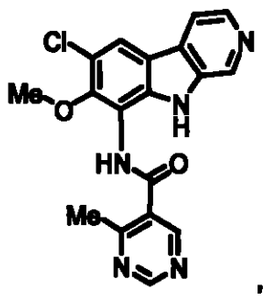
R^5 es hidrógeno o un grupo alifático C_{1-6} que está opcionalmente sustituido con halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$ o $-N(R^7)C(O)N(R^7)_2$;

cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;
cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4}; y
cada R^9 es independientemente hidrógeno o un grupo alifático C_{1-3}.

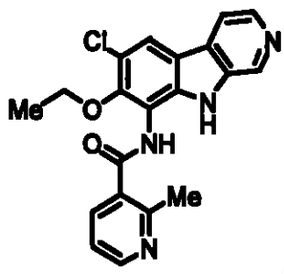
16. Un compuesto seleccionado entre el grupo compuesto por:



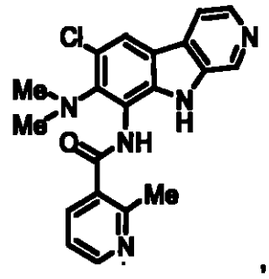
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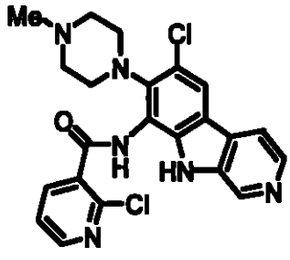
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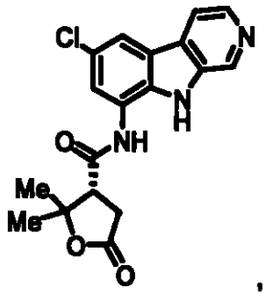
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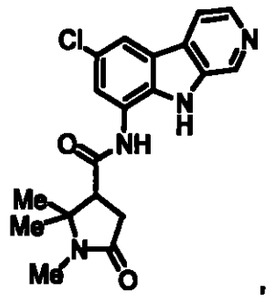
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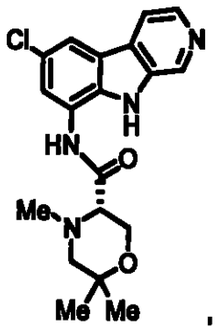
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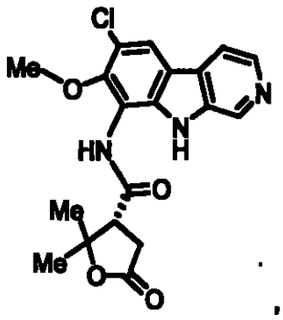
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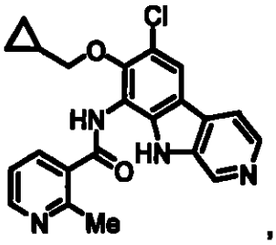
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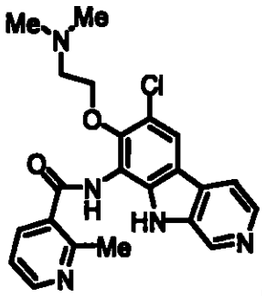
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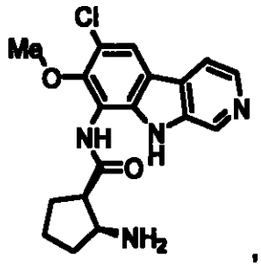
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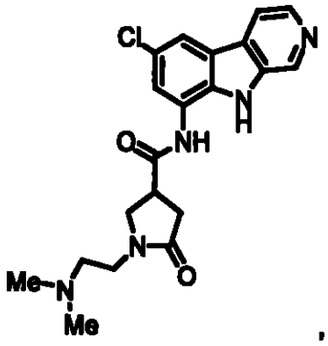
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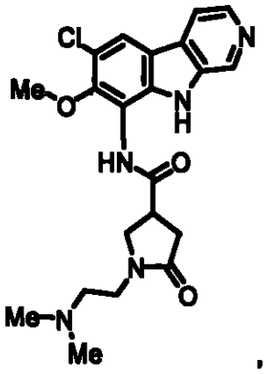
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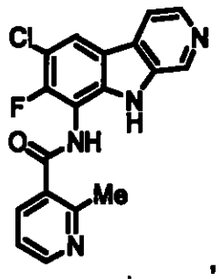
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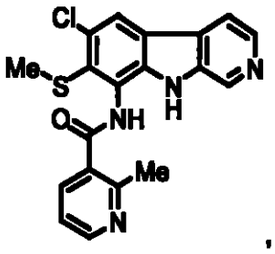
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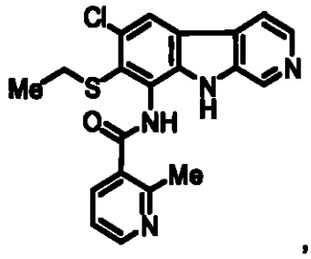
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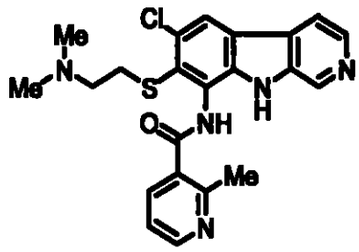
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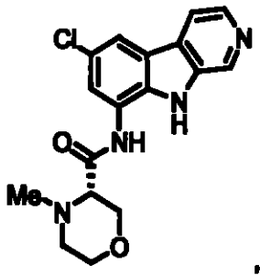
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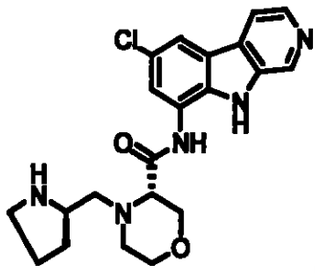
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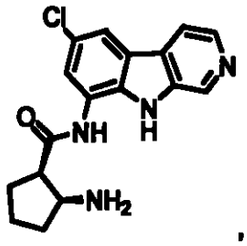
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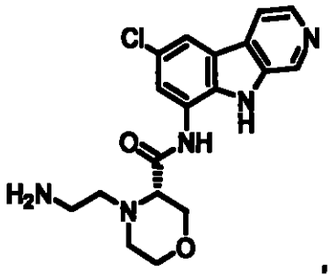
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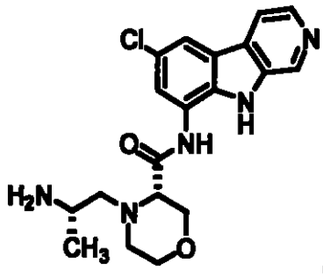
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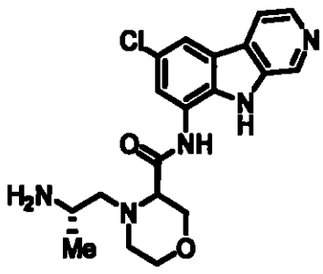
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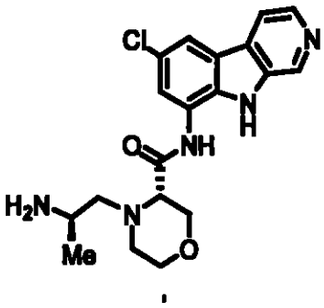
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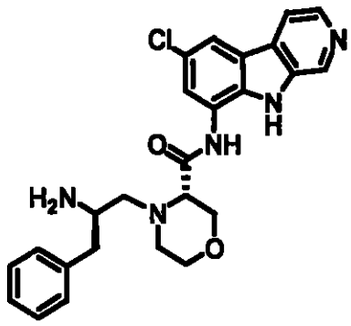
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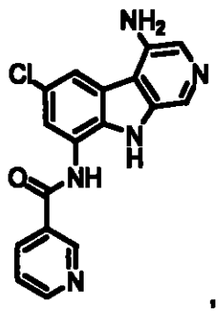
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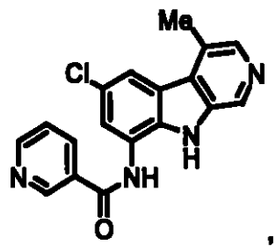
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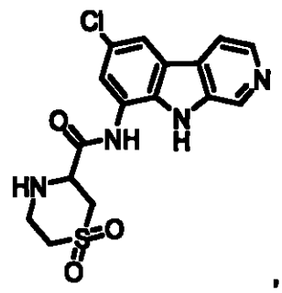
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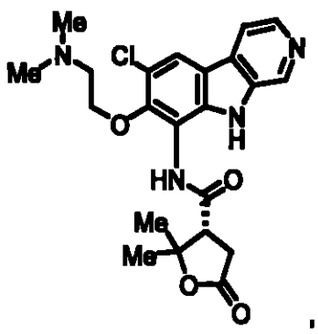
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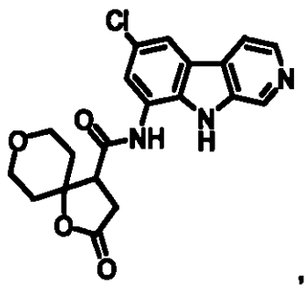
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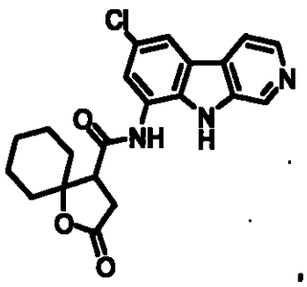
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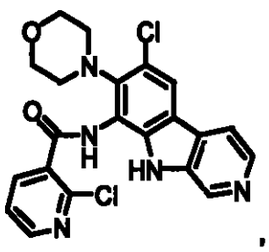
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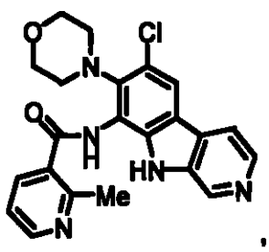
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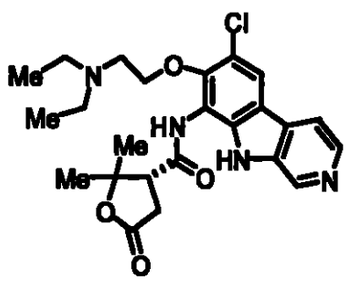
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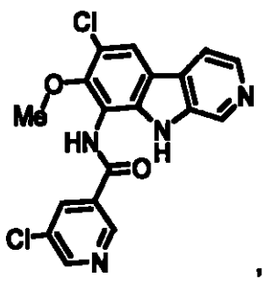
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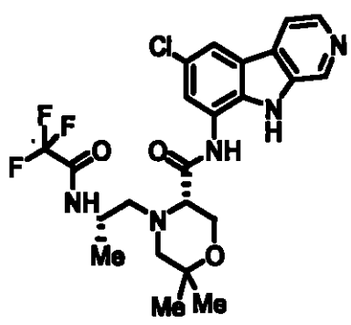
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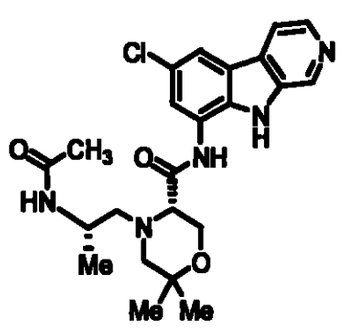
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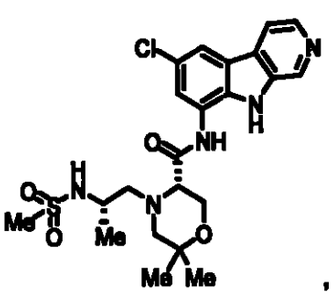
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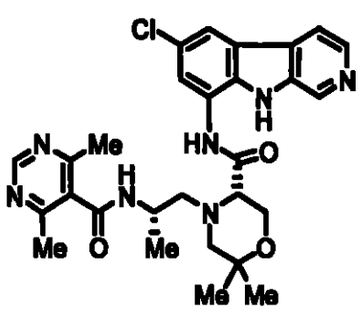
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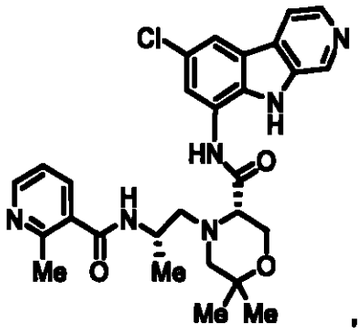
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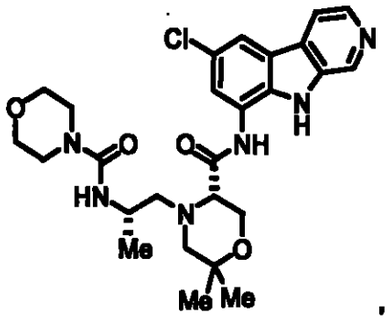
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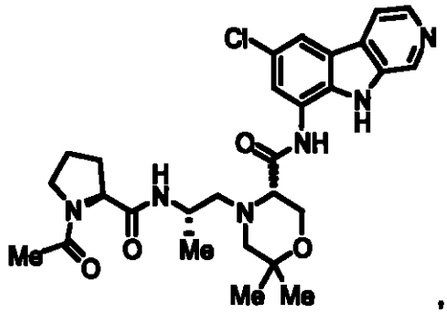
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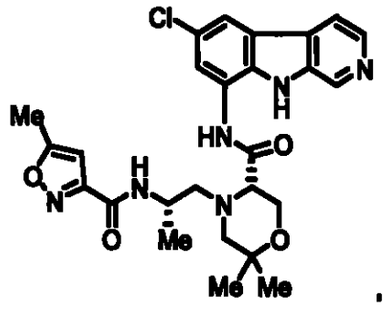
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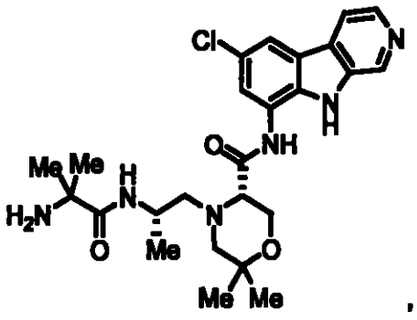
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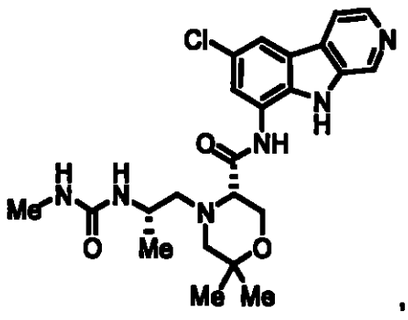
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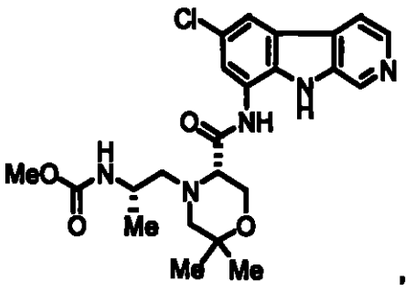
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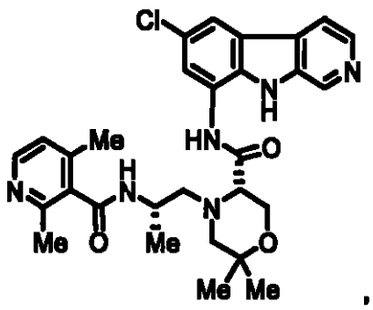
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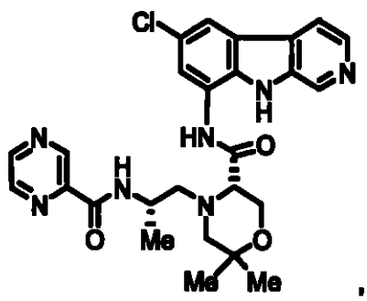
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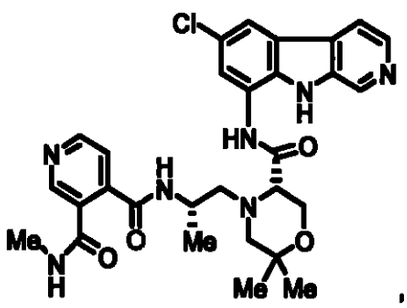
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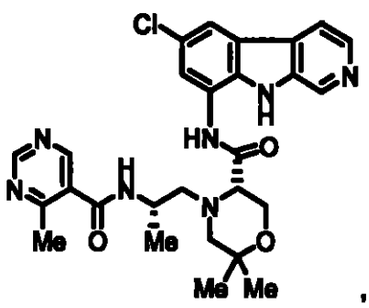
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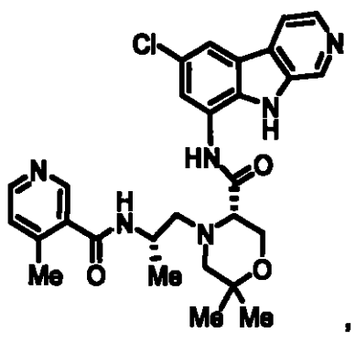
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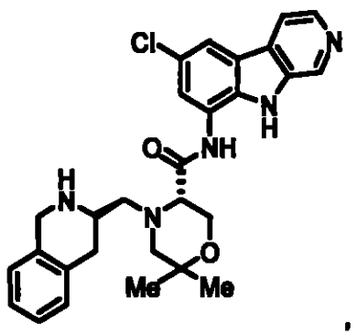
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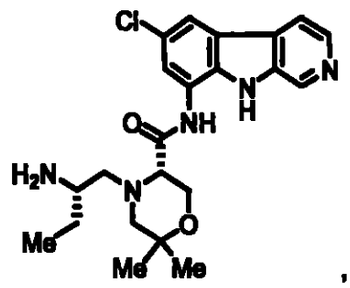
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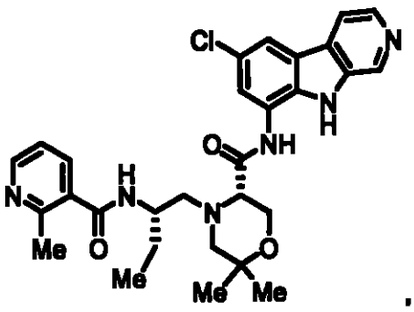
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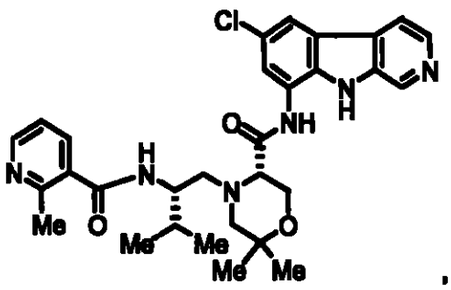
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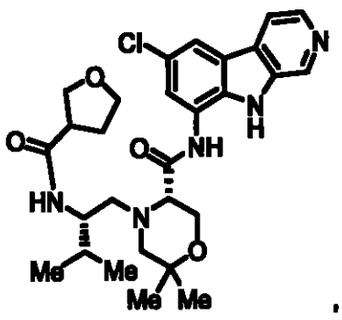
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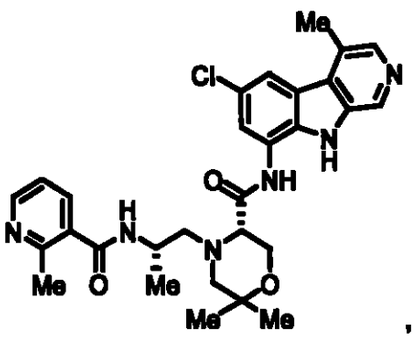
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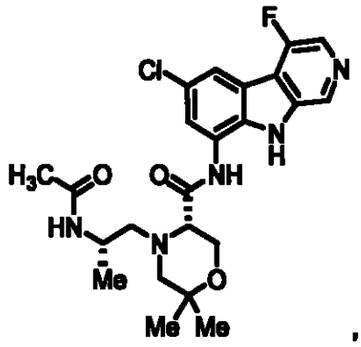
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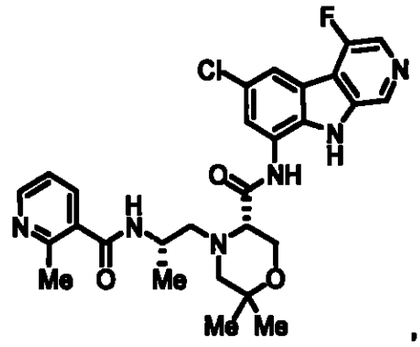
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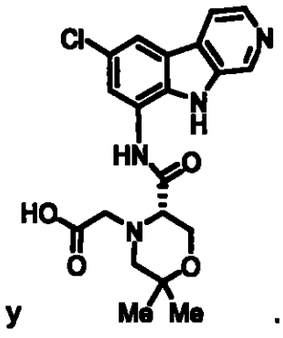
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y

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17. Una composición farmacéutica que comprende un compuesto de la reivindicación 1 y un vehículo farmacéuticamente aceptable.

18. Una composición farmacéutica que comprende un compuesto de la reivindicación 16 y un vehículo farmacéuticamente aceptable.

19. Un método para tratar una enfermedad mediada por IKK que comprende administrar a un paciente en necesidad de dicho tratamiento una cantidad terapéuticamente eficaz de un compuesto de la reivindicación 1.

20. El método de la reivindicación 19, donde la enfermedad es una enfermedad inflamatoria o una enfermedad relacionada con el sistema inmune.

21. El método de la reivindicación 19, donde la enfermedad se selecciona entre el grupo compuesto por artritis reumatoide, asma, psoriasis, artritis psoriática, enfermedad pulmonar obstructiva crónica, enfermedad inflamatoria del intestino o esclerosis múltiple.

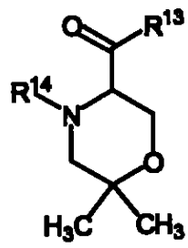
22. El método de la reivindicación 19, donde la enfermedad es cáncer.

23. El método de la reivindicación 22, donde el cáncer se selecciona entre linfoma, mieloma múltiple, metástasis ósea osteolítica, cáncer de cabeza o cuello, cáncer de pulmón, cáncer de próstata o cáncer pancreático.

24. El método de la reivindicación 23, donde el cáncer es un linfoma.

25. Un método para inhibir IKK en un paciente en necesidad del mismo que comprende administrar al paciente un compuesto de la reivindicación 1.

26. Un compuesto de fórmula 3a:



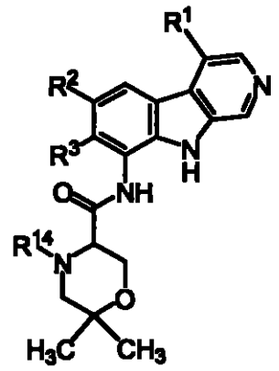
3a

en la que R^{13} es halo, OH, OR^{15} o un grupo protector de ácido carboxílico;
R^{15} es un grupo alifático, arilo, heteroarilo, aralquilo o heteroaralquilo;

R^{14} es un grupo protector de amino, hidrógeno o -W-G;
W es –Q-, –Q-C(O)-, $-C(R^9)_2-C(R^9)(R^{12})-$ o $-C(R^9)_2-[C(R^9)(R^{12})]_{1-2}-$;
Q es $-C(R^9)_2-$ o $-C(R^9)_2C(R^9)_2-$;
G es –OH, $-NR^4R^5$, $-N(R^9)CONR^4R^5$, $-N(R^9)SO_2$(un grupo alifático C_{1-3}), $-N(R^9)COCF_3$, $-N(R^9)CO$(un grupo alifático C_{1-6}), $-N(R^9)CO$(heterociclilo), $-N(R^9)CO$(heteroarilo), $-N(R^9)CO$(arilo), un anillo heterociclilo de 3-7 miembros o un heteroarilo de 5-6 miembros, donde cada uno de los restos heteroarilo, arilo y heterociclilo de G está opcionalmente sustituido con 1-3 R^{10};
R^4 es hidrógeno, heterociclo de 3-7 miembros o un grupo alifático C_{1-6};
R^5 es hidrógeno, un grupo alifático C_{1-6} o un anillo heterocíclico de 3-7 miembros que tiene 1-2 heteroátomos en el anillo seleccionados entre N, O o S, donde R^5 está opcionalmente sustituido con halo, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^8$ o $-N(R^7)C(O)N(R^7)_2$;
cada R^7 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-4} o dos R^7 sobre el mismo átomo de nitrógeno se toman junto con el nitrógeno para formar un anillo heteroarilo o heterociclilo de 5-6 miembros;
cada R^8 se selecciona independientemente entre un grupo alifático C_{1-4};
cada R^9 se selecciona independientemente entre hidrógeno o un grupo alifático C_{1-3};
cada R^{10} se selecciona independientemente entre oxo, $-R^{11}$, $-T-R^{11}$ o $-V-T-R^{11}$;
cada R^{11} se selecciona independientemente entre un grupo alifático C_{1-6}, halo, $-S(O)_2N(R^7)_2$, $-OR^7$, -CN, $-SR^8$, $-S(O)_2R^8$, $-C(O)R^7$, $-CO_2R^7$, $-N(R^7)_2$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)R^7$, $-N(R^7)CO_2R^7$ o $-N(R^7)C(O)N(R^7)_2$;
T es una cadena alquileno C_{1-4} lineal o ramificada;
V es –O-, $-N(R^7)-$, -S-, -S(O)-, $-S(O)_2-$, -C(O)- o $-CO_2-$; y
R^{12} es hidrógeno o una cadena lateral de aminoácidos.

27. El compuesto de la reivindicación 26 que es (*S*)-3a.

28. Un compuesto de fórmula IV:



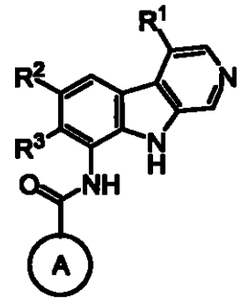
IV

en la que R^{14} es un grupo protector de amino o hidrógeno;
R^1 es hidrógeno, halo, un grupo alifático C_{1-3}, amino, ciano, (alquil C_{1-3})$_{1-2}$ amino, alcoxi C_{1-3}, -$CONH_2$, -$NHCOCF_3$ o -CH_2NH_2;
R^2 es hidrógeno, halo, un grupo alifático C_{1-3}, -CF_3; y
R^3 es hidrógeno, halo, un grupo alifático C_{1-6}, haloalquilo C_{1-6}, alcoxi C_{1-6}, hidroxi, amino, ciano o (alquil C_{1-6})$_{1-2}$ amino.

El compuesto de la reivindicación 28 que es (*S*)-IV.

Resumen de la Invención

Esta invención proporciona compuestos beta-carbolina de fórmula I:



I

donde el Anillo A es un anillo piridinilo, pirimidinilo, morfolinilo, piperidinilo, piperazinilo, pirrolidinilo, piranilo, tetrahidrofuranoilo, ciclohexilo, ciclopentilo o tiomorfolinilo sustituido y R^1, R^2 y R^3 son como se han definido en la memoria descriptiva. Los compuestos son inhibidores de IKK-2 que son útiles para tratar enfermedades mediadas por IKK-2 tales como enfermedades inflamatorias y cáncer.

TRANSMITTAL LETTER TO THE UNITED STATES RECEIVING OFFICE

Date	09 April 2004
International Application No.	
Attorney Docket No.	MPI03-043

EV375839255US

I. Certification under 37 CFR 1.10 (if applicable)

EV375839255US	09 April 2004
Express Mail Mailing Number	Date of Deposit

I hereby certify that the application/correspondence attached hereto is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Box PCT, Assistant Commissioner for Patents, Washington, D.C. 20231.

Beverly Sotiropoulos	Beverly Sotiropoulos
Signature of Person Mailing Correspondence	Typed or Printed Name of Person Mailing Correspondence

II. ☑ New International Application

TITLE	BETA-CARBOLINES USEFUL FOR TREATING INFLAMMATORY DISEASE	Earliest priority date (Day/Month/Year)
		09 April 2003

SCREENING DISCLOSURE INFORMATION: In order to assist in screening the accompanying international application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied. (Note: check as many boxes as apply):

A. ☐ The invention disclosed was not made in the United States.

B. ☐ There is no prior U.S. application relating to this invention.

C. ☑ The following prior U.S. application(s) contain subject matter which is related to the invention disclosed in the attached international application. (*NOTE: priority to these applications may or may not be claimed on form PCT/RO/101 (Request) and this listing does not constitute a claim for priority*)

application no.	60/461,468	filed on	09 April 2003	application no.		filed on	
application no.		filed on		application no.		filed on	

E. ☑ The present international application ☑ contains additional subject matter not found in the prior U.S. application(s) identified in paragraph C above. The additional subject matter is found on pages throughout and ☑ **DOES NOT ALTER** ☐ **MIGHT BE CONSIDERED TO ALTER** the general nature of the invention in a manner which would require the U.S. application to have been made available for inspection by the appropriate defense agencies under 35 U.S.C. 181 and 37 CFR 5.1. See 37 CFR 5.15.

III. ☐ **A response to an Invitation from the RO/US. The following document(s) is(are) enclosed:**

A. ☐ A Request for an Extension of Time to File a Response

B. ☐ A Power of Attorney (General or Regular)

C. ☐ Replacement pages:

D. ☐ Submission of Priority Documents

E. ☐ Fees as specified on attached Fee Calculation sheet form PCT/RO/101 annex

IV. ☐ **A Request for Rectification under PCT Rule 91** ☐ **A Petition** ☐ **A Sequence Listing Diskette**

V. ☑ **Other (please specify):** PCT Request Form (7 pages-in duplicate), PCT Fee Calculation Sheet (1 page), 193 pages of Specification (pp. 1-169), 23 pages of Claims (pp. 170-192), 1 page of Abstract (p. 193), General Power of Attorney for Millennium Pharmaceuticals, Inc. by Scott Brown (1 page); General Power of Attorney for Michael E. Hepperle (1 page); General Power of Attorney for Julie Fields Liu (1 page); General Power of Attorney for Francois Soucy (1 page); General Power of Attorney for Yingchun Ye (1 page); General Power of Attorney for Robert S. Murray (1 page); General Power of Attorney for Raman Prakash (1 page); General Power of Attorney for Jeremy D. Little (1 page); General Power of Attorney for Alfredo Castro (1 page); General Power of Attorney for Hormoz Mazdiyasni (1 page); General Power of Attorney for Paul E. Fleming (2 pages); General Power of Attorney for Dominic Reynolds (1 page); and return postcard

The person signing this form is the:		
	☐ Applicant	Ian Robert SILVERMAN
	☑ Attorney/Agent (Reg. No.) 37,443	Typed name of signer
	☐ Common Representative	Ian Robert Silverman
		Signature

PTO-1382 (Rev 08-1997) U.S. Department of Commerce: Patent and Trademark Office

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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving office use only
International Application No.
International Filing Date
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* MPI03-043

Box No. I TITLE OF INVENTION

BETA-CARBOLINES USEFUL FOR TREATING INFLAMMATORY DISEASE

Box No. II APPLICANT ☐ This person is also inventor

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MILLENNIUM PHARMACEUTICALS, INC.
40 Landsdowne Street
Cambridge, MA 02139
United States of America

Telephone No.: (617) 679-7000

Facsimile No.: (617) 551-8820

Applicant's Registration No. with the Office:

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

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

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HEPPERLE, Michael E.
280 Newbury Street #4
Boston, MA 02116
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office:

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

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

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

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

SILVERMAN, Ian Robert
MILLENNIUM PHARMACEUTICALS, INC.
40 Landsdowne Street
Cambridge, MA 02139
United States of America

Telephone No.: (617) 551-8604

Facsimile No.: (617) 551-8820

Applicant's Registration No. with the Office: 37,443

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

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Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LIU, Julie Fields
1 Fox Lane
Lexington, MA 02420
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SOUCY, Francois
39 Ellen Road
Stoneham, MA 02180
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*.

YE, Yingchun
241 Forest Park
Durham, NH 03824
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MURRAY, Robert S.
37 Lyman Avenue
Medford, MA 02155
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PRAKASH, Raman
125 Summer Street
Acton, MA 01720
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LITTLE, Jeremy D.
62 Valley Street
Wakefield, MA 01880
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CASTRO, Alfredo
81 Middlesex Street
Winchester, MA 01890
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MAZDIYASNI, Hormoz
19 Old Farm Road
Douglas, MA 01516
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

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Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

FLEMING, Paul E.
7 Sunnyside Avenue
Wellesley, MA 02482
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

REYNOLDS, Dominic
27 Chalk Street, Apt. 1F
Cambridge, MA 02139
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's Registration No. with the Office

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

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

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

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Box No. V DESIGNATION OF STATES *Mark the applicable check-boxes below; at least one must be marked*

The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

- ☐ DE Germany is not designated for any kind of national protection
- ☐ KR Republic of Korea is not designated for any kind of national protection
- ☐ RU Russian Federation is not designated for any kind of national protection

(The check-boxes above may be used to exclude (irrevocably) the designations concerned in order to avoid the causing of the effect, under the national law of an earlier national application from which priority is claimed. See the Notes to Box No. V as to the consequences of such national law provisions in these and certain other States.)

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1) 09 April 2003 (09.04.04)	60/461,468	US		
item (2)				
item (3)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box

** Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):*.....

.....

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / EP .....

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos. VIII(i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*: Number of declarations

☐	Box No. VIII(i)	Declaration as to the identity of the inventor	:
☐	Box No. VIII(ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:
☐	Box No. VIII(iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:
☐	Box No. VIII(iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:
☐	Box No. VIII(v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	

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Supplemental Box

CONTINUATION of Box No. IV (AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE)

BROWN, Scott A., Reg. No. 32,724
SILVERI, Jean M., Reg. No. 39,020
KUTZENCO, Allan N., Reg. No. 41,578
KLUNDER, Janice M., Reg. No. 41,121
SCHRAY, Kerri Pollard, Reg. No. 47,866
SIOUSSAT, Tracy M., Reg. No. 50,609

All of MILLENNIUM PHARMACEUTICALS, INC., 40 Landsdowne Street, Cambridge, MA, 02139, United States of America

CONTINUATION of Box No. VI (PRIORITY CLAIM):

The priority of the following earlier application(s) is also hereby claimed:

item (4)						
item (5)						
item (6)						

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Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application contains:

(a) In paper form, the following number of sheets:

request (including declaration sheets)	:	7
description (excluding sequence listing part and/or tables related thereto)	:	169
Claims	:	23
Abstract	:	1
Drawings	:	
Sub-total number of sheets	:	200
sequence listing	:	
tables related thereto	:	
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (b) below)		
Total number of sheets	:	200

(b) ☐ only in computer readable form (Section 801(a)(i))

(i) ☐ sequence listing

(ii) ☐ tables related thereto

(c) ☐ also in computer readable form (Section 801(a)(ii))

(i) ☐ sequence listing

(ii) ☐ tables related thereto

Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the

☐ sequence listing.....

☐ tables related thereto.....

(additional copies to be indicated under item 9(ii), and/or 10(ii), in right column)

This international application is accompanied by the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item)*:

			Number of items
1.	☒	fee calculation sheet	: 1
2.	☐	original separate power of attorney	:
3.	☐	original general power of attorney	:
4.	☒	copies of general powers of attorney (12); reference number, if any	: 12
5.	☐	statement explaining lack of signature	:
6.	☐	priority document(s) identified in Box No. VI as item(s):	:
7.	☐	translation of international application into *(language)*	:
8.	☐	separate indications concerning deposited microorganism or other biological material	:
9.	☐	sequence listing in computer readable form (indicate type and number of carriers)	:
	(i) ☐	Copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)	:
	(ii) ☐	*(only where check-box (b)(i) or (c)(i) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule13ter	:
	(iii) ☐	Together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in the left column	:
10	☐	Tables in computer readable form related to sequence listing *(indicate type and number of carriers)*	:
	(i) ☐	copy submitted for the purposes of international search under Section 802 (b-quarter) only (and not as part of the international application)	:
	(ii) ☐	*(only where check-box (b)(ii) or (c)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-quarter)	:
	(iii) ☐	together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column	:
11	☒	other *(specify)* *Transmittal Letter/Postcard*	: 2

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

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

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

Ian Robert Silverman

Ian Robert SILVERMAN, Registration No. 37,443
Agent for Applicants

For receiving Office use only

1. Date of actual receipt of the purported international application:		2. Drawings:
3. corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		☐ received
4. Date of timely receipt of the required corrections under PCT Article 11(2):		
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid	☐ not received:

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

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PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office only

International application No.

Applicant's or agent's file reference MP103-043

Date stamp of the receiving Office

Applicant MILLENNIUM PHARMACEUTICALS, INC. et al.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE ..... | USD 300 [T]

2. SEARCH FEE ..... | 1920 [S]
International search to be carried out by IB/EP
(If two or more International Searching Authorities are competent to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Where items (b) and/or (c) of Box No. IX apply, enter Sub-Total number of Sheets
Where items (b) and (c) of Box No. IX do not apply, enter Total number of Sheets 200

[i1] First 30 sheets ..... | 1053 [I1]

[i2] 170 (number of sheets in excess of 30) x 12 (fee per sheet) = | 2040 [I2]

[i3] additional components (only if sequence listing and/or tables related thereto are filed in computer readable form under Section 801(a)(i), or both in that form and on paper, under Section 801(a)(ii).

400 x _____ = | _____ [I3]

Add amounts entered at I1, I2 and I3 and enter total at I ..... | 3093 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international filing fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the international filing fee.)

4. FEE FOR PRIORITY DOCUMENT (if applicable) ..... | 20 [P]

5. TOTAL FEES PAYABLE TOTAL | 5333
Add amounts entered at T, S, I and P, and enter total in the Total box

MODE OF PAYMENT

- ☒ Authorization to charge deposit account (see below)
- ☐ bank draft
- ☐ coupons
- ☐ cheque
- ☐ cash
- ☐ other *(specify)*:
- ☐ postal money order
- ☐ revenue stamps

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT *(this mode of payment may not be available at all receiving Offices)*

Receiving Office: RO/US

☒ Authorization to charge the total fees indicated above.

Deposit Account No.: 501668

☒ (This check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit) Authorization to charge any deficiency or credit any over payment in the total fees indicated above.

Date: April 9, 2004

Name: Jan Robert SILVERMAN, Reg. No. 37,443

☒ Authorization to charge the fee for the priority document.

Signature: Jan Robert Silverman

INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY (IPEA)

International Application No.:	PCT/US2004/011080
International Filing Date:	09 APRIL 2004 (09.04.2004)
Applicant:	MILLENNIUM PHARMACEUTICALS, INC. et al.
Title:	BETA-CARBOLINES USEFUL FOR TREATING INFLAMMATORY DISEASE
Receiving Office:	RO/US
Priority Date Claimed:	09 APRIL 2003 (09.04.2003)
Attorney's Docket No.	MPI03-043P1RNWOM

TRANSMITTAL OF DEMAND FOR INTERNATIONAL PRELIMINARY EXAMINATION AND REQUEST FOR DETAILED SUBSTANTIVE PRELIMINARY EXAMINATION

VIA FEDERAL EXPRESS INTERNATIONAL

European Patent Office
Chapter II
D-80298, Munich, GERMANY

Sir:

Chapter II Demand for International Preliminary Examination

Please find enclosed herewith a Chapter II PCT Demand for International Preliminary Examination (IPE) (6 pages) and Fee Calculation Sheet (1 page) with Debit Order to Deposit Account 2830.0337 in the amount of EUR 1,659, which account is in the name of Millennium Pharmaceuticals, Inc.

Request for Detailed Substantive Preliminary Examination

Applicant respectfully requests that the International Preliminary Examining Authority conduct a detailed substantive examination of the referenced application.

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Please acknowledge receipt of the Demand for IPE and Request for Detailed Substantive Preliminary Examination by dating and returning the attached postcard.

Respectfully submitted,

MILLENNIUM PHARMACEUTICALS, INC.

By Ian Robert Silverman

Ian Robert SILVERMAN
Registration No. 37,443
Agent for Applicants
Millennium Pharmaceuticals, Inc.
40 Landsdowne Street
Cambridge, MA 02139
Telephone: (617) 551-8604
Facsimile: (617) 551-8820

Dated: 22 October 2004

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

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IPEA/EP
Federal Express No. 790313704239

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examination Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference MPI03-043
International application No. PCT/US04/011080	International filing date *(day/month/year)* 09 April 2004 (09.04.2004)	(Earliest) Priority date *(day/month/year)* 09 April 2003 (09.04.2003)
Title of Invention BETA-CARBOLINES USEFUL FOR TREATING INFLAMMATORY DISEASE		

Box No. II APPLICANT(S)

Name and Address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*	Telephone No.: (617) 679-7000
MILLENNIUM PHARMACEUTICALS, INC. 40 Landsdowne Street Cambridge, MA 02139 United States of America	Facsimile No.: (617) 551-8820 Teleprinter No.:
State *(i.e. country)* of nationality: US	State *(i.e. country)* of residence: US

Name and Address: *(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country.)*	
HEPPERLE, Michael E. 280 Newbury Street #4 Boston, MA 02116 United States of America	
State *(i.e. country)* of nationality: DE	State *(i.e. country)* of residence: US

Name and Address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*	
LIU, Julie Fields 1 Fox Lane Lexington, MA 02420 United States of America	
State *(i.e. country)* of nationality: US	State *(i.e. country)* of residence: US

☒ Further applicants are indicated on a continuation sheet.

International applicatio[illegible]
PCT/US04/011080

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Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

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

SOUCY, Francois
39 Ellen Road
Stoneham, MA 02180
United States of America

State *(that is, country)* of nationality: CA	State: *(that is, country)* of residence: US

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

YE, Yingchun
241 Forest Park
Durham, NH 03824
United States of America

State *(that is, country)* of nationality: CN	State: *(that is, country)* of residence: US

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

MURRAY, Robert S.
37 Lyman Avenue
Medford, MA 02155
United States of America

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

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

PRAKASH, Raman
125 Summer Street
Acton, MA 01720
United States of America

State *(that is, country)* of nationality: IN	State: *(that is, country)* of residence: US

☒ Further applicants are indicated on another continuation sheet.

Sheet No. 3	International application no. PCT/US04/011080

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

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

LITTLE, Jeremy D.
62 Valley Street
Wakefield, MA 01880
United States of America

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

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

CASTRO, Alfredo
81 Middlesex Street
Winchester, MA 01890
United States of America

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

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

MAZDIYASNI, Hormoz
19 Old Farm Road
Douglas, MA 01516
United States of America

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

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

FLEMING, Paul E.
7 Sunnyside Avenue
Wellesley, MA 02482
United States of America

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

☒ Further applicants are indicated on another continuation sheet.

Sheet No. 4	International application No. PCT/US04/011080

Continuation of Box No. II APPLICANT(S)
If none of the following sub-boxes is used, this sheet should not be included in the demand.

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

REYNOLDS, Dominic
27 Chalk Street, Apt. 1F
Cambridge, MA 02139
United States of America

State *(that is, country)* of nationality: GB	State: *(that is, country)* of residence: US

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

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

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

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

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

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

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/US04/011080

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

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) of (an) agent(s) for international preliminary examination

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

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

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

SILVERMAN, Ian Robert
MILLENNIUM PHARMACEUTICALS, INC.
40 Landsdowne Street
Cambridge, Massachusetts 02139
United States of America

Telephone No.: (617) 551-8604
Facsimile No.: (617) 551-8820
Teleprinter No.:

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

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

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

☒ the international application as originally filed

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

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

the drawings: ☒ as originally filed (no drawings filed); ☐ as amended under Article 34

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

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

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

Language for the purposes of international preliminary examination: English

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

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

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

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

Box No. V ELECTION OF STATES

☒ The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)* except .....
(If the applicant does not wish to elect certain eligible States, the name(s) or country code(s) of those States must be indicated above.)

International application No.
PCT/US04/011080

Box No. VI CHECK LIST

The demand is accompanied by the following documents for the purposes of international preliminary examination:			For International Preliminary Examining Authority use only: received	not received
1 Translation of international application	:	sheets	☐	☐
2 amendments under Article 34	:	sheets	☐	☐
3 copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4 copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5 letter	:	2 sheets	☐	☐
6 other *(specify)*	:	sheets	☐	☐

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

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☒ fee calculation sheet (1 sheet)
5. ☒ other *(specify)* *Return receipt postcard*

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

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

Ian Robert Silverman

Ian Robert SILVERMAN
Registration No. 37,443
Agent for Applicants

Dated: 22 October 2004

For International Preliminary Examination Authority use only

1. Date of actual receipt of DEMAND.
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PCT

CHAPTER II

FEE CALCULATION SHEET

Annex to the demand for international preliminary examination

For International Preliminary Examining Authority use only

International Application No. PCT/US04/011080

Date stamp of the IPEA

Applicant's or agent's file reference MPI03-043

Applicant
MILLENNIUM PHARMACEUTICALS, INC. et al.

Calculation of prescribed fees

1. Preliminary examination fee ..... EUR 1,530 (P)

2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)* ..... EUR 129 (H)

3. Total of prescribed fees
Add the amounts entered at P and H and enter total in the TOTAL box..... EUR 1,659 TOTAL

Mode of Payment

- [X] authorization to charge deposit account with the IPEA (see below)
- [ ] cash
- [ ] cheque
- [ ] revenue stamps
- [ ] postal money order
- [ ] coupons
- [ ] bank draft
- [ ] other *(specify)*:

Deposit Account Authorization *(this mode of payment may not be available to all IPEAS)*

The IPEA/ EP [X] is hereby authorized to charge the total fees indicated above to my deposit account (2830.0337)

[X] *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account (2830.0337)

2830.0337	22 October 2004	Ian Robert Silverman
Deposit Account Number	Date *(day/month/year)*	Signature Ian Robert SILVERMAN Registration No. 37,443

See Notes to the fee calculation sheet

PATENT COOPERATION TREATY

RECEIVED
MILLENNIUM PHARMACEUTICALS
APR 13 2005
314
PCT DOCKETING DEPARTMENT
DOCKETED BY:

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

SILVERMAN, Ian, Robert
Millennium Pharmaceuticals, Inc.
40 Landsdowne Street
Cambridge, MA 02139
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	05.04.2005
Applicant's or agent's file reference: MP103-043	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/011080	09.04.2004	09.04.2003

Applicant
MILLENIUM PHARMACEUTICALS, INC. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

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

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

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

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

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office - Gitschiner Str. 103 D-10958 Berlin Tel. +49 30 25901 - 0 Fax: +49 30 25901 - 840	HALBARTSCHLAGER, M Tel. +49 30 25901-714

Form PCT/IPEA/416 (January 2004)

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference MP103-043	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US2004/011080	International filing date *(day/month/year)* 09.04.2004	Priority date *(day/month/year)* 09.04.2003
International Patent Classification (IPC) or national classification and IPC C07D471/04, C07D265/30, A61K31/437		
Applicant MILLENIUM PHARMACEUTICALS, INC. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the International application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.10.2004	05.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - Gitschiner Str. 103 D-10958 Berlin Tel. +49 30 25901 - 0 Fax +49 30 25901 - 840	Authorized Officer Telephone No. +49 30 25901-

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/011080

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-169 as originally filed

   Claims, Numbers

   1-29 as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No. PCT/US2004/011080

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

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

☐ the entire international application,

☒ claims Nos. 19-25

because:

☒ the said international application, or the said claims Nos. 19 to 25 with regard to industrial application relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

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

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No. PCT/US2004/011080

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-16,18,27-29
	No:	Claims	1,2,17,26
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-18,26-29
Industrial applicability (IA)	Yes:	Claims	1-18,26-29
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Re Item I.

Unclarities and inconsistencies

1. Claims

Formula II-C is claimed in claim 3. Due to the dependency on claim 2 (and consequently on claim 1), definitions of R^1, R^2 and R^3 are unnecessarily repeated.

Formula III-A of claim 10 does not correspond to formula III-A of the description. The compound claimed in claim 10, although clearly part of general formula I, has no exact support in the description. This particular embodiment should be transferred in the specification among the preferred embodiments. A consequence is that the dependence of the following claims is irregular.

Claim 11 appears to find a support in page 16 of the description. But another irregularity is seen in claim 12 where the group R^{12} has not the same meaning as in claim 10 on which claim 12 is said to be dependent. The same remark applies to claim 13.

Claim 14 finds a support in description, page 19.

Claim 16 is directed to compounds of examples 1-31, 39-43, 45-47, 49-63. Compounds of figures Nos. 32-38, 47 are not examplified but disclosed on pages 30, 32 resp.
The figure No. 44 does not correspond to compound of example 44 which shows a morpholine ring in place of a pyran ring. According to the preparation, a morpholine ring should be expected as shown in figure 44.
Example 47, page 126 corresponds to figure No. 48. A second example 47 (?) disclosed on page 130 has no corresponding figure in the description or in claim 16. If the nomenclature is erroneous because of a possibly missing part after "-propionyl-", this second example 47 could correspond to figure No. 47 of page 32 which also appears in claim 16.
According to the preparation, the name of example 71 should probably read "-piperazin-1-yl-" instead of "-piperdin-1-yl-".

The compounds (S)-III-A-a of claim 15 have no support in the description. Moreover the given dependency on claim 14 is erroneous because of several discrepancies in the

substituent definitions.

Support for claim 26 directed to intermediate compounds of formula 3a is partly on page 57 of the description. But R^{15} is there defined as "an organic radical" which is nowhere specified in the specification. The "restricted" definition given in claim 26 in general terms "aliphatic", "aryl", etc, is not more admissible as also lacking support. For an appropriate support, the text of claim 26 should be introduced in the description including correct definitions already disclosed for the unspecific expressions like "aliphatic", "aryl", derivatives thereof and "carboxylic acid and amino protecting groups".

Claims 27, 28 and (implicitely) 29 are supported by the description, pages 58 and 59 with the reservations made above.

2. Description

Definitions given in the description for expressions which do not appear in the claims (like "linker group") are superfluous and may eventually be misleading for the plain understanding of the invention.

Considering the biological testing, essentially assay procedures are given without specific results. Only in the measurement of the IKB kinase phosphotransferase activity description is said that the compounds were active inhibitors of the IKK complex and that the IKB kinase inhibition average IC_{50} values were "generally" below "about" 10 microM. The "inventive compounds" (compounds of the invention or compounds which show an inventive effect ?) are also said to be selective for inhibiting IKK-2 as opposed to IKK-1. There appear mere statements which may not be sufficiently substantiated.

In the test on rat models for rheumatoid arthritis, "certain compounds were found to be active". This test cannot be taken into consideration for the evaluation of an inventive step as not all claimed compounds appear to be concerned. Furthermore, it is said that "compounds of formula II-C were found to be surprisingly superior compared to other compounds where ring A is a pyridine ring". This is quite obscure since, firstly, compounds of formula II-C are compounds wherein ring A is a pyridine ring and, secondly, the said "other compounds" are not defined. Same remark applies to the next sentence about compounds of formula III-A-a.

Re Item III.

Claims 19 to 25 are directed to methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods. They relate to subject-matter considered by this authority to be covered by the provisions of Rule 67.1(iv) PCT.

No opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34 (4) (a)(i) PCT). For the assessment on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treament and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Under the terms of Rule 39.1(iv) PCT, the ISA was not required to carry out a search of such claims, but as indicated in the ISR, the search was carried out and based on the alleged effects of the compounds. Similarly, the IPEA (which is the ISA) is not required to carry out an international preliminary examination of such claims, but as for the ISR, the IPER will be based on the alleged effects of the compounds (Rule 67.1 (iv) PCT).

Re Items V. and VI.

1. **Cited documents**

The following documents are referred to in this communication:

D1 WO 01/68648
D2 LEE MICHAEL ET AL.: CANCER LETTERS, vol. 101, no. 1, 1996, pages 53-57
D3 PALANKI M S S: CURRENT MEDICINAL CHEMISTRY, vol. 9, no. 2, January 2002, pages 219-227
D4 HOTTELET M ET AL: IMFLAMMATION RESEARCH, vol. 49, no. Supplement 2, 24 September 2000 , page S910
D5 DE 199 51 360
D6 WO 03/024936
D7 WO 03/039545 (intermediate document)
D8 CASTRO ALFREDO C. ET AL.: BIOORGANIC & MEDICINAL CHEMISTRY

LETTERS, vol. 13, no. 14, 2003, pages 2419-2422

D9 WO 00/09492

The intermediate documents D7 and D8 are relevant for the purposes of Rules 33.1 c, 64.3 and 70.10 PCT, but since the priority documents are not available at the time of establishing the written opinion, they are not taken into account. It is based on the assumption that all claims enjoy priority rights from the filing date of the priority document(s). If it later turns out that this assumption is not correct, the intermediate document in the International Search Report could become relevant in order to assess whether the claims satisfy the criteria set forth in Article 33(1) PCT.
If the priority date is not valid for the complete claimed subject-matter, this document may become relevant prior art in a possible regional/national phase.

2. Novelty

2.1 Final compounds

D1 destroys the novelty of the final compounds which the disclosure of the examples 83, 118 and 121 which are also claimed for the same biological properties.

Due to the extreme closeness of the prior art, the question arises of which feature should distinguish unambiguously the present subject-matter from the state of the art. The distinguishing feature must be clearly common for all the claimed compounds in order to satisfy the unity criterion (Rule 13 PCT).

2.2 Intermediate compounds

D9 is novelty destroying for the compounds claimed in claim 26 which the disclosure of the examples 23 to 25. It is noted that this is essentially due to the unspecificity of the expressions "carboxylic acid protecting group" or "amino protecting group" (actually they are functional definitions which are not admissible when they can be better defined).

Intermediate compounds of formula IV of claim 28 appear to be novel.

3. Inventive step

3.1 The problem underlying the present application is, according to the description, to provide beta-carbolines useful for treating inflammatory diseases and cancer, and more specifically for treating IKK-2 mediated diseases. D1 discloses novelty destroying compounds which have the same activities.

3.2 Before prosecuting with the substantive examination, the novelty must be clearly established, *i.e.* by at least one characterising feature which complies with the unity requirements.

It is thus necessary that this "novelty feature" be well-defined, since the acknowledgement of the inventive step should be based on an unexpected effect that this particular difference brings to the novel invention (see also item I.2 above the remarks concerning the assays described in the specification).

Citations D2 to D6 belong to the background art which may be useful in the examination process.

PATENT COOPERATION TREATY

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From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

SILVERMAN, Ian, Robert
Millennium Pharmaceuticals, Inc.
40 Landsdowne Street
Cambridge, MA 02139
United States of America

Date of mailing (day/month/year) 13 July 2004 (13.07.2004)	
Applicant's or agent's file reference MP103-043	**IMPORTANT NOTIFICATION**
International application No. PCT/US2004/011080	International filing date (day/month/year) 09 April 2004 (09.04.2004)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 09 April 2003 (09.04.2003)
Applicant MILLENIUM PHARMACEUTICALS, INC. et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. (If applicable) The letters "NR" appearing in the right-hand column denote a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

3. (If applicable) An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b) (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
09 April 2003 (09.04.2003)	60/461,468	US	09 June 2004 (09.06.2004)

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

Facsimile No. (41-22) 338.89.70

Authorized officer

Katty CASTILLO (Fax 338 8970)

Telephone No. (41-22) 338 9223

3004
325

SUPERINTENDENCIA Y COMERCIO
Radicacion : 05113041 00000000 Folios: 323
Fecha (HOY): 2005-11-04 17:24:47
Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCION PCT MODELO DE UTILIDAD	( )	( )
- Indicación de que se solicita la concesión de una patente.	( )	(X)
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	( )	(X)
- Descripción de la invención.	( )	(X)
- Dibujos de ser estos pertinentes.	( )	(X)
- Comprobante de Pago de las tasas establecidas.		
COMPLETA (X) INCOMPLETA	( )	( )
DISEÑO INDUSTRIAL ( )		
- Indicación de que se solicita el registro de Diseño Industrial	( )	( )
- Datos de identificación del solicitante o de la persona que presenta	( )	( )
- la solicitud.		
- Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	( )	( )
Comprobante de pago de las tasas establecidas	( )	( )
COMPLETA ( ) INCOMPLETA	( )	( )
ESQUEMA DE TRAZADO ( )		
- Indicación de que solicita el registro de un Esquema de trazado.	( )	( )
- Datos de identificación del solcitante o de la persona que presenta La solicitud.	( )	( )
Representación gráfica del esquema de trazado	( )	( )
Comprobante de pago de las tasas establecidas.	( )	( )
COMPLETA ( ) INCOMPLETA	( )	( )

Firma Responsable:

Fecha:

325
326

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
PATIÑO LEYVA LUIS

REFERENCIA	Radicación No.	5 113041
	Solicitud internacional	US2004/011080
	Fecha inicio fase nacional	09/11/2005
	Trámite	11
	Evento	379
	Actuación	430
	Oficio No.	1164

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

1. La solicitud no contiene la traducción de las reivindicaciones tal como fueron presentadas. (Numeral 5.2.5, capítulo quinto del título décimo de la Circular Única de la SIC).

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

3. La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

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

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 03 FEB. 2006
adpal