

Señora Jefe
DIVISIÓN DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Bogotá, D.C.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 07-007670- -00003-0000
FOLIO 2008-11-28-14-48-11 Dep. 2020 NUECREACION
Ha 11 PATENTE/II Eve 378 FASENACIONAL
Act 5.35 PETICION Folios: 2

Re.: Expediente No. 07 7.670
Fase Nacional Solicitud de Patente PCT
a nombre de
AMGEN SF, LLC
Código 011 - 378 - 538

En relación con la solicitud contenida en el Expediente de la referencia y, en particular, con la publicación del extracto de la misma en la Gaceta de la Propiedad Industrial No. 593 de 27 de Junio de 2008, por medio del presente escrito muy respetuosamente me permito solicitarle se sirva proceder a efectuar el **EXAMEN DE PATENTABILIDAD** de la solicitud aquí contenida.

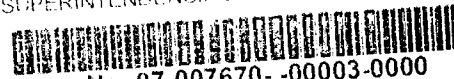
Para tal efecto, acompaño el comprobante de pago por concepto de la tasa respectiva, Recibo No. 012, 409

De Usted Atentamente,

GERMAN CASTILLO GRAU
T.P. # 2.978 del C.S.J.

134

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-007670- -00003-0000

Fecha 2008-11-26 14:48:11 Dep 2020 NUECREACION
Lto 11 PATENTE(YII) Evt 378 FASENACIONAL
Act 536 PETICION Folios 2

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 92,409
FECHA : NOVIEMBRE 26 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CASTILLO GRAU Y ASOCIADOS	CONSIGNACION	BANCO DE BOGOTA	062754387	98927592	26/11/2008	1,167,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
		85 EXAMEN DE PATENTABILIDAD DE INVEN	420,000.00
		TOTAL :	\$ 420,000.00

ON: CUATROCIENTOS VEINTE MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

DIVISIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 07-07670

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **593** DE

FECHA **27 DE JUNIO DE 2008** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **24 DE SEPTIEMBRE DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____ NO X



US 20030055054A1

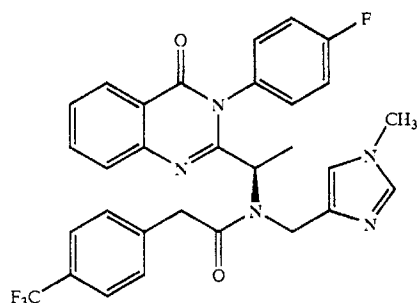
(19) **United States**(12) **Patent Application Publication**
Medina et al.(10) **Pub. No.: US 2003/0055054 A1**(43) **Pub. Date: Mar. 20, 2003**(54) **CXCR3 ANTAGONISTS**(75) Inventors: **Julio C. Medina**, San Carlos, CA (US);
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Marcus, San Francisco, CA (US)

Correspondence Address:

PENNIE AND EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 100362711(73) Assignee: **Tularik, Inc.**(21) Appl. No.: **10/231,895**(22) Filed: **Aug. 29, 2002****Related U.S. Application Data**(63) Continuation of application No. 10/015,532, filed on
Dec. 11, 2001.(60) Provisional application No. 60/255,241, filed on Dec.
11, 2000. Provisional application No. 60/296,499,
filed on Jun. 6, 2001.**Publication Classification**(51) **Int. Cl.⁷** **C07D 417/02**; C07D 413/02;
C07D 43/02; C07D 41/02
(52) **U.S. Cl.** **514/227.8**; 514/228.5; 514/231.5;
514/252.11; 514/254.01; 514/253.01;
514/252.12; 514/326; 514/365;
514/374; 514/396; 544/59;
544/60; 544/359; 544/360;
544/111; 546/186; 546/207;
548/215; 548/198; 548/311.1(57) **ABSTRACT**Compounds, compositions and methods that are useful in
the treatment of inflammatory and immune conditions and
diseases are provided herein. In particular, the invention
provides compounds which modulate the expression and/or
function of a chemokine receptor. The subject methods are
useful for the treatment of inflammatory and immunoregu-
latory disorders and diseases, such as multiple sclerosis,
rheumatoid arthritis and type I diabetes.

Synthesis of Compound 10.04

[0509]

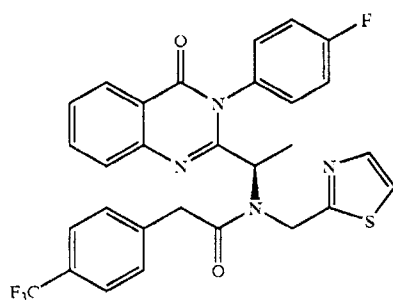


10.04

[0510] Compound 10.04 was prepared following the synthetic procedure for compound 10.02, described above. MS (ESI⁺) 564.2 [MH]⁺.

Synthesis of Compound 10.05

[0511]



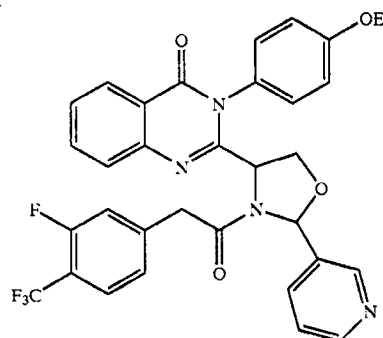
10.05

[0512] Compound 10.05 was prepared following the synthetic procedure of compound 10.01, described above. ¹H NMR (d₆-DMSO, T=140° C.) δ 8.11 (d, J=8.0 Hz, 1H), 7.82 (t, J=8.1 Hz, 1H), 7.68 (d, J=8.1 Hz, 1H), 7.60-7.40 (m, 6H), 7.38-7.15 (m, 5H), 5.33 (bs, 1H), 5.02 (dd, J=11.4 Hz, 2H), 3.60 (bm, 2H), 1.45 (d, J=7.0 Hz, 3H). m.p. 173-174° C. MS (ESI⁺) 567.2 [MH]⁺. Anal. Calcd. for C₂₉H₂₂F₄N₄O₂S: C, 61.48; H, 3.91; N, 9.89. Found: C, 61.36; H, 4.08; N, 9.75.

Example 11

Preparation of Compound 11.01

[0513]

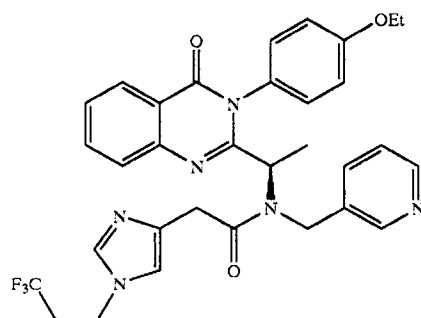


11.01

[0514] The synthesis of compound 11.01 is shown in FIG. 14. ¹H NMR (d₆-DMSO, T=120° C.) δ 8.8-7.0 (m, 17H), 6.35 (s, 1H), 5.00 (m, 1H), 4.35 (m, 1H), 4.25-4.00 (m, 4H), 3.65 (m, 1H), 1.40 (t, 3H). MS (ESI⁺) 619.1 [MH]⁺.

Preparation of Compound 11.02

[0515]

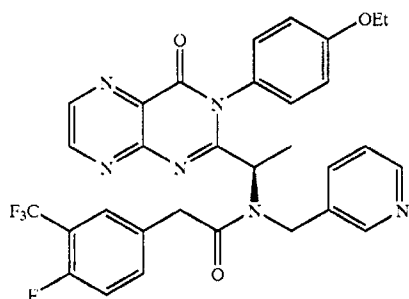


11.02

[0516] The synthesis of compound 11.02 is shown in FIG. 17. ¹H NMR (d₆-DMSO, T=150° C.) δ 8.38 (m, 2H), 8.09 (m, 1H), 7.84 (m, 2H), 7.68 (m, 1H), 7.54 (m, 2H), 7.40-7.00 (m, 6H), 5.25 (m, 1H), 4.74 (m, 2H), 4.25 (m, 1H), 4.14 (m, 3H), 3.62 (m, 1H), 3.31 (m, 1H), 2.78 (m, 2H), 1.37 (m, 6H). MS (ESI⁺) 605.3 [MH]⁺.

Preparation of Compound 11.03

[0517]

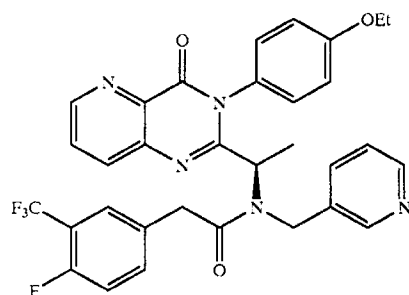


11.03

[0518] The synthesis of compound 11.03 is shown in FIG. 18. ^1H NMR (d_6 -DMSO, $T=150^\circ\text{C}$) δ 9.02 (s, 1H), 8.85 (s, 1H), 8.40 (s, 1H), 8.38 (m, 1H), 7.57 (m, 1H), 7.41 (m, 3H), 7.33–7.15 (m, 5H), 5.30 (q, 1H), 4.80 (dd, 2H), 4.13 (q, 2H), 3.64 (d, 1H), 3.21 (bs, 1H), 1.46 (d, 3H), 1.37 (t, 3H). MS (ESI $^+$) 607.2 [MH] $^+$.

Preparation of Compound 11.04

[0519]

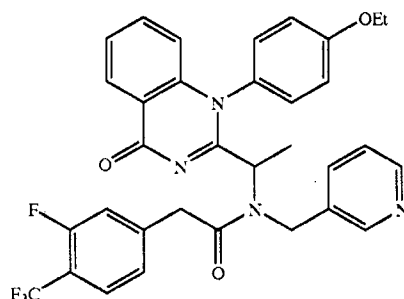


11.04

[0520] The synthesis of compound 11.04 is shown in FIG. 18. ^1H NMR (d_6 -DMSO, $T=150^\circ\text{C}$) δ 8.81 (d, 1H), 8.38 (s, 1H), 8.35 (m, 1H), 8.03 (m, 1H), 7.81 (m, 1H), 7.54 (m, 1H), 7.40 (m, 3H), 7.33–7.05 (m, 5H), 5.28 (q, 1H), 4.75 (dd, 2H), 4.13 (q, 2H), 3.60 (d, 1H), 3.19 (bs, 1H), 1.44 (d, 3H), 1.37 (t, 3H). MS (ESI $^+$) 606.2 [MH] $^+$.

Preparation of Compound 11.05

[0521]

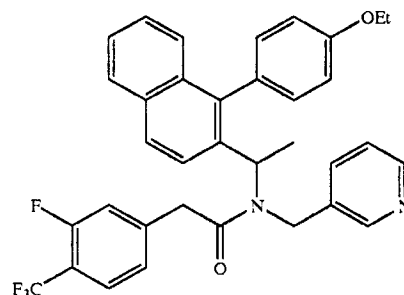


11.05

[0522] The synthesis of compound 11.05 is shown in FIG. 11. ^1H NMR (d_6 -DMSO, $T=150^\circ\text{C}$) δ 8.47 (s, 1H), 8.37 (d, 1H), 8.13 (d, 1H), 7.66–7.25 (m, 7H), 7.25–7.10 (m, 6H), 6.59 (d, 1H), 5.25 (q, 1H), 4.86 (dd, 2H), 4.18 (q, 2H), 3.70 (d, 1H), 3.31 (bd, 1H), 1.41 (m, 6H). MS (ESI $^+$) 605.2 [MH] $^+$.

Preparation of Compound 11.06

[0523]

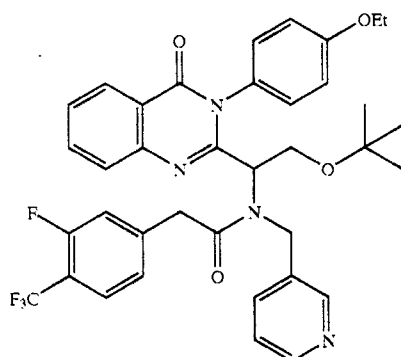


11.06

[0524] The synthesis of compound 11.06 is shown in FIG. 3. ^1H NMR (d_6 -DMSO, $T=150^\circ\text{C}$) δ 8.30 (m, 1H), 8.22 (s, 1H), 7.95 (m, 2H), 7.83 (m, 1H), 7.57 (m, 1H), 7.48 (m, 1H), 7.36 (m, 2H), 7.24 (m, 2H), 7.15–6.95 (m, 6H), 5.45 (q, 1H), 4.50 (dd, 2H), 4.14 (q, 2H), 3.57 (d, 1H), 3.05 (bd, 1H), 1.54 (d, 3H), 1.38 (t, 3H). MS (ESI $^+$) 587.3 [MH] $^+$.

Preparation of Compound 11.07

[0525]



11.07

[0526] The synthesis of compound 11.07 is shown in FIG. 1. ^1H NMR (CDCl_3) δ 8.30 (m, 3H), 7.85 (m, 2H), 7.52 (m, 3H), 7.23 (m, 1H), 7.11 (m, 1H), 7.03 (m, 2H), 6.82 (d, 1H), 6.75 (d, 1H), 6.54 (m, 1H), 5.07 (q, 1H), 4.60 (dd, 2H), 4.05 (m, 3H), 3.82 (m, 2H), 1.85 (d, 1H), 1.45 (t, 3H), 1.17 (s, 9H). MS (ESI^+) 677.3 $[\text{MH}]^+$.

Example 12

[0527] This example illustrates a CXCR3 binding assay that can be used for evaluating the compounds of the present invention.

[0528] Unless otherwise noted, all reagents used are available from commercial sources (e.g., Sigma). Test compounds are diluted in DMSO to a concentration that is 40-times the intended final assay concentration; 5 μL are transferred to each well of a 96-well flat-bottomed polypropylene plate (e.g., from Greiner, Inc.). CXCR3-expressing cells obtained from ChemoCentryx were used in the assays to generate the data set forth in the Table provided in FIG. 12. The cells were resuspended in assay buffer (25 mM Hepes, 80 mM NaCl, 1 mM CaCl_2 , 5 mM MgCl_2 , 0.2% bovine serum albumin, pH 7.1, stored at 4 ° C.) at 5 million cells per mL; 100 μL of this cell suspension is then transferred to each well of a 96-well plate containing the diluted test compounds. ^{125}I -labelled chemokine (purchased from commercial sources, e.g., Amersham, PE Life Sciences) is diluted in assay buffer to a concentration of approximately 60 pM; 100 μL of this chemokine solution is transferred to each well of a 96-well plate containing compounds and cell suspension. The plates are sealed with commercially available foil plate seals (e.g., from E&K Scientific), and stored at 4 ° C. for 2 to 4 h, shaking gently. At the end of this incubation period, the contents of the assay plates are transferred to GF/B filter plates (Packard) that have been pre-coated by dipping into a solution containing 0.3% polyethyleneimine (Sigma), using a cell harvester (Packard), and washing twice with wash buffer (25 mM Hepes, 500 mM NaCl, 1 mM CaCl_2 , 5 mM MgCl_2 , pH 7.1, stored at room temperature). The filter plates are sealed on the bottom with plate seals (Packard), 50 μL of Microscint-20 scintillation fluid (Packard) is added to each well, and the top of the plates are sealed with clear plastic (TopSeal A, Packard). The plates are counted on a scintillation counter, such as a

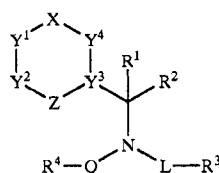
Packard TopCount. To measure non-specific binding, 4 wells containing unlabelled "cold" chemokine were included on each 96-well plate. To measure maximum binding, 4 wells containing 5 μL of DMSO, 100 μL of cell suspension and 100 μL of ^{125}I -labelled chemokine solution were included on each 96-well plate. Data were analyzed using commercially available software (e.g., Excel from Microsoft, Prism from GraphPad Software Inc.).

[0529] Other assays may be used to identify compounds that modulate CXCR3 chemokine receptor activity, for example, binding assays (see, e.g., Weng et al. (1998) *J. Biol. Chem.* 273:18288-18291, Campbell et al. (1998) *J. Cell Biol.* 141:1053-1059, Endres et al. (1999) *J. Exp. Med.* 189:1993-1998 and Ng et al. (1999) *J. Med. Chem.* 42:4680-4694), calcium flux assays (see, e.g., Wang et al. (2000) *Mol. Pharm.* 57:1190-1198 and Rabin et al. (1999) *J. Immunol.* 162:3840-3850) and chemotaxis assays (see, e.g., Albanesi et al. (2000) *J. Immunol.* 165:1395-1402 and Loetscher et al. (1998) *Eur. J. Immunol.* 28:3696-3705).

[0530] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. A compound having the formula (I):



wherein

X is a member selected from the group consisting of a bond, $-\text{C}(\text{O})-$, $-\text{C}(\text{R}^5)(\text{R}^6)-$, $-\text{C}(\text{R}^5)=$, $-\text{S}(\text{O})_2-$ and $-\text{N}=-$;

Z is a member selected from the group consisting of a bond, $-\text{N}=-$, $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}^{17})-$ and $-\text{C}(\text{R}^7)=$, with the proviso that X and Z are not both a bond;

L is a member selected from the group consisting of a bond, $\text{C}(\text{O})-(\text{C}_1-\text{C}_8)\text{alkylene}$, $(\text{C}_1-\text{C}_8)\text{alkylene}$ and $(\text{C}_2-\text{C}_8)\text{heteroalkylene}$;

Q is a member selected from the group consisting of a bond, $(\text{C}_1-\text{C}_8)\text{alkylene}$, $(\text{C}_2-\text{C}_8)\text{heteroalkylene}$, $-\text{C}(\text{O})-$, $-\text{OC}(\text{O})-$, $-\text{N}(\text{R}^8)\text{C}(\text{O})-$, $-\text{CH}_2\text{CO}-$, $-\text{CH}_2\text{SO}-$ and $-\text{CH}_2\text{SO}_2-$;

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403/06, 413/14

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(21) International Application Number: PCT/US01/47850

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CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VN, YU, ZA, ZM, ZW.

(71) Applicant (*for all designated States except US*): **TU-
LARIK INC.** [US/US]; Two Corporate Drive, South San
Francisco, CA 94080 (US).

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

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Burlingame, CA 94010 (US). **MARCUS, Andrew, P.**

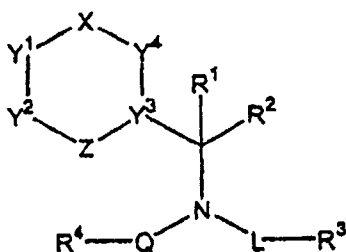
Published:

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

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

WO 02/083143 A1

(54) Title: CXCR3 ANTAGONISTS



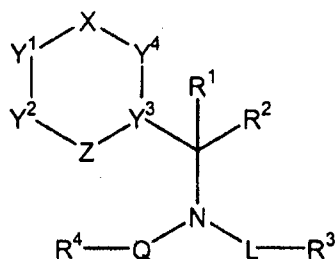
(57) Abstract: Compounds, compositions and methods that are useful in the treat-
ment of inflammatory and immune conditions and diseases are provided herein. In
particular, the invention provides compounds which modulate the expression and/or
function of a chemokine receptor. The subject methods are useful for the treatment
of inflammatory and immunoregulatory disorders and diseases, such as multiple
sclerosis, rheumatoid arthritis and type I diabetes.

The highly selective expression of CXCR3 makes it an ideal target for intervention to interrupt inappropriate T cell trafficking. The clinical indications for such intervention are in T-cell mediated autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and type I diabetes. Inappropriate T-cell infiltration also occurs in psoriasis and other pathogenic skin inflammation conditions, although the diseases may not be true autoimmune disorders. In this regard, up-regulation of IP-10 expression in keratinocytes is a common feature in cutaneous immunopathologies. Inhibition of CXCR3 can be beneficial in reducing rejection in organ transplantation. Ectopic expression of CXCR3 in certain tumors, especially subsets of B cell malignancies indicate that selective inhibitors of CXCR3 will have value in tumor immunotherapy, particularly attenuation of metastasis.

In view of the clinical importance of CXCR3, the identification of compounds that modulate CXCR3 function represents an attractive avenue into the development of new therapeutic agents. Such compounds are provided herein.

SUMMARY OF THE INVENTION

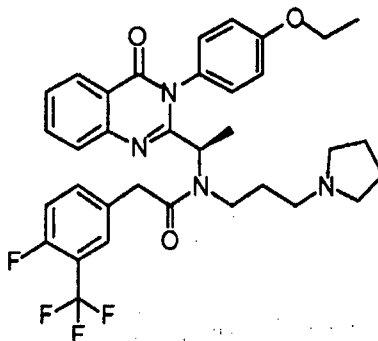
The present invention provides compounds which are useful in the treatment or prevention of certain inflammatory and immunoregulatory disorders and diseases, including asthma and allergic diseases, as well as autoimmune pathologies such as rheumatoid arthritis and atherosclerosis. The compounds provided herein have the general formula (I):



25

wherein X represents a bond, -C(O)-, -C(R⁵)(R⁶)-, -C(R⁵)=, -S(O)-, -S(O)₂- or -N=; Z represents a bond, -N=, -O-, -S-, -N(R⁷)- or -C(R⁷)=, with the proviso that X and Z are not both a bond; L represents a bond, C(O)-(C₁-C₈)alkylene, (C₁-C₈)alkylene or (C₂-C₈)heteroalkylene; Q represents a bond, (C₁-C₈)alkylene, (C₂-C₈)heteroalkylene, -C(O)-,

Synthesis of Compound 3.42•HCl



N-{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-2-(4-fluoro-3-trifluoromethyl-phenyl)-*N*-(3-pyrrolidin-1-yl-propyl)-acetamide

5

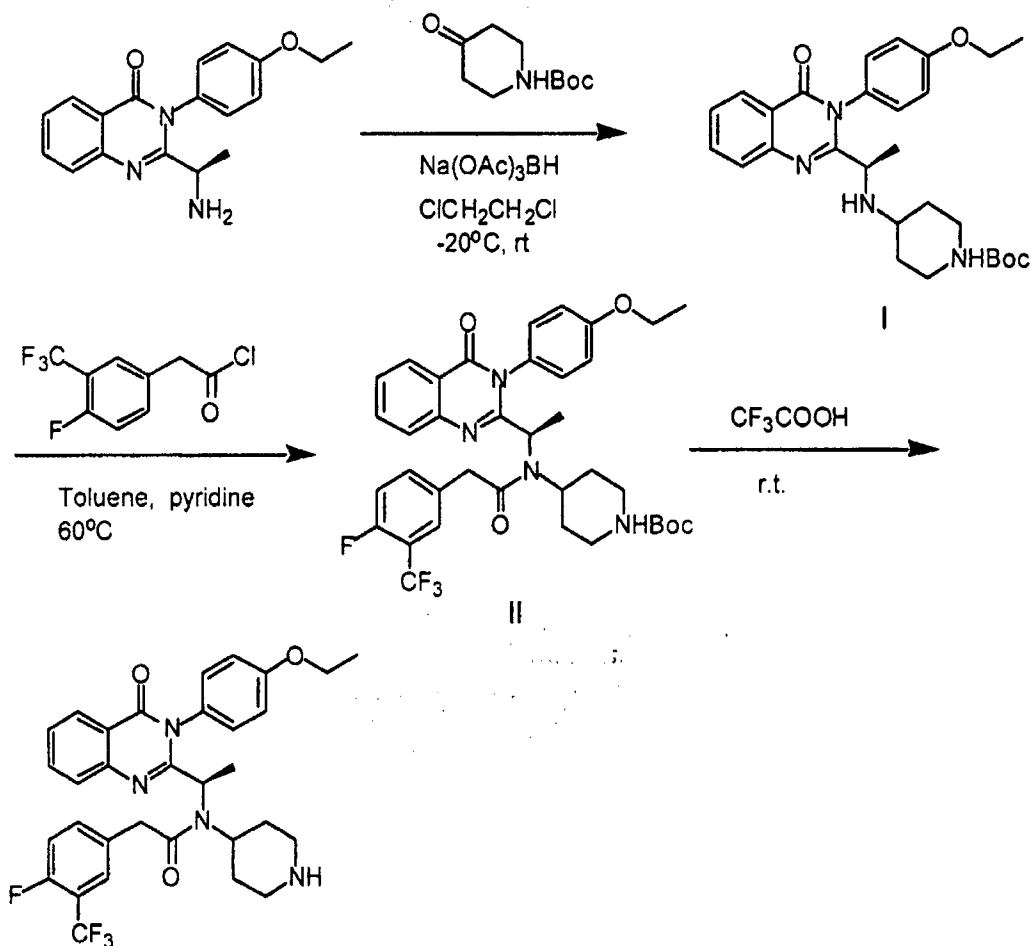
3.42

Compound 3.42 was prepared following the synthesis of compound 3.02.

White solid. ^1H NMR (DMSO, $T=120^\circ\text{C}$) 1.35 (t, 3H, $J = 6.93$ Hz), 1.52 (m, 3H), 1.9 (m, 6H), 2.85-3.05 (m, 3H), 3.36-3.57 (m, 4H), 3.95 (m, 3H), 4.08 (q, 2H, $J = 6.93$ Hz),
10 5.17 (m, 1H), 7.02-7.10 (m, 2H), 7.24-7.46 (m, 5H), 7.58 (t, 1H, $J = 7.6$ Hz), 7.75 (d, 1H, $J = 7.73$ Hz), 7.88 (t, 1H, $J = 7.73$ Hz), 8.15 (d, 1H, $J = 8$ Hz). MS(ESI^+) 625..3 (MH^+).

143

Synthesis of Compound 3.43



T0913409

5

3.43

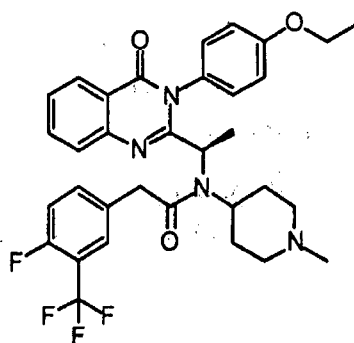
4-{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-
ethylamino}-piperidine-1-carboxylic acid *tert*-butyl ester (I). 4-Oxo-Piperidine-1-
carboxylic acid *tert*-butyl ester (0.468 g, 2.35 mmol) was added to a solution of amine
(0.6g, 1.96mmol) in dichloroethane (10ml) at -10°C , followed by $\text{Na}(\text{OAc})_3\text{BH}$ (0.602 g,
10 2.84 mmol). The mixture was kept at that temperature for 1.5 h, then warmed slowly to
room temperature and stirred overnight. The solution was diluted with DCM, washed by
sat. NaHCO_3 , water, brine and dried over Na_2SO_4 . The solvent was evaporated and a white
solid (1.1g) was obtained, which was used in the next step. $\text{MS}(\text{ESI}^+)$ 493.3 (MH^+).

4-{{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-
15 [(4-fluoro-3-trifluoromethyl-phenyl)-acetyl]-amino}-piperidine-1-carboxylic acid
ester (II). Pyridine (0.289 g, 3.66 mmol) was added to a mixture of I (0.6 g, 1.22 mmol)

and phenylacetyl chloride (0.44 g, 1.83 mmol) in toluene (15 ml). The solution was heated to 60°C for 3 h, and then poured into 1N HCl. The aqueous layer was extracted with EtOAc three times, the combined organic layer was then washed by sat. NaHCO₃, water, brine and dried over Na₂SO₄. The solvent was evaporated and the crude oil was subjected by flash column to afford a yellow solid (540 mg). MS(ESI⁺) 697.3 (MH⁺).

{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-2-(4-fluoro-3-trifluoromethyl-phenyl)-piperidin-4-yl-acetamide (3.43). Trifluoroacetic acid (1.77 g, 15.5 mmol) was added to a solution of II (0.54 g, 0.77 mmol) in dichloromethane. The mixture was stirred at room temperature for 3 h. The solvent was evaporated, the residue was dissolved in EtOAc and washed with sat. NaHCO₃, water, brine and dried over Na₂SO₄. The solvent was evaporated and the crude oil was subjected by flash column to afford a white solid (440 mg). ¹H NMR (DMSO, T=120°C) 1.21 (m, 1H), 1.34 (m, 4H), 1.48 (d, 3H, *J* = 6.8 Hz), 2.16 (m, 3H), 2.70-2.97 (m, 4H), 3.28 (d, 1H, *J* = 16 Hz), 3.64 (m, 1H), 4.09 (q, 2H, *J* = 6.8 Hz), 5.04 (m, 1H), 7.07-7.20 (m, 3H), 7.30-7.44 (m, 4H), 7.58 (t, 1H, *J* = 7.33 Hz), 7.76 (d, 1H, *J* = 8 Hz), 7.88 (d, 1H, *J* = 7.07 Hz), 8.14 (d, 1H, *J* = 7.73 Hz), MS(ESI⁺) 597.3 (MH⁺).

Synthesis of Compound 3.44



***N*-{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-2-(4-fluoro-3-trifluoromethyl-phenyl)-*N*-(1-methyl-piperidin-4-yl)-acetamide**

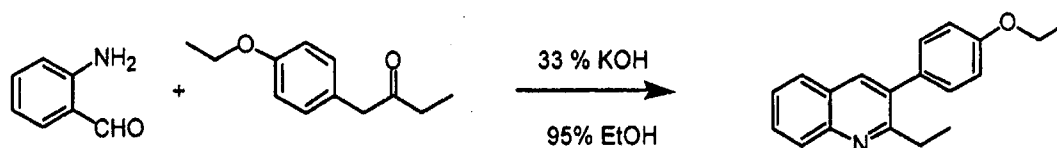
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Formaldehyde (37% in water) (0.016g, 0.20 mmol) was added to a solution of T0913409 (0.06 g, 0.1mmol) in dichloroethane (5 ml), followed by Na(OAc)₃BH (0.127 g, 0.60 mmol) at room temperature. The mixture was stirred

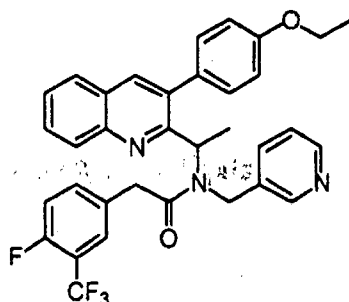
overnight. The solution was diluted with DCM, washed by sat. NaHCO₃, water, brine and dried over Na₂SO₄. The solvent was evaporated and the residue was purified by flash column to afford a white solid (58 mg). ¹H NMR (DMSO, T=120°C) 1.18 (m, 1H), 1.33 (t, 3H, J = 6.8 Hz), 1.48 (d, 3H, J = 6.67 Hz), 1.61 (m, 1H), 1.95 (m, 1H), 2.10 (s, 3H), 2.38 (m, 2H), 2.64 (m, 1H), 2.76 (m, 1H), 2.88 (s, 2H), 3.26 (d, 1H, J = 16 Hz), 3.58 (m, 1H), 4.09 (d, 2H, J = 6.8 Hz), 5.04 (m, 1H), 7.08-7.45 (m, 7H), 7.58 (m, 1H), 7.76 (d, 1H, J = 8 Hz), 7.89 (m, 1H), 8.14 (d, 1H, J = 7.73 Hz). MS(ESI⁺) 611.3 (MH⁺).

Synthesis of Compound 3.45

10



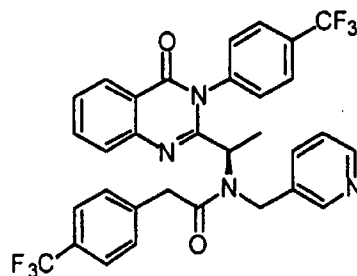
3-(4-Ethoxyphenyl)-2-ethyl-quinoline. 33% Potassium hydroxide (1.3 ml) was added to a mixture of o-amino aldehyde (0.31 g, 2.6 mmol) and ketone (0.5 g, 2.6 mmol) in 95% EtOH. The solution was heated to reflux for 2 h and then poured into water. The aqueous layer was extracted with EtOAc three times, the combined organic layer was then washed by water, brine and dried over Na₂SO₄. The solvent was evaporated and the crude oil was subjected by flash column to afford a white solid (170 mg). ¹H NMR (CDCl₃) 1.23 (t, 3H, J = 7.5 Hz), 1.47 (t, 3H, J = 6.93 Hz), 2.98 (q, 2H, J = 7.47 Hz), 4.11 (q, 2H, J = 6.93 Hz), 6.98 (m, 2H, J = 6.8 Hz), 7.31 (m, 2H), 7.49 (m, 1H), 7.68 (m, 1H), 7.77 (d, 1H, J = 8 Hz), 7.92 (s, 1H), 8.08 (d, 1H, J = 8 Hz). MS(ESI⁺) 278.3 (MH⁺).



N-{1-[3-(4-Ethoxy-phenyl)-quinolin-2-yl]-ethyl}-2-(4-fluoro-3-trifluoromethyl-phenyl)-*N*-pyridin-3-ylmethyl-acetamide

Starting from 3-(4-ethoxy-phenyl)-2-ethyl-quinoline, compound **3.45** was prepared following the synthesis of compound **1.01** (IV - 1.01). yellow solid. ^1H NMR (DMSO, T=120°C) 1.35 (t, 3H, $J = 6.80$ Hz), 1.57 (m, 3H), 2.94 (br., 1H), 3.63 (d, 1H, $J = 16$ Hz), 4.11 (q, 2H, $J = 6.80$ Hz), 4.63 (m, 2H), 5.88 (m, 1H), 7.07 (m, 5H), 7.22-7.33 (m, 3H), 7.58 (m, 2H), 7.76 (t, 1H, $J = 7.6$ Hz), 7.90 (d, 1H, $J = 8$ Hz), 7.80-8.12 (m, 3H), 8.24 (m, 1H). MS(ESI $^+$) 588..3 (MH $^+$).

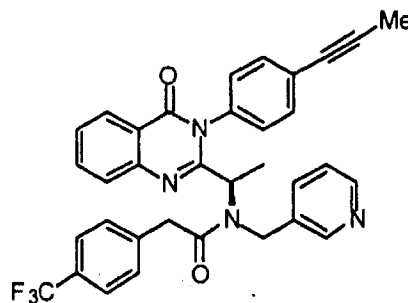
Synthesis of Compound 3.46



3.46

(R)-2-((N-3-Picolyl)-N-(4-trifluoromethylphenylacetyl)-1-aminoethyl)-3-(4-trifluoroethyl)-3H-quinazoline-4-one (**3.46**). Compound **1** was synthesized using the method described in Figure 4, except that 4-trifluoromethylaniline was used in place of p-phenetidine. ^1H NMR (DMSO- d_6 , T=120 °C) 1.40 (m, 3H), 2.89 (m, 1H), 3.58 (m, 1H), 4.78 (m, 2H), 5.24 (m, 1H), 7.21 (d, $J = 4.0$ Hz, 1H), 7.26 (m, 2H), 7.55 (m, 5H), 7.71 (m, 1H), 7.80 (br s, 1H), 7.90 (m, 3H), 8.12 (d, $J = 7.8$ Hz, 1H), 8.38 (t, $J = 5.1$ Hz, 2H) ppm. MS (ESI $^+$) m/z 611.3 [M+H] $^+$.

Synthesis of Compound 3.47



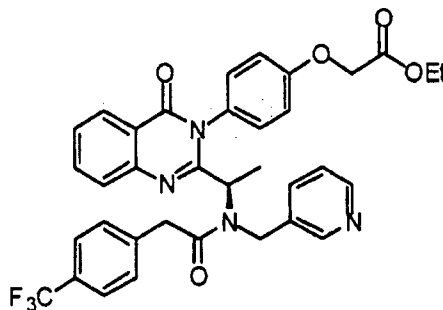
3.47

147

(R)-2-((N-3-Picolyl)-N-(4-trifluoromethylphenylacetyl)-1-aminoethyl)-3-(4-(1-propynyl))-3H-quinazoline-4-one (3.47). Compound 3.47 was synthesized using the method described for the synthesis of compound 3.07, except that excess amount of propyne gas was used in place of trimethylsilylacetylene. ¹H NMR (DMSO-*d*₆, T=120 °C) 1.41 (m, 3H), 2.09 (s, 3H), 2.89 (m, 1H), 3.55 (m, 1H), 4.72 (m, 2H), 5.23 (m, 1H), 7.18 (m, 1H), 7.28 (m, 2H), 7.55 (m, 8H), 7.71 (m, 1H), 7.87 (m, 1H), 8.11(d, J = 7.8 Hz, 1H), 8.37 (m, 2H) ppm. MS (ESI⁺) m/z 581.2 [M+H]⁺.

10

Synthesis of Compound 3.48

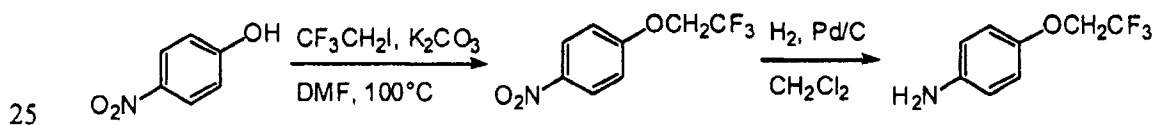


3.48

(R)-2-((N-3-Picolyl)-N-(4-trifluoromethylphenylacetyl)-1-aminoethyl)-3-(4-carboethoxymethoxy)-3H-quinazoline-4-one (3.48). Compound 3.48 was synthesized using the method described in Figure 4, except that 4-(carboethoxymethoxy)aniline was used in place of p-phenetidine. ¹H NMR (DMSO-*d*₆, T=120 °C) 1.24 (t, J = 7.1 Hz, 3H), 1.39 (d, J = 5.4 Hz, 3H), 3.51 (br s, 1H), 4.21 (q, J = 7.1 Hz, 2H), 4.72 (br s, 2H), 4.78 (s, 2H), 5.22 (m, 2H), 7.16 (m, 3H), 7.29 (d, J = 7.7 Hz, 2H), 7.44 (m, 1H), 7.54 (m, 4H), 7.68 (m, 2H), 7.86 (t, J = 7.0 Hz, 1H), 8.10 (d, J = 7.0 Hz, 1H), 8.36 (br s, 2H) ppm. MS (ESI⁺) m/z 645.2 [M+H]⁺.

20

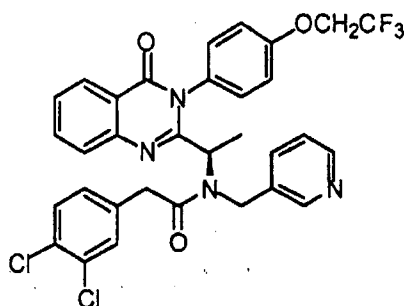
Synthesis of Compound 3.49



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4-(2,2,2-trifluoroethoxy)aniline. To a mixture of 4-nitrophenol (1.39 g, 10 mmol, 1.0 equiv), and K_2CO_3 (1.8 g, 13 mmol, 1.3 equiv) in 10 mL of dry DMF was added 1-iodo-2,2,2-trifluoroethane (2.31 g, 11 mmol, 1.1 equiv). The mixture was heated in an oil bath at 100 °C for 24 h. Half of the initial amount of K_2CO_3 and 1-iodo-2,2,2-trifluoroethane were added. The mixture was stirred for another 24 h at 100 °C. This was repeated once more on the third day. At the end of this 72 h reaction, the mixture was cooled to room temperature and poured into 40 mL of water. The mixture was extracted twice with 20 mL of diethyl ether. The combined ether extract was washed once with 40 mL of brine, dried over anhydrous Na_2SO_4 , filtered to remove drying agent, and evaporated *in vacuo* to yield 1.6 g of a crude product as light yellow solid. ^1H NMR (DMSO- d_6) 4.98 (q, $J = 8.8$ Hz, 2H), 7.30 (d, $J = 9.2$ Hz, 2H), 8.26 ((d, $J = 9.2$ Hz, 2H) ppm.

To a solution of the crude 4-(2,2,2-trifluoroethoxy)nitrobenzene (1.6 g, 7.2 mmol, 1.0 equiv) in 40 mL of dichloromethane was added 0.4 g of a 5% palladium on activated carbon (0.19 mmol, 0.026 equiv). Hydrogen gas was introduced using a balloon while the mixture was stirred vigorously for 48 h at room temperature. After all starting material had been consumed, the mixture was filtered through a pad of Celite to remove the palladium catalyst. The filtrate was evaporated *in vacuo* to give a crude product as a brown liquid. This crude product was purified by distillation at reduced pressure to give 1.3 g of pure 4-(2,2,2-trifluoroethoxy)aniline as a colorless liquid, which solidified upon cooling to 0 °C. b.p. 81-83 °C at 0.5 torr; ^1H NMR (DMSO- d_6) δ 4.53 (q, $J = 9.1$ Hz, 2H), 4.76 (br s, 2H), 6.52 (d, $J = 7.6$ Hz, 2H), 6.76 (d, $J = 7.6$ Hz, 2H) ppm.

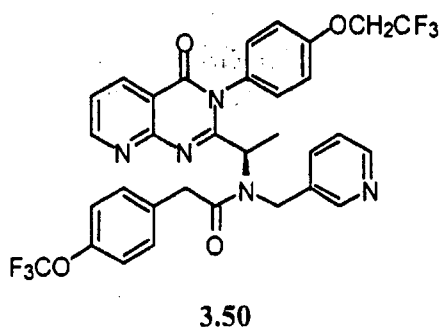


3.49

(R)-2-((N-3-Picolyl)-N-(3,4-dichlorophenylacetyl)-1-aminoethyl)-3-(4-(2,2,2-trifluoroethoxy)phenyl)-3H-quinazoline-4-one trifluoroacetate

(3.49•CF₃COOH). The trifluoroacetic acid salt of compound 3.49 was synthesized using the method described in Figure 4, except that 4-(2,2,2-trifluoroethoxy)aniline was used in place of p-phenetidine, and that 3,4-dichlorophenylacetic acid was used in place of 4-trifluoromethylphenylacetic acid. ¹H NMR (DMSO-*d*₆, T=120 °C) δ 1.42 (br s, 3H), 3.50 (m, 1H), 4.75 (m, 5H), 5.22 (m, 1H), 7.03 (d, J = 7.9 Hz, 1H), 7.27 (m, 5H), 7.43 (d, J = 8.2 Hz, 1H), 7.50 (m, 1H), 7.56 (t, J = 7.4 Hz, 1H), 7.67 (t, J = 8.4 Hz, 2H), 7.87 (t, 1H), 8.11 (d, J = 7.6 Hz, 1H), 8.42 (br s, 2H) ppm. MS (ESI⁺) m/z 641.2 [M+H]⁺.

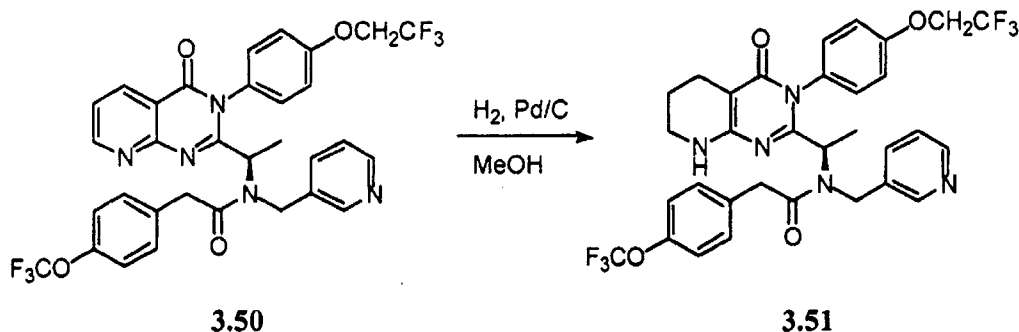
Synthesis of Compound 3.50



(R)-2-((N-3-Picolyl)-N-(4-trifluoromethoxyphenylacetyl)-1-aminoethyl)-3-(4-(2,2,2-trifluoroethoxy)phenyl)-3H-8-azaquinazoline-4-one (5).

Compound 3.50 was synthesized using the method described in Figure 13, except that 4-(2,2,2-trifluoroethoxy)aniline was used in place of p-phenetidine. ¹H NMR (DMSO-*d*₆, T=120 °C) δ 1.42 (m, 3H), 3.51 (m, 1H), 4.17 (m, 1H), 4.77 (q, J = 8.7 Hz, 2H), 4.89 (m, 2H), 5.25 (m, 1H), 7.18 (m, 4H), 7.28 (m, 3H), 7.47 (m, 1H), 7.59 (m, 2H), 7.90 (m, 1H), 8.50 (m, 3H), 9.01 (m, 1H) ppm. MS (ESI⁺) m/z 658.2 [M+H]⁺.

Synthesis of Compound 3.51



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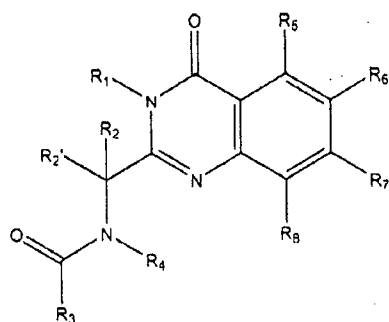
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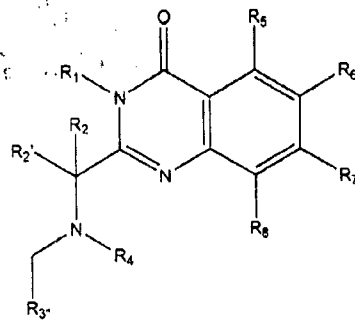
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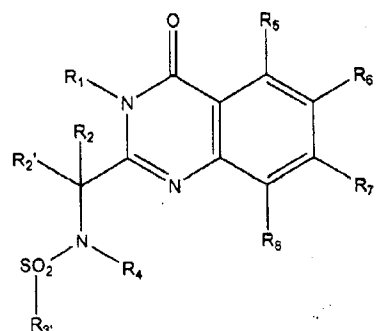
(54) Title: METHODS AND COMPOSITIONS UTILIZING QUINAZOLINONES



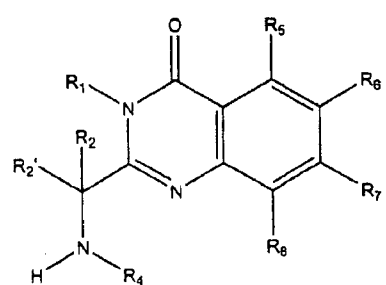
(a)



(b)



(c)



(d)

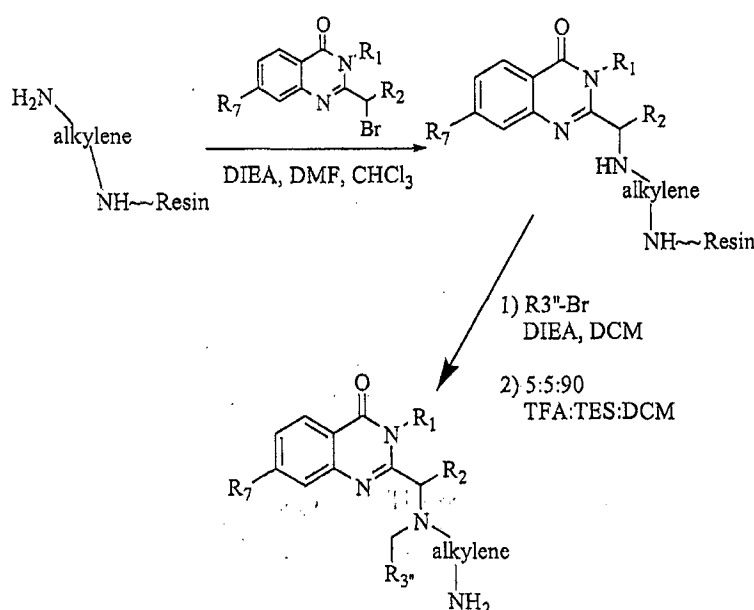
WO 01/98278 A1

(57) Abstract: Quinazolinones of formulae (a, b, c and d) are disclosed. They are useful for treating cellular proliferative diseases and disorders associated with KSP kinesin activity.

vessel using a Beckman Biomet 2000 automated liquid dispenser. The reaction mixture was shaken using a mechanical shaker, sonicated in an ultrasonic water bath, and then incubated overnight at room temperature. The mixture was diluted in CHCl_3 (300 μL) and washed with 5% aqueous NaHCO_3 and water. The solvent was removed in vacuo to provide compound 6 (65%). The purity of the compound was analyzed by TLC eluted with CH_2Cl_2 -ethanol-concentrated aqueous NH_3 , 100:10:1.

Examples 2 and 3

Synthesis of compounds of General Formula 1d



- 10 All anhydrous solvents were purchased from Aldrich chemical company in SureSeal® containers. Most reagents were purchase from Aldrich Chemical Company. Abbreviations: DCM, dichloromethane; DIEA, N,N-diisopropylethylamine; DMF, N,N-dimethylformamide; TES, triethylsilane; TFA, trifluoroacetic acid. Array synthesis was conducted in 15 x 75 mm glass round bottom screw-cap vials contained
- 15 in a 4 x 6 array aluminum synthesis block, sealed with a Teflon-lined rubber membrane. Reagents were added and aqueous extractions performed with single or multichannel pipettors. Filtrations were performed using Whatman/Polyfiltronics 24 well, 10 mL filtration blocks. Evaporation of volatile materials from the array was performed with a Labconco Vortex-Evaporator or by sweeping with a 4 x 6 nitrogen
- 20 manifold.

Example 2 (solid phase synthesis of a single compound)

STEP 1: 1,3-Diaminopropane trityl resin (Novabiochem, 1.2 mmol/g) (0.20 g, 0.24 mmol) was weighed into a screw-cap vial and 3 mL of a 1:1 mixture of DMF and chloroform was added. DIEA (0.130 mL, 0.72 mmol) and 2-(1'-bromopropyl)-3-benzylquinazolin-4-one (from Example 1) (0.188 g, 0.48 mmol) were added. The vial was sealed, heated to 70 °C and shaken overnight. The resin was filtered and washed (3 x DCM, 2 x MeOH, 1 x DCM, 2 x ether) and dried under vacuum. A 27 mg aliquot of resin was treated with 5:5:90 TFA:TES:DCM for 15 min and the mixture was filtered and evaporated, resulting in 8 mg (64% yield) of the quinazolinone-diamine intermediate. LCMS analysis showed >80 % purity.

STEP 2: The resin from Step 1 was swelled in 3 mL of DCM. DIEA (0.130 mL, 0.72 mmol) and 4-bromobenzyl bromide (0.12 g, 0.48 mmol) were added. The vial was sealed and shaken overnight. LCMS analysis of a cleaved aliquot revealed an approximate 1:1 mixture of starting material and product. Another 0.130 mL of DIEA and 0.12 g of 4-bromobenzyl bromide were added and the mixture was shaken at 70 °C for 8 h. The resin was filtered, washed (as above), and dried under vacuum.

STEP 3: The resin from Step 2 was twice shaken for 30 min with 5:5:90 TFA:TES:DCM and filtered. The filtrates were combined and evaporated, yielding 140 mg of an orange oil. This material was purified by reverse phase preparative HPLC (acetonitrile-water gradient) to provide 27 mg (17% for 3 steps) of the mono-TFA salt.

Example 3 (combinatorial synthesis of multiple compounds)

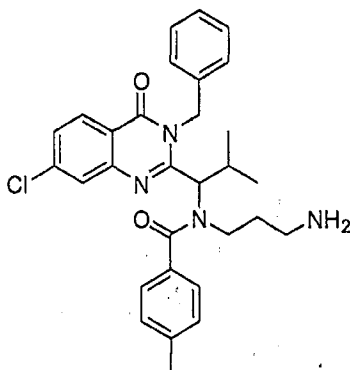
STEP 1: 1,2-Diaminoethane trityl resin (Novabiochem, 0.95 mmol/g) (200 g, 1.9 mmol) and 1,3-Diaminopropane trityl resin (Novabiochem, 1.14 mmol/g) (2.0 g, 2.28 mmol) were each placed in different 10 mL polypropylene fritted tubes (Bio-Rad). To each were added 4 mL of DMF, 4 mL of chloroform, 3 eq. of DIEA (1.0 mL and 1.2 mL, respectively) and 2 eq. of 2-(1'-bromopropyl)-3-benzylquinazolin-4-one (from Example 1) (1.5 g and 1.8 g, respectively). The mixtures were shaken at 70 °C overnight. Each mixture was washed (3 x DCM, 2 x MeOH, 1 x DCM, 2 x ether) and dried under vacuum. Analysis of a cleaved aliquot revealed the presence of the appropriate quinazolinone-diamine for each in >90 % purity.

- STEP 2: The quinazolinone ethyl-diamine resin (105 mg, 0.10 mmol) was placed into each of the vials in the first 2 rows of the array, and the quinazolinone propyl-diamine resin (88 mg, 0.10 mmol) was placed into each vial of the last 2 rows of the array. To each vial was added DIEA (0.131 mL, 0.75 mmol). Into each vial of the first 2 rows of the array was added a different amine, and the additions were repeated for the last two rows of the array. The reaction block was shaken at 70 °C overnight. Liquid was removed from each vial by multichannel pipette using fine-pointed gel-well tips, and the resins were washed (2 x DCM, 1 x MeOH, 1 x DCM) and dried under vacuum.
- STEP 3: To each vial of the array was added 2 mL of a 10:5:85 TFA:TES:DCM solution. The reaction block was shaken for 45 min and the mixtures were transferred to a filter block, filtered, and washed twice with 0.75 mL DCM. The solutions were evaporated to yield yellow-to-red oils. These thick oils were triturated twice with ether, dissolved in DCM and treated with 4 M HCl in dioxane to provide the HCl salts (unknown number of salts per compound) as tan-to-white powdery or amorphous solids. Analysis by LCMS showed all to be >75 % pure.

Examples 4-6

- Six racemic quinazolinones were separated into their enantiomers by chiral chromatography. The chiral chromatography of three of these compounds is described below:

Example 4

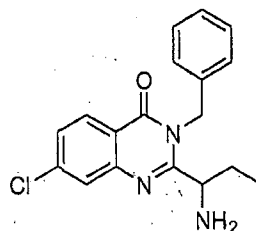


- Column - Chiralpak AD, 250 x 4.6 mm (Diacel Inc.). Sample - 0.5 mg/mL in EtOH.

Conditions – 15 min at 60% EtOH in Hexane, enantiomer 1 elutes at 4.5 min,
enantiomer 2 elutes at 4.9 min.

Example 5

5

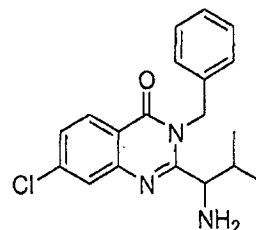


Column - Chiralcel OJ, 250 x 4.6 mm (Diacel Inc.). Sample – 0.5 mg/mL in EtOH.

Conditions - 15 min at 10% EtOH in Hexane, (R)-enantiomer elutes at 8.4 min, (S)-

10 enantiomer elutes at 9.6 min.

Example 6



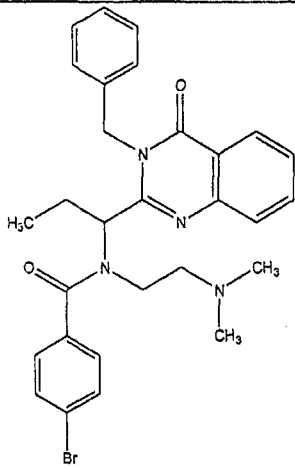
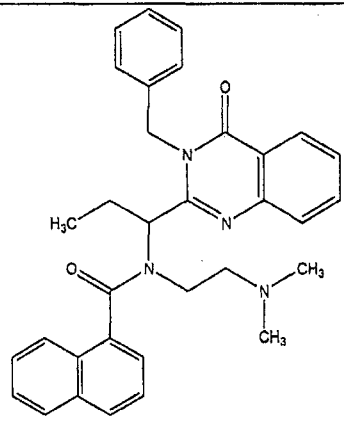
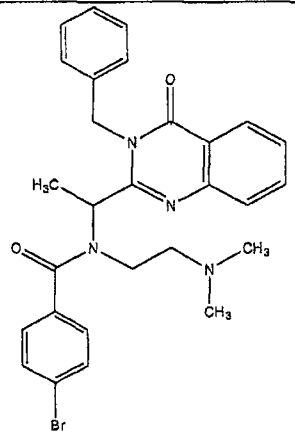
15

Column - Chiralpak AD, 250 x 4.6 mm (Diacel Inc.). Sample – 0.5 mg/mL in EtOH.

Conditions - 15 min at 70% EtOH in Hexane, enantiomer 1 elutes at 6.5 min,

enantiomer 2 elutes at 8.8 min.

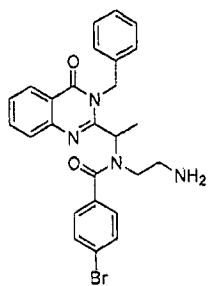
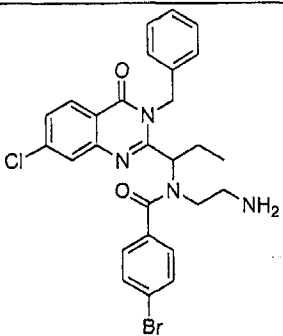
20 The table below depicts the IC₅₀ activity of the racemate and the enantiomers of three
other compounds separated as above. In all three cases, one enantiomer was
significantly more potent than the other. By independent chiral synthesis, it appears
that the more active enantiomer is the R enantiomer.

	IC ₅₀ (μM) Racemate	IC ₅₀ (μM) Enantiomer 1	IC ₅₀ (μM) Enantiomer 2
	0.06	0.28	0.03
	12.7	>>40	6.6
	2.6	>>40	1.3

Examples 7 and 8

The following two compounds were synthesized as single enantiomers by the route shown in Figure 4. The data indicate that the more active enantiomer is the R enantiomer.

5

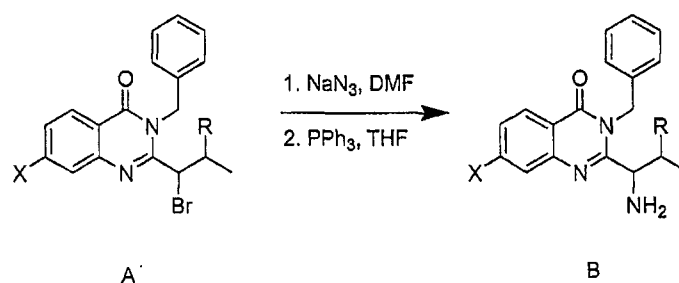
	K_i (μ M)	K_i (μ M)
	S enantiomer	R enantiomer
	2	<0.1
	>0.5	<0.05

Example 9

Chiral Resolution by Recrystallization with Tartaric Acid

10

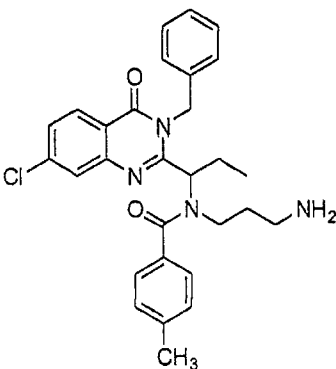
Intermediate A, prepared in Example 1, can be converted to an intermediate B, which, upon resolution, provides an alternative to the first five steps shown in Figure 4. The process is shown in the scheme below:



The *R* enantiomer of B can be crystallized selectively by heating a mixture of B with 1.1 equivalents of *D*-tartaric acid in a mixture of isopropanol and methanol and then
5 letting the mixture return to room temperature.

Example 9: X = Cl, R = H

Racemic intermediate B (1.5 g), dissolved in 100 mL of boiling isopropanol, was mixed with 0.8 g of *D*-tartaric acid in 100 mL of boiling methanol. The mixture was
10 allowed to slowly reach room temperature. After standing overnight, the solid was removed by filtration and rinsed with ethyl acetate and hexanes, and allowed to air dry. The dried solid (0.8 g) was then dissolved in a boiling mixture of 50 mL of isopropanol and 50 mL of methanol and allowed to slowly cool to room temperature. After standing overnight, the resulting solid was removed by filtration and rinsed with
15 ethyl acetate and hexanes, and allowed to air dry. The dried solid was then stirred with saturated sodium bicarbonate for 30 min and extracted with ethyl acetate. The organics were dried (MgSO₄), filtered and evaporated to dryness. The resulting clear oil weighed 345 mg. Chiral purity of >95% was determined by conversion of a portion to the S-Mosher amide and examination of the product by ¹HNMR.
20 The enantiomerically pure compounds below were prepared, according to the remaining steps in Figure 4, from material resulting from the procedure described above using both *D*- and *L*-tartaric acid.

	Racemic I C ₅₀ (uM)	R Isomer IC ₅₀ (uM)	S Isomer IC ₅₀ (uM)
	<0.05	<0.05	>0.5

Example 10

Induction of Mitotic Arrest in Cell Populations Treated with a Quinazolinone KSP Inhibitor

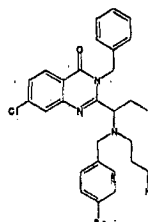
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FACS analysis to determine cell cycle stage by measuring DNA content was performed as follows. Skov-3 cells (human ovarian cancer) were split 1:10 for plating in 10cm dishes and grown to subconfluence with RPMI 1640 medium containing 5% fetal bovine serum (FBS). The cells were then treated with either 10nM paclitaxel, 10 400nM quinazolinone 1, 200nM quinazolinone2, or 0.25% DMSO (vehicle for compounds) for 24 hours. Cells were then rinsed off the plates with PBS containing 5mM EDTA, pelleted, washed once in PBS containing 1% FCS, and then fixed overnight in 85% ethanol at 4°C. Before analysis, the cells were pelleted, washed once, and stained in a solution of 10µg propidium iodide and 250µg of ribonuclease 15 (RNase) A per milliliter at 37°C for half an hour. Flow cytometry analysis was performed on a Becton-Dickinson FACScan, and data from 10,000 cells per sample was analyzed with Modfit software.

weight x 100% ($\Delta T/\Delta C$) was subtracted from 100% to give the tumor growth inhibition (TGI) for each group.

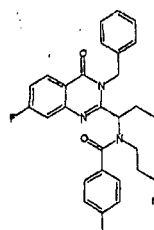
Compounds 1-5 (below) were tested by the above-described method, giving the results summarized below in Tables A - D. Other compounds of the present invention show comparable activities when tested by this method.

Compound 1



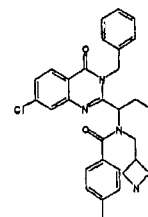
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Compound 2

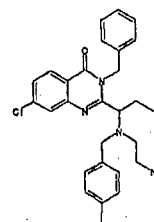


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Compound 3

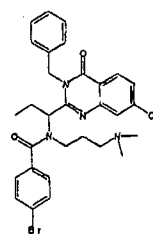


Compound 4



20

Compound 5



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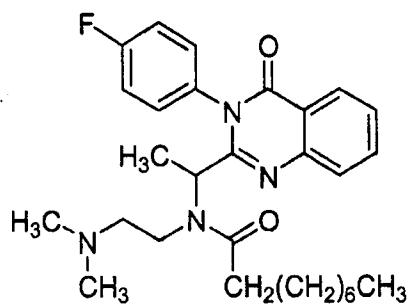
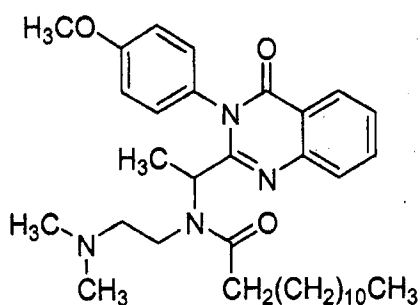
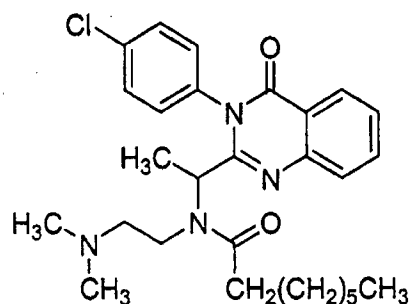
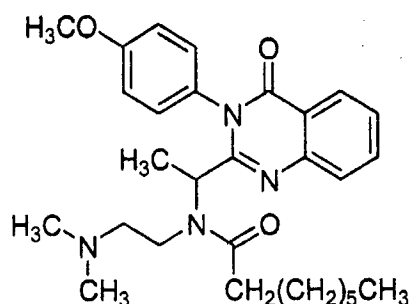
WO 01/16114 A2

(54) Title: COMPOUNDS AND METHODS FOR MODULATING CXCR3 FUNCTION

(57) Abstract: Compounds and compositions are provided that bind to the CXCR3 chemokine receptor and which are useful for treating diseases associated with CXCR3 activity, such as multiple sclerosis.

ethylene and Z is dimethylamino. Most preferably, n is 0, X is N, R¹ is a (C₈-C₁₄)acyl group, R² is unsubstituted (C₁-C₄)alkyl, Y is ethylene, Z is dimethylamino and Ar is a 4-substituted phenyl group, more preferably, 4-fluorophenyl, 4-chlorophenyl, 4-bromophenyl, 4-methylphenyl, and 4-methoxyphenyl.

- 5 In a group of most preferred embodiments, the compound has a formula selected from the group:



and

