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Industria y Comercio
SUPERINTENDENCIA

PCT

DELEGATURA DE PROPIEDAD INDUSTRIAL
DIVISION DE NUEVAS CREACIONES

08-129815

SOLICITUD DE PATENTE DE
INVENCION

TITULO: INHIBIDORES DE AKT (PROTEINA CINASA B)

SOLICITANTE: ELI LILLY AND COMPANY

DIRECCION: Lilly Corporate Center, Indianápolis, Indiana 46285 E.U.A.

APODERADO: CARLOS R. OLARTE

BOGOTÁ 05 de Diciembre de 2008

99

OLARTE RAISBECK



SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 08-129815-00000-0000

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Act: 411 PRESENTACION

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Folios: 116

PETITORIO - FORMULARIO ÚNICO DE SOLICITUD PATENTE PCT

ENTRADA EN FASE NACIONAL

P2008/000180

SOLICITUD DE:

1

☒

Patente de Invención.

☐

Patente de Modelo de Utilidad.

☒

Capítulo I.

☐

Capítulo II.

2

**SOLICITANTE
(71)**

Nombre: ELI LILLY AND COMPANY

Dirección: Lilly Corporate Center,
Indianápolis, Indiana 46285 E.U.A.

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

Teléfono: _____ **Fax:** _____
E-mail: _____

IDENTIFICACIÓN

C.C. ☐ **NIT** ☐

C.E. ☐ **Otro** ☐

Cual _____

Número _____

**REPRESENTANTE
O APODERADO**

Nombre: CARLOS R. OLARTE

Dirección: World Trade Center Torre A,
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Teléfono: 601-7700 **Fax:** 601-7799
E-mail: office@olarteraisbeck.com

IDENTIFICACIÓN

C.C. ☒ **NIT** ☐

C.E. ☐ **Otro** ☐

Cual _____

Número 79.782.747Bta
TP 74.295

**INVENTOR (ES)
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Dirección: VER PAGINA ADJUNTA

Nacionalidad o Domicilio: VER PAGINA ADJUNTA

5

PCT (86)

Solicitud Internacional No. PCT/US2007/070945 Fecha Junio 12, 2007

Publicación Internacional No. WO 2007/149730 Fecha Diciembre 27, 2007

6

Título (54) INHIBIDORES DE AKT (PROTEINA CINASA B)

Clasificación Internacional (51) C07D 217/02, A61K 31/435, A61P 35/00, A61P 31/18

Prioridad
SI ☒ **NO** ☐

(33) País de Origen

US
US

(32) Fecha

Junio 20, 2006
Enero 19, 2007

(31) Número de Solicitud

60/805,260
60/885,765

9

Comprobante de pago No. 08-93,855

Fecha Diciembre 2 de 2008

10

ANEXOS☒

Comprobante de pago de la tasa de presentación de la solicitud.

☒

Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.

☒

Poderes, si fuere el caso. Adjunto copia simple ver expediente No. 07-128.249

☐

Documento de cesión del inventor al solicitante o a su causante.

☐

Declaraciones.

☒

Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos).

☒

Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos).

☐

Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).

☐

Traducción del examen preliminar internacional efectuado por la IPEA.

☐

De ser el caso, copia del contrato de acceso.

☐

De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.

☐

De ser el caso, certificado de depósito del material biológico.

☐

Arte final 12 x 12.

☐

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.

☒

Otros. Búsqueda Internacional, Secuencias.

11

FIGURA CARACTERÍSTICA:

12

Solicito la concesión de la patente,

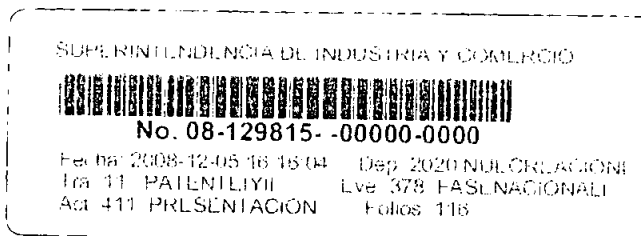
NOMBRE: CARLOS R. OLARTEFIRMA: C.C. 78.782.747 Btá T.P. 74.295

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

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PCT/US2007/070945

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Estado Unidos
Nacionalidad: Estadounidense
6. **Christopher Randall SCHMID,**
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Nacionalidad: Estadounidense



SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 93,855
FECHA : DICIEMBRE 2 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
OLARTE RAISBEK	CONSIGNACION	BANCO DE BOGOTA	062754387	14445211	02/12/2008	8,195,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
		1 TRAMITES DE SOL. DE PATENTE DE IN	501,000.00
		TOTAL :	\$ 501,000.00

SON: QUINIENTOS UN MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

ELI LILLY AND COMPANY

Todos Rokita Secretario de Estado

Secretario de Estado de Indiana Estado de Indiana

(Convención de la Haya del 5 de octubre de 1961)

- 1963
Lico
Circuito erry para
to en y para el estado

5. En Indianapolis, Indiana 6. El 22 de julio de 2003

7. Por el Secretario de Estado de Indiana 8. No. A2003-11279

9. Gran Sello del Estado de Indiana

10. Firma legible de Todd Rokita- Secretario de Estado de Indiana

1279
SECRETARIO DE ESTADO

The Statehouse, Indianapolis, Indiana 46204, (317) 232-6531, fax (317) 233-3283

CERTIFICACION NOTARIAL

Bilingüe inglés- español, el 17 de julio de 2003 el suscrito notario Catherine Michel certifica que Peter G. Stringer, domiciliado en 7620 Castleton Farms, West Drive, Indianapolis, Indiana - 46256 Estados Unidos ejercía funciones de Director Ejecutivo y Patentes Internacionales de Ely Lilly & Company, con sede en Indianapolis, Indiana, Estados Unidos.

Marlene F. Castro
MARLENE CASTRO
Inductora e Intérprete Oficial
Inglés - Español - Inglés
Resolución No. 2117 del 7 Oct 1987

El otorgante fundamenta sus certificaciones en este documento: Delegación de Autoridad concerniente a Ciertas Materias de Patentes, suscrito el 20 de enero de 2003: conforme al poder otorgado al Asesor General de la Compañía por el Comité ejecutivo de la Junta Directiva de Eli Lilly and Company.

Firma del notario público Catherine Michel certifica

Sus funciones vencen el 21 de octubre de 2008 - residente en el condado Johnson

Sello de notario público de Indiana

--

Es traducción fiel del inglés, con copia archivo para confrontación. Bogotá 28 de julio-2003.

Marlen Neira C



MARLEN NEIRA CASTRO

Traductor e Intérprete Oficial

Inglés - Español - Inglés

Resolución No. 2117 del 7 Oct 1987

OLARTE RAISBECK

OLARTE RAISBECK & FRIERI, LTDA.
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Power of Attorney

The undersigned,

domiciled in

ELI LILLY AND COMPANY

El suscrito,

domiciliado en

Lilly Corporate Center, Indianapolis, Indiana 46285 US

by means of these presents hereby grants to **Carlos R. Olarte, J. Ian Raisbeck and Ana María Frieri** full and sufficient Power of Attorney to file, prosecute, obtain, defend and enforce any and all of the company's **Patents, Utility Models and Industrial Designs** in the Republic of Colombia, to which end it empowers them to take all steps necessary before any administrative or judicial authority of any class for the objects stated, to file petitions, appeals, replies and oppositions, pay taxes and annuities, request testimonies, receive documents and securities, abandon applications and collect refunds. Sufficient power is hereby granted to receive notifications on behalf of the Company, to confer powers on those who are to represent the company judicially and extrajudicially, to answer in court in the matter of all claims and demands that may be presented in connection with the objects stated, giving them likewise power to substitute these presents and revoke such substitutions.

por medio del presente otorga a **Carlos R. Olarte, J. Ian Raisbeck y Ana María Frieri** poder especial, amplio y suficiente para presentar, tramitar, obtener, defender y hacer respetar todas y cualquiera de **Patentes, Modelos de Utilidad y Diseños Industriales** de la compañía en la República de Colombia, a cuyo efecto los faculta para dar todos los pasos necesarios ante cualquier autoridad administrativa o judicial de cualquier jerarquía para cumplir con lo encomendado, elevar solicitudes, apelaciones, respuestas y oposiciones, pagar impuestos y anualidades, solicitar testimonios, recibir documentos y valores, desistir y percibir. Se concede poder bastante para recibir notificaciones a nombre de la compañía, otorgar poderes a quienes vayan a representar a la compañía judicial y extrajudicialmente, responder en juicio a todas las reclamaciones o demandas que por motivo de lo encomendado se presentaren, dándoles asimismo facultad para sustituir el presente y revocar dichas sustituciones.

Signature/Firma



Date/Fecha: 17 July 2003

City/Country/Ciudad,País: Indianapolis, Indiana, United States of America

OLARTE RAISBECK

OLARTE RAISBECK & FRIERI, LTDA.
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Notarial Certification

On this date,

/ 7 July 2003

The undersigned Notary Public certifies:

1. That

of legal age and domiciled in

7620 Castleton Farms, West Drive, Indianapolis, Indiana 46256 US
signed the foregoing document.

2. That the grantor has executed the foregoing
document as Executive Director, International
Patents
of ELI LILLY AND COMPANY

3. That ELI LILLY AND COMPANY
having its principal place of business located in
Indianapolis, Indiana, United States of
America

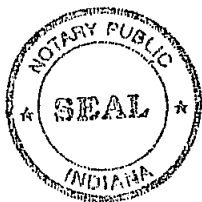
is duly incorporated, is currently in existence, and
that the purposes for which the Power of Attorney is
granted are within the scope of its corporate
purposes.

4. That the grantor has the authority to execute the
Power of Attorney and that his/her representation is
legal.

5. The foundation for my certifications contained in
points 2, 3 and 4 was the exhibition of the following
authentic document(s):

Delegation of Authority Concerning Certain
Patent Matters, executed January 20, 2003;
pursuant to the authority granted to the
General Counsel of the Company by the
executive Committee of the Board of
Directors of Eli Lilly and Company

NOTARY PUBLIC (Signature):



En esta fecha,

el Notario Público suscrito certifica:

1. Que

Peter G. Stringer
mayor de edad y domiciliado
suscribió el documento que antecede.

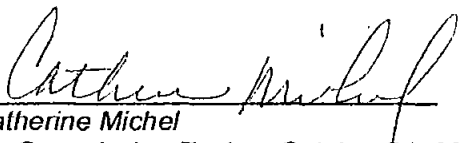
2. Que el otorgante ha suscrito el referido
documento en su carácter de
de

4. Que
con sede principal ubicada en

está legalmente constituida, que tiene existencia
legal vigente y que los propósitos para los cuales
se otorga el poder se encuentra dentro de los que
comprenden su objeto social.

4. Que el otorgante tiene la representación para
suscibir el poder, y que ésta es legítima.

5. El fundamento de mis certificaciones
contenidas en los puntos 2, 3 y 4 fue la exhibición
de los (del) siguiente(s) documento(s) auténtico(s):


Catherine Michel
My Commission Expires October 21, 2008
Resident of Johnson County

X17330

5/8

PCT REQUEST

Original (for SUBMISSION)

VIII-2-1	Declaration: Entitlement to apply for and be granted a patent Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent (Rules 4.17(ii) and 51bis.1(a)(ii)), in a case where the declaration under Rule 4.17(iv) is not appropriate: Name (LAST, First)	in relation to this international application ELI LILLY AND COMPANY is entitled to apply for and be granted a patent by virtue of the following:
VIII-2-1(i)		ELI LILLY AND COMPANY is entitled as employer of the inventor, JOSEPH, Sajan
VIII-2-1(i)		ELI LILLY AND COMPANY is entitled as employer of the inventor, LI, Renhua
VIII-2-1(i)		ELI LILLY AND COMPANY is entitled as employer of the inventor, MYERS, Michael, Ray
VIII-2-1(i)		ELI LILLY AND COMPANY is entitled as employer of the inventor, ABURUB, Aktham
VIII-2-1(i)		ELI LILLY AND COMPANY was entitled as employer of the inventor, DAI, Jenny, Pingqi
VIII-2-1(i)		ELI LILLY AND COMPANY is entitled as employer of the inventor, SCHMID, Christopher, Randall

X17330

6/8

PCT REQUEST

Original (for SUBMISSION)

VIII-3-1	Declaration: Entitlement to claim priority Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application specified below, where the applicant is not the applicant who filed the earlier application or where the applicant's name has changed since the filing of the earlier application (Rules 4.17(iii) and 51bis.1(a)(iii)) Name	in relation to this international application ELI LILLY AND COMPANY is entitled to claim priority of earlier application No. 60/805,260 by virtue of the following:
VIII-3-1(i v)		an assignment from JOSEPH, Sajan to ELI LILLY AND COMPANY, dated 11 January 2007 (11.01.2007)
VIII-3-1(i v)		an assignment from MYERS, Michael, Ray to ELI LILLY AND COMPANY, dated 11 January 2007 (11.01.2007)
VIII-3-1(i v)		an assignment from LI, Renhua to ELI LILLY AND COMPANY, dated 26 January 2007 (26.01.2007)

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

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27 December 2007 (27.12.2007)

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(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/805,260 ✓ 20 June 2006 (20.06.2006) ✓ US
60/885,765 ✓ 19 January 2007 (19.01.2007) US

(71) Applicant (for all designated States except US): ELI
LILLY AND COMPANY [US/US]; Lilly Corporate
Center, Indianapolis, Indiana 46285 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): ✓ JOSEPH, Sajjan
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46228 (US) ✓ LI, Renhua [US/US]; J0493 Hollowood
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North Alabama Street #406, Indianapolis, Indiana 46204
(US) ✓ DAI, Jenny, Pingqi [US/US]; 87 Hawthorne
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(74) Agents: HOSTETTLER, Danica et al.; Eli Lilly And
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Indiana 46206-6288 (US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH,
CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG,
ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL,
IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK,
LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW,
MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL,
PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA,
ZM, ZW.

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

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii))

Published:

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

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



WO 2007/149730 A2

(54) Title: INHIBITORS OF AKT (PROTEIN KINASE B)

(57) Abstract: 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof as Akt inhibitors that are antineoplastic and/or antiviral agents as well as compositions comprising these compounds and methods of using these compounds.

-1-

INHIBITORS OF AKT (PROTEIN KINASE B)

The present invention provides compounds that are inhibitors of Akt, compositions comprising these compounds, and methods of using these compounds.

5

BACKGROUND OF THE INVENTION

Protein kinases are involved in the signal transduction pathways linking growth factors, hormones and other cell regulation molecules to cell growth, survival and metabolism under both normal and pathological conditions. One such protein kinase, protein kinase B (also known as Akt), is a serine/threonine kinase that plays a central role in promoting the proliferation and survival of a wide range of cell types, thereby protecting cells from apoptosis (programmed cell death).

A number of protein kinases and phosphatases regulate the activity of Akt. For instance, activation of Akt is mediated by phosphatidylinositol 3-kinase (PI3-K), which initiates the binding of second messenger phospholipids to the pleckstrin homology (PH) binding domain of Akt. The binding anchors Akt to plasma membrane and results in phosphorylation and activation of the enzyme. Amplifications of the catalytic subunit of PI3-K, p110 α , or mutations in the PI3-K regulatory subunit, p85 α , lead to activation of Akt in several types of human cancer. Recent studies have also demonstrated the role of the PI3-K/AKT pathway in the life cycle of numerous viruses.

WO 01/91754 pertains to protein kinase inhibitors. WO 2005/011697 involves protein kinase A and B inhibitors. WO 2005/054202 pertains to AKT inhibitors.

Because of its pivotal role in the regulation of cell survival, Akt provides a novel therapeutic target for the effective treatment of various disorders, particularly cancer and viral infections. However, such treatment requires the development of potent, selective, bioavailable inhibitors of Akt. A need exists for alternative Akt inhibitors. Thus, the present invention provides novel inhibitors of Akt that exhibit increased potency, selectivity, and/or bioavailability, compositions comprising these compounds, and methods of using these compounds.

Additionally, pharmaceutical compositions containing novel inhibitors of Akt when diluted or in solution necessarily must be free from precipitation in order to be

-2-

delivered to a patient. The present invention provides pharmaceutical compositions comprising novel Akt inhibitors at particular pH ranges that lack such precipitation.

SUMMARY OF THE INVENTION

5 The present invention provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof.

 The present invention also provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride.

10 The present invention further provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate.

 The present invention also provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-
15 hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, for use as a medicament.

 Additionally, the present invention provides the use of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-
20 isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, for the manufacture of a medicament for treating multiple myeloma, non small cell lung cancer, glioblastoma, neuroblastoma, melanoma or neoplasms of the prostate, breast, ovaries, primary stomach, intestinal-type, endometrium, thyroid, pancreas, lung, or bladder.

25 The present invention also provides the use of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, for the manufacture of a medicament for treating
30 multiple myeloma, non small cell lung cancer, glioblastoma or neoplasms of the prostate, breast, or ovaries.

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The present invention additionally provides the use of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, for the manufacture of a medicament for treating non small cell lung cancer or glioblastoma.

The present invention further provides the use of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, for the manufacture of a medicament for treating hepatitis C, rubella, human immunodeficiency virus (HIV), hepatitis B, or human cytomegalovirus (HCMV).

Additionally, the present invention provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, for the treatment of multiple myeloma, non small cell lung cancer, glioblastoma, neuroblastoma, melanoma or neoplasms of the prostate, breast, ovaries, primary stomach, intestinal-type, endometrium, thyroid, pancreas, lung, or bladder.

The present invention also provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, for the treatment of multiple myeloma, non small cell lung cancer, glioblastoma or neoplasms of the prostate, breast, or ovaries.

The present invention additionally provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol

-4-

monohydrochloride hemihydrate, for the treatment of non small cell lung cancer or glioblastoma.

The present invention further provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, for the treatment of hepatitis C, rubella, human immunodeficiency virus (HIV), hepatitis B, or human cytomegalovirus (HCMV).

The present invention additionally provides a method of treating multiple myeloma, non small cell lung cancer, glioblastoma, neuroblastoma, melanoma or neoplasms of the prostate, breast, ovaries, primary stomach, intestinal-type, endometrium, thyroid, pancreas, lung, or bladder in a patient in need thereof comprising administering an effective amount of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate.

The present invention also provides a method of treating multiple myeloma, non small cell lung cancer, glioblastoma or neoplasms of the prostate, breast, or ovaries in a patient in need thereof comprising administering an effective amount of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate.

The present invention additionally provides a method of treating non small cell lung cancer or glioblastoma in a patient in need thereof comprising administering an effective amount of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate.

The present invention further provides a method of treating hepatitis C, rubella, human immunodeficiency virus (HIV), hepatitis B, or human cytomegalovirus (HCMV) in a patient in need thereof comprising administering an effective amount of 4-[5-(2-

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amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate.

5 The present invention also provides a pharmaceutical composition comprising 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, and a
10 pharmaceutically acceptable carrier, diluent or excipient.

 The present invention additionally provides a lyophilized pharmaceutical composition comprising 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-
15 [5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, and a pharmaceutically acceptable carrier, diluent or excipient wherein the pH of said composition when diluted with aqueous diluent is less than 4.2 and greater than 2.0, less than 3.2 and greater than 2.0, or less than 2.8 and greater than 2.0.

 The present invention also provides a pharmaceutical composition comprising 4-
20 [5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt thereof, including 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride or 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate, and a pharmaceutically acceptable carrier, diluent or excipient in solution wherein the pH of
25 said composition is less than 4.2 and greater than 2.0, less than 3.2 and greater than 2.0, or less than 2.8 and greater than 2.0.

 The present invention further provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate in crystalline form having an X-ray powder diffraction pattern with intense peaks at $2\theta = 4.9, 14.8, \text{ and } 10.2$.

30 The present invention also provides 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or hydrate thereof.

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DETAILED DESCRIPTION OF THE INVENTION

Compounds of the present invention are inhibitors of Akt and are believed to be useful in the treatment of disorders related to Akt activity. Thus, compounds of the present invention are antineoplastic and/or antiviral agents.

5 The compounds of the present invention are useful for the treatment of neoplasms that exhibit defects in PTEN, neoplasms with deregulated PI3-Kinase activity, or neoplasms that exhibit elevated Akt activity. Akt inhibitors are believed to be useful in treating multiple myeloma (Hsu *et al.*, *Blood* (2001) 98(9) 2853-2855); non small cell lung cancer (Balsara, *Carcinogenesis* (2004) 25(11) 2053-2059); glioblastoma (Koul, *et al.*, *Mol. Cancer Ther.* (2006) 5: 637-644); neuroblastoma (Li, *et al.*, *Cancer Res.* (2005) 10 65(6), 2070-2075); melanoma (Dai, *et al.*, *J. of Clin. Oncology* (2005) 23(7), 1473-1482) and neoplasms of the prostate (Majumder, *et al.*, *Oncogene* (2005) 24, 7465-7474); breast (Tokunaga, *et al.*, *J. of Clin. Oncology* (Meeting Abstracts) (2005) 23(16S), 9500); ovaries (Cheung, *et al.*, *PNAS* (1992) 89, 9267-9271; Yuan *et al.* (2000); Hu *et al.* 15 (2000)); primary stomach or intestinal-type (Ang, *et al.*, *Cancer Lett.* (2005) 225(1), 53-59); endometrium (Jin, *et al.*, *British J. of Cancer* (2004) 91 1808-1812); thyroid (Ringel, *et al.*, *Cancer Res.* (2001) 61(16), 6105-6111; De Vita, *et al.*, *Cancer Res.* (2000) 60, 3916-3920); pancreas (Schliemen, *et al.*, *Brit. J. of Cancer* (2003) 89, 2110-2115); lung (Massion, *et al.*, *Am. J. Resp. Crit. Care Med.* (2004) 170, 1088-1094); or bladder 20 (Rieger-Christ, *et al.*, *Oncogene* (2004) 23(27), 4745-4753). Akt inhibitors are also believed to be useful in treating viruses such as hepatitis C and NS5A of hepatitis C (Mannova, *et al.*, *J. Virol.* (2005) 79(14), 8742-8749; He *et al.* (2002)); rubella (Cooray, *et al.*, *Virology J.* (2005) 2(1), 1-12); Tat protein of human immunodeficiency virus (HIV) (Borgatti *et al.* (1997)); Protein X of hepatitis B (Lee *et al.* (2001)), or human 25 cytomegalovirus (HCMV) (Johnson *et al.* (2001)).

4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol forms pharmaceutically-acceptable acid addition salts with, for example, the physiologically-acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Pharmaceutically acceptable salts and common methodology for preparing 30 them are well known in the art. See, e.g., P. Stahl, *et al.*, *Handbook of Pharmaceutical Salts: Properties, Selection and Use*, (VCHA/Wiley-VCH, 2002); S.M. Berge, *et al.*,

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"Pharmaceutical Salts," *Journal of Pharmaceutical Sciences*, Vol. 66, No. 1, January 1977.

Preferable salts for 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol include the monohydrochloride and di-hydrochloride.

5 The intermediates described herein can form salts.

In addition to salts, the compounds of the present invention and the intermediates described herein can form a hydrate or a hydrate of the pharmaceutically acceptable salt.

A preferable compound is 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol. Yet another preferable compound is 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride. A more preferable compound is 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate.

As used herein, the term "patient" refers to a mammal that is afflicted with one or more disorders associated with elevated Akt activity. It will be understood that the most preferred patient is a human. It is also understood that this invention relates specifically to the inhibition of mammalian Akt/PKB.

The terms "treatment," "treat," "treating," and the like, are meant to include effects such as decreasing and/or inhibiting the growth of neoplasms, the amplification and/or overexpression of Akt 1, Akt2, and/or Akt3, cell proliferation and survival, and/or viral replication.

20 As used herein, the term "effective amount" refers to an amount that inhibits Akt to an extent that provides a pharmacological effect.

The present invention also provides a pharmaceutical composition comprising 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol, or a pharmaceutically acceptable salt thereof, or hydrate thereof, and a pharmaceutically acceptable carrier, diluent, or excipient. Pharmaceutical compositions for the present invention include forms prior to dilution, such as a lyophilized form, and forms subsequent to dilution, such as a form ready for administration to a patient. The pH of these pharmaceutical compositions is from less than about 4.2 to greater than about 2.0. More preferably, the pH is from less than about 3.2 to greater than about 2.0. The most preferable pH is from less than about 2.8 to greater than about 2.0. A "pharmaceutically acceptable carrier, diluent, or excipient" is a medium generally accepted in the art for the delivery of biologically active agents to patients, e.g., mammals, preferably humans. Such carriers, diluents, or

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excipients are generally formulated according to a number of factors well within the purview of those of ordinary skill in the art to determine and account for. These include, without limitation: the type and nature of the active agent being formulated; the subject to which the agent-containing composition is to be administered; the intended route of administration of the composition; and the therapeutic indication being targeted.

Pharmaceutically acceptable carriers and excipients include both aqueous and non-aqueous liquid media, as well as a variety of solid and semi-solid dosage forms. Such carriers, diluents, or excipients can include a number of different ingredients and additives in addition to the active agent, such additional ingredients being included in the formulation for a variety of reasons, e.g., stabilization of the active agent, well known to those of ordinary skill in the art. Descriptions of suitable pharmaceutically acceptable carriers, diluents, or excipients, and factors involved in their selection, are found in a variety of readily available sources. *See, e.g.,* Remington: The Science and Practice of Pharmacy (A. Gennaro, *et al.*, eds., 19th ed., Mack Publishing Co., 1995).

The compounds of the present invention may be administered systemically, such as intravenously (e.g. by bolus or infusion), in dosage unit formulations of pharmaceutical compositions containing conventional non-toxic pharmaceutically acceptable carriers, diluents, or excipients.

For pharmaceutical compositions of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol, the active ingredient will ordinarily be present in an amount of about 0.5 to 95% by weight based on the total weight of the composition. Appropriate coatings may be applied to increase palatability or to delay adsorption.

The therapeutically effective amounts of the compounds of the invention for treating the disorders described herein in a patient can be determined in a variety of ways known to those of ordinary skill in the art. It will be understood, however, that the specific dose levels for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease. Frequency of dosage may also vary depending on the compound used and the particular disease treated. For example, a typical daily dose may contain from about 1 mg to 1 g of the active ingredient.

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The compounds of this invention may be prepared by employing the information provided herein, in addition to other standard manipulations that are known in the literature or exemplified in the experimental procedures.

The terms and abbreviations used herein have their normal meanings unless otherwise designated. For example "LC" refers to liquid chromatography; "dppb" refers to bis(diphenylphosphino)butane; Pd(OAc)₂ refers to palladium acetate; "DMF" refers to N,N-dimethylformamide; "DMSO" refers to dimethylsulfoxide; "Et₂O" refers to diethyl ether; "EtOAc" refers to ethyl acetate; "TFA" refers to trifluoroacetic acid; MeOH refers to methanol.

10

PREPARATIONS

Preparation 1

2-[7-(4-Methoxy-phenyl)-isoquinoline-5-sulfonyl]-ethylamine di-hydrochloride

Treat a slurry of {2-[7-(4-methoxy-phenyl)-isoquinoline-5-sulfonyl]-ethyl}-
15 carbamic acid tert-butyl ester (11.00 g, 24.85 mmol) in anhydrous MeOH (150 mL) with 4N HCl in dioxane (350 mL). Stir the resulting reaction mixture at room temperature overnight, then concentrate *in vacuo* to 1/2 volume and treat with excess EtOAc, causing a yellow solid to precipitate. Recover the resulting solid by vacuum filtration under an N₂ atmosphere; wash with EtOAc and dry under vacuum (under an N₂ atmosphere) to afford
20 the title compound (10.23 g, 99% yield) as a yellow solid: MS (ES): m/z 343.0 (M⁺+H).

Preparation 2

7-Bromo-isoquinoline-5-sulfonic acid

Add fuming H₂SO₄ (2,000 mL, 21.33 mol; 26-29.5% free SO₃) to a 5-L round
25 bottom flask, equipped with a mechanical stirrer, reflux condenser, N₂ line, and thermometer. Cool the fuming H₂SO₄ to ~10 °C with an ice/acetone bath, then add 7-bromoisoquinoline HCl (500.00 g, 2.04 mol) portion-wise, keeping the temperature of the reaction mixture below ~15-20 °C. Upon complete addition of 7-bromoisoquinoline HCl, heat the resulting reaction mixture at ~100 °C overnight. Cool the reaction mixture to
30 room temperature, then slowly pour into a stirring solution of ice H₂O. Recover the resulting precipitate by vacuum filtration, washing with H₂O, then Et₂O, and dry under vacuum filtration, followed by drying in a drying oven under reduced pressure at ~35 °C

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to afford the title compound (501.42 g, 85% yield) as an off-white solid: TOF-MS [ES⁺; m/z] 287.9331/287.9330.

Preparation 3

5 7-(4-(Methoxy-phenyl)-isoquinoline-5-sulfonic acid.

Treat a solution of 7-bromo-isoquinoline-5-sulfonic acid (150.00 g, 0.520 mol) and 4-methoxyphenylboronic acid (90.87 g, 0.598 mol) in DMF (1,400 mL) and MeOH (375 mL) with 2M aqueous Na₂CO₃ (652 mL). Deoxygenate the resulting slurry 3X with N₂, then add Pd(OAc)₂ (2.33 g, 0.0104 mol) and dppb (5.54 g, 0.0130 mol). Heat the
10 resulting reaction mixture at ~70 °C for 3 hr, then allow to cool to room temperature overnight. Dilute the reaction mixture with H₂O (4,000 mL) and adjust the pH to ~pH 2 with 5N aqueous HCl. Allow the resulting slurry to stir at room temperature for 30 min, then recover the brown solid by vacuum filtration, washing with H₂O and drying under vacuum filtration. Dissolve the brown solid in DMF (1,000 mL) and 2M aqueous
15 Na₂CO₃ (650 mL) and filter the resulting solution through a pad of Celite®, washing with DMF (400 mL)/H₂O (400 mL). Treat the filtrate with 5N aqueous HCl to adjust the pH to ~pH 2. Allow the resulting slurry to stir at room temperature for 30 min, then recover the solid by vacuum filtration, washing with H₂O and drying under vacuum filtration overnight. Dissolve the solid in DMF (1,000 mL) and 2M aqueous Na₂CO₃
20 (1,000 mL) again and treat the resulting solution with Celite® to create a slurry. Stir the resulting slurry at room temperature for 30 min, then filter through a pad of Celite®, washing with H₂O. Treat the filtrate with 5N aqueous HCl to adjust the pH to ~pH 2. Stir the resulting slurry at room temperature for 30 min, then recover the solid by vacuum filtration, washing with H₂O and drying under vacuum filtration overnight. Crush the
25 solid, then wash with EtOAc, drying under vacuum filtration overnight, followed by drying in a drying oven under reduced pressure at ~35 °C to afford the title compound (136.79 g, 83% yield) as a yellow solid: TOF-MS [ES⁺; m/z] 316.0624/316.0643. Anal. Calcd. For C₁₆H₁₃NO₄S: C 60.94; H 4.15; N 4.44; S 10.16. Found C 60.76; H 4.13; N 4.50; S 9.90.

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Preparation 4

7-(4-Methoxy-phenyl)-isoquinoline-5-sulfonyl chloride

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To a slurry of 7-(4-methoxy-phenyl)-isoquinoline-5-sulfonic acid (12.13 g, 38.5 mmol) in 1,2-dichloroethane (200 mL) and DMF (2.99 mL, 38.5 mmol) add oxalyl chloride (26.8 mL, 308 mmol) dropwise. Mechanically stir the slurry under nitrogen atmosphere while heating at 60-65 °C for 4 hr. Cool the slurry to -10 °C. Wait for 30 min then filter. Wash the yellow solid with 20% ether/dichloromethane and dry under a nitrogen atmosphere affording the title compound (14.8 g) as a yellow powder.

Preparation 5

7-(4-Methoxy-phenyl)-isoquinoline-5-thiol sodium salt

10 A. To a slurry of 7-(4-methoxy-phenyl)-isoquinoline-5-sulfonyl chloride (4.0 g, 12 mmol) in dioxane (40 mL) add tricarboxy ethyl phosphine hydrochloride (13.73 g, 48 mmol) and water (10 mL). Heat the mixture to 100 °C and stir for 3 hr. Cool the mixture in an ice bath, and add NaOH (5 N, 60 mL) slowly via a pipette. Filter and air dry the pale yellow precipitate to afford the title compound (3.2 g, 95%). Mass spectrum (LCMS) m/z = 266.2 ($M-Na^+$).

Alternatively, the title compound can be made as follows:

B. (i) Deoxygenate a solution of 7-(4-methoxy-phenyl)-isoquinoline-5-sulfonic acid (100.00 g, 0.317 mol) in anhydrous toluene (2,500 mL) 3X with N_2 , then treat with Ph_3P (332.58 g, 1.268 mol), I_2 (80.46 g, 0.317 mol), and Bu_3N (152.00 mL, 0.638 mol).
20 Allow the resulting reaction mixture to reflux for 1 hour under N_2 , then cool to room temperature overnight while bubbling air into the reaction mixture. Treat the reaction mixture with an aqueous solution of 1N NaOH (500 mL), then stir the resulting reaction mixture at room temperature overnight while bubbling air into the reaction mixture. Recover the resulting tan precipitate by vacuum filtration. Wash with a 1/1 solution of
25 THF/ Et_2O , then dry under vacuum filtration to afford bis-(7-(4-methoxy-phenyl)-isoquinoline-5)-disulfide (72.50 g, 86% yield) as a light tan solid: 1H NMR (400 MHz, DMSO) δ 9.39 (s, 2H), 8.51 (d, 2H), 8.41 (s, 2H), 8.11 (d, 2H), 8.00 (d, 2H), 7.53 (d, 4H), 6.94 (d, 4H), 3.78 (s, 6H).

B. (ii.) Treat a slurry of bis-(7-(4-methoxy-phenyl)-isoquinoline-5)-disulfide in
30 anhydrous THF (850 mL) with $NaBH_4$ (2.84 g, 75.07 mmol). Heat the resulting reaction mixture at ~35 °C under N_2 for ~1 hour, then heat at ~45 °C under N_2 for 1 hour.

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Preparation 6

7-(4-Methoxy-phenyl)-isoquinoline-5-sodium sulfinate

To 7-(4-methoxy-phenyl)-isoquinoline-5-sulfonyl chloride (1.48 g, 4 mmol) in water (10 mL), add Na_2SO_3 (1.01 g, 8 mmol) and NaHCO_3 (1.01 g, 12 mmol). Heat the mixture to 100 °C and stir for 1 hr. Cool the reaction mixture to room temperature, and remove water under reduced pressure. Add methanol (40 mL) to the residue, stir for 10 min. Filter the white solid, wash with methanol, and combine filtrates. Concentrate to give the title compound (1.1 g).

10

Preparation 7

(3,5-Dichloro-benzylidene)-(2,2-diethoxy-ethyl)-amine

The 2,2-Diethoxy-ethylamine (1852.5 g; 1.00 equiv; 13.63 moles), 3,5-dichloro-benzaldehyde (2453 g; 1.00 equiv; 13.60 moles), and Toluene (12 L) is charged to a 22 L flask equipped with a Dean Stark trap, condenser, nitrogen inlet, overhead stirring, and thermocouple. The light yellow reaction is warmed to reflux. Solvent begins distilling at 88°C. A total of ~ 650mL distillate (~ 240mL of water) is collected. The temperature is increased to 114°C during the distillation. NMR after 2 hr at reflux shows the product. The heat is shut off after 2.5 hr. The solution is gravity filtered into a carboy through fluted filter paper to remove a few particulates (including a small section of glass tubing, which is most likely from the 3,5-dichloro-benzaldehyde). The filtered solution is concentrated using Buchi flask with the water bath set at 45°C. Once the solvent stops coming over, the temperature is increased to 70°C with full vacuum and held for ~1.5 hr to remove any residual toluene. The weight of (3,5-Dichloro-benzylidene)-(2,2-diethoxy-ethyl)-amine is 4059.3 g (102.9% of theory). Mass Spectrum (LCMS) $m/z = 291.2$ (M+H⁺)

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Preparation 8

5,7-Dichloro-isoquinoline hydrochloride

Triflic acid (2.97 L; 33.52 moles; 5.03 kg) is charged to a 12 L flask equipped with a Dean Stark trap, overhead stirring, condenser, nitrogen inlet, 3 L addition funnel

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(buffered from the flask via a condenser), and thermocouple. The triflic acid is heated to 120°C. (3,5-Dichloro-benzylidene)-(2,2-diethoxy-ethyl)-amine (1350.5 g; 1.00 equiv; 4.65 moles) is diluted with dichloromethane (1350 mL) and is charged to the addition funnel. The addition is started with the temperature at 119°C. The addition is completed
5 over 90 minutes holding the temperature at 120°C. Approximately 1500 mL of distillate is collected during the addition. Area % HPLC 45 minutes after the addition is completed and indicates 94.14% product and 4.86% (3,5-Dichloro-benzylidene)-(2,2-diethoxy-ethyl)-amine. The heat is shut off after 1.5 hr. The reaction is cooled to ~80°C. At this point the flask is placed in an ice water bath and is cooled further. Add methanol (2.7 L)
10 with the reaction at 9°C. The addition is completed over 60 minutes. The maximum temperature is 27°C. Some solid forms during the methanol addition. This slurry is transferred in portions to a 2 L addition funnel and is added to a solution of ammonium hydroxide (5.1 L; 36.67 moles; 4.59 kg) and water (5.1 L) in a 22 L flask, chilled in ice water to <2°C. The addition funnel has a Teflon® tube extension to prevent the material
15 from running down the side of the flask, which can lead to a more oily solid adhering to the flask wall. The material is added in small slugs to prevent the addition funnel from plugging due to the solids. The addition is completed over 35 minutes including a methanol (900 mL) rinse of the flask and addition funnel. The maximum temperature is 26°C. A brown solid forms. The slurry is cooled to 14°C. The solid is filtered onto a
20 polypropylene pad. The solid is washed with 2 x 4 L and 1 x 2 L of water. The solid is then washed with 2 L of heptane to aid drying. The solid is dried *in vacuo* at 50°C to a weight of 999.0 g (108.4% of theory). The 5,7-dichloro-isoquinoline (997 g; 1.00 equiv; 5.03 moles) and ethanol (14.97 L) are charged to a 22 L flask equipped with an addition funnel, nitrogen inlet, overhead stirring, and thermocouple. The brown slurry is stirred at
25 ambient temperature (22°C). The acetyl chloride (1180 mL; 16.58 moles; 1.30 kg) is charged to the addition funnel. This is added to the flask dropwise. The brown slurry darkens. The solid appears to mostly dissolve by adding 100-200 mL of the acetyl chloride. A complete solution does not form. Solid reappears as the addition continues. The addition is completed over 45 minutes. The maximum temperature is
30 43°C, which is reached about halfway into the addition and is maintained with cool water in the bath. A sample of the reaction is removed after ~3/4 of the acetyl chloride is added for NMR. Since the sample appears to be the salt, excess acetyl chloride is likely not

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needed. The slurry is stirred and is allowed to cool. After 40 minutes, the temperature is 30°C. The slurry is cooled to <2°C and is held for 1.5 hr in an ice-water bath. The slurry is filtered. The solid is washed with 2 x 1.3 L of chilled ethanol. The wet cake weight is 811.4 g. The solid is dried in vacuo at 50°C. The dry weight is 648.4 g. This represents a 61.0% yield. Mass Spectrum (LCMS) m/z = 199.05) (M+H⁺)

Preparation 9

[2-(7-Chloro-isoquinolin-5-ylsulfanyl)-ethyl]-carbamic acid tert-butyl ester

Potassium carbonate (1952 g; 14.12 moles) is charged to a 22 L flask equipped with overhead stirring, addition funnel, nitrogen inlet, and thermocouple. Dimethylformamide (4 L) is added. The 5,7-dichloro-isoquinoline hydrochloride (646.1 g; 1.00 equiv; 2.76 moles) is added in portions and along with DMF (330 mL). The Boc-Cysteamine (514 g; 1.05 equiv; 2.90 moles) is dissolved in dimethylformamide (1520 mL) and is charged to the addition funnel. The mixture is warmed to 60°C. Nitrogen is purged through the head space for 15 minutes while the mixture is warming. The Boc-Cysteamine solution is added over 2 hrs and 15 minutes at 60°C. HPLC 1 hour and 20 minutes after the addition indicates 28.1% starting material, 67.26% product, 4.22% isomer, and 0.25% bis. The reaction is warmed to 75°C. The HPLC data in Table A is collected at 75°C.

Time at 75°C	% 5,7-dichloro-isoquinoline HCl	% [2-(7-Chloro-isoquinolin-5-ylsulfanyl)-ethyl]-carbamic acid tert-butyl ester	% isomer	% bis substituted
30 minutes	2.75	90.27	5.54	1.44

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1 hour	0.93	91.39	5.52	2.16
1.5 hours	0.20	91.25	5.32	3.23

Table A. Reaction HPLC Data for the Preparation of [2-(7-chloro-isoquinolin-5-ylsulfanyl)-ethyl]-carbamic acid tert-butyl ester

After 1.5 hours the mantle is replaced with a cooling bath. The reaction is cooled
 5 to 20-25°C. The solid is filtered and is washed with DMF (2 x 810 mL). The DMF
 solution is diluted with MTBE (5.15 L) and 5% LiCl (5.15 L). After stirring in a 50 L
 bottom outlet flask, the layers are separated. The aqueous layer is extracted with MTBE
 (2.9 L). The MTBE layers are combined and washed with 5% LiCl (2 x 2.9 L). The
 MTBE layer is gravity filtered through fluted paper and is concentrated using Buchi flask
 10 with the water bath set at 35°C to give 892.7 g (95.6% of theory, uncorrected) of [2-(7-
 chloro-isoquinolin-5-ylsulfanyl)-ethyl]-carbamic acid tert-butyl ester. Mass Spectrum
 (LCMS) $m/z = 339.85$ ($M+H^+$).

Preparation 10

15 [2-(7-Chloro-isoquinoline-5-sulfonyl)-ethyl]-carbamic acid tert-butyl ester

The [2-(7-Chloro-isoquinolin-5-ylsulfanyl)-ethyl]-carbamic acid tert-butyl ester
 (700 g; 1.00 equiv; 2.07 moles) is dissolved in isopropyl alcohol (10.53 L) by rotating on
 Buchi flask with the water bath set at 50°C. Mequinol (12.29 g; 98.01 mmols; 12.29 g),
 sodium tungstate dihydrate (31.4 g; 95.20 mmols), the [2-(7-Chloro-isoquinolin-5-
 20 ylsulfanyl)-ethyl]-carbamic acid tert-butyl ester solution, isopropyl alcohol (2.77 L),
 water (3.38 L), and acetic acid (123 mL; 2.15 moles; 128.90 g) are charged to a 22 L flask
 equipped with a condenser, nitrogen inlet, overhead stirring, addition funnel, and
 thermocouple. Addition of the water causes the brown solution to become slightly milky
 looking. The reaction is warmed to 50°C using a hot water bath. Hydrogen peroxide
 25 (607 mL; 5.94 moles; 673.77 g) is added over 1 hour. During the addition, the
 temperature is maintained at 50-55°C by the hydrogen peroxide addition rate and by
 adding cool or warm water to the bath as needed. The reaction is still milky brown. The
 reaction temperature is 54°C at the end of the addition and is maintained at 53-57°C using

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the warm water bath. HPLC 10 minutes after the addition indicates 24.69% peak at 2.89 min., 65.57% product, 5.03% isomer, 4.33% bis, and 0.18% peak at 3.05 min. HPLC 2 hours after the addition indicates 0.06% sulfoxide, 1.27% peak at 3.046 min., 88.10% product, 4.61% isomer, and 5.79% bis. The bath is drained and replaced with ice water to cool the reaction. When the reaction temperature reaches 27°C, the peroxide is quenched by adding 9% sodium bisulfite solution (250 mL). No increase in temperature is observed. Peroxide test strips and starch iodide paper indicate no peroxide remaining. Water (5.64 L) is added over 15-20 minutes. The temperature increases from 18 to 24°C during the addition. The mixture is stirred at ambient temperature overnight. The slurry is cooled in an ice water bath to <5°C and held. Quantitative HPLC of the supernate over time indicated the crystallization is complete after 1.5 hours at <5°C. The solid is filtered and washed with water (2 x 2 L). The wet cake weight was 831 g. The solid is dried in vacuo at 50°C. The weight of dry [2-(7-Chloro-isoquinoline-5-sulfonyl)-ethyl]-carbamic acid tert-butyl ester is 531 g (69.31% of theory, uncorrected). Mass Spectrum (LCMS) $m/z = 371.85$ ($M+H^+$)

Preparation 11

(2-{7-[4-(Tetrahydro-pyran-2-yloxy)-phenyl]-isoquinoline-5-sulfonyl}-ethyl)-carbamic acid tert-butyl ester

The 60 L reactor is charged with PEPPSI™ (Pyridine-Enhanced Precatalyst Preparation Stabilization and Initiation; 51.0 g; 74.9 mmol), [2-(7-Chloro-isoquinoline-5-sulfonyl)-ethyl]-carbamic acid tert-butyl ester (1.383 kg; 3.729 moles), phenyl boronic acid (895 g; 4.03 moles), K_2CO_3 (1.039 kg; 7.518 moles), and ethanol (15 L) under a nitrogen purge. Initially 12 L of ethanol is added to the reactor and the remaining 3 L was used to rinse in the other charges. The reaction is warmed to reflux (78°C). HPLC indicates a complete reaction after ½ hour. The reaction is cooled to 59°C using the Huber® circulator. Water (8.7 L) is added to the reaction over 18 minutes. The temperature is decreased from 59°C to 42°C during the water addition. The reaction is cooled using the Huber® circulator from 42°C to 0°C over 1 hour. The slurry is held at 0°C for 1 hour. The solid is filtered using a polypropylene pad and washed with 1:1 ethanol:water (3.7 L; chilled). The solid is dried on the filter overnight. The solid is then dried in vacuo at 60°C for 22 hours. The dry weight of the solid is 2.171 kg. The Pd

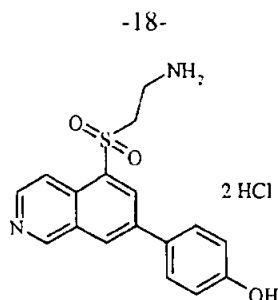
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level in the crude (2-{7-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-isoquinoline-5-sulfonyl}-ethyl)-carbamic acid tert-butyl ester is 2196 mcg/g and the solid contains 6.9% water by KF. A 50 L bottom outlet flask is charged with dichloromethane (4 L). The crude (2-{7-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-isoquinoline-5-sulfonyl}-ethyl)-carbamic acid tert-butyl ester is charged to the 50 L flask and rinsed in with dichloromethane (1 L). Na₂SO₄ (1.9 kg) is charged and rinsed in with dichloromethane (0.1 L). Darco G-60® (3.8 kg) is charged to a 20 L Büchi flask and slurried in dichloromethane (12 L). This slurry is transferred to the 50 L flask and rinsed in with dichloromethane (4.9 L). The mixture is stirred for 2 hrs at ambient temperature. A 50 cm stainless steel filter is fitted with a polypropylene pad and charged with Hyflo Super Cel® (1.7 kg). The Hyflo Super Cel® pad is rinsed with dichloromethane (2 L). The contents of the 50 L flask are filtered onto the Hyflo Super Cel® pad. The flask and filter cake are rinsed with dichloromethane (22 L). The filtrate is charged to the 60 L reactor through a 0.45 micron cartridge filter. The contents of the reactor are warmed and concentrated by distillation under slight vacuum (~640 mm Hg). Approximately 33 L of distillate is collected over 2 hrs at 35-37°C. Ethanol (10 L) is added. The vacuum is adjusted slowly down to 280 mm Hg while continuing the distillation. Once the vacuum is reached, the temperature is increased to maintain the distillation. Once the volume in the reactor reaches ~10 L, ethanol (6.5 L) is added at such a rate to maintain the volume in the reactor while distilling out solvent. The distillation is stopped once all the ethanol is added. The final volume is ~10 L. The temperature at the end of the distillation is 56°C. The slurry that forms during the solvent exchange is cooled to 20°C and held overnight. The slurry is filtered. The solid is washed with 1:1 ethanol:water (1.8 L). The wet cake weight is 1.878 kg. The solid is dried in vacuo at 50°C to give 1.2306 kg (64.36% yield) (2-{7-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-isoquinoline-5-sulfonyl}-ethyl)-carbamic acid tert-butyl ester. Mass Spectrum (LCMS) m/z = 513.62) (M+H).

EXAMPLES

Example 1

4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride salt



A. {2-[7-(4-Methoxy-phenyl)-isoquinolin-5-ylsulfany]-ethyl}-carbamic acid tert-butyl ester

- To 7-(4-methoxy-phenyl)-isoquinoline-5-thiol sodium salt (0.29 g, 1 mmol) in acetone (10 mL), add (2-bromo-ethyl)-carbamic acid tert-butyl ester (0.22 g, 1 mmol) and potassium carbonate (0.14 g, 1 mmol). Stir the mixture at room temperature for 4 hr then load onto a silica gel column, and elute with 65% ethyl acetate in hexane to afford the title compound (0.35 g, 86%). Mass spectrum (LCMS) $m/z = 355.2$ ($M+H^+$).

10 B. {2-[7-(4-Methoxy-phenyl)-isoquinoline-5-sulfonyl]-ethyl}-carbamic acid tert-butyl ester

- To a solution of {2-[7-(4-methoxy-phenyl)-isoquinolin-5-ylsulfany]-ethyl}-carbamic acid tert-butyl ester (0.35 g, 0.85 mmol) in acetic acid (8 mL), add water (2 mL). Cool the mixture to 0 °C in an ice bath. Add a solution of $KMnO_4$ (0.27 g, 1.7 mmol) in water (2 mL) dropwise over a period of 5 min. After the addition, stir the reaction mixture at 0 °C for 30 min. Add H_2O_2 (30% in water) dropwise until the brown color disappears. Partition the mixture between ethyl acetate (50 mL) and saturated sodium bicarbonate (40 mL). Separate the organic layer, dry over sodium sulfate; filter and concentrate. Chromatograph the residue on a silica gel column with 66% ethyl acetate in hexane to afford the title compound (0.245 g) as white solid. Mass spectrum (LCMS) $m/z = 433.2$ ($M+H^+$).

C. 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride

- To {2-[7-(4-methoxy-phenyl)-isoquinoline-5-sulfonyl]-ethyl}-carbamic acid tert-butyl ester (0.22 g, 0.51 mmol) in dichloroethane (10 mL), add BBr_3 (1.0 M in dichloromethane, 3.0 mL) at -20 °C. After the addition, stir the reaction mixture at -20 °C for 2 hr, slowly warm to room temperature and stir at room temperature for 2 hr. Add

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methanol (5 mL) to quench excess BBr_3 . Evaporate the solvent under reduced pressure, and purify the residue via Reverse Phase HPLC (0-100% acetonitrile:water with 0.01% TFA) to afford 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol as the TFA salt (0.18 g). Then, convert 0.12 g of the TFA salt to the di-hydrochloride salt by adding 2 mL of saturated aqueous sodium bicarbonate to the TFA salt and stir overnight in order to convert the TFA salt to the free base. Filter the free base. Wash the free base, first with water, then with diethyl ether. Dry the solid. Then, suspend the solid in MeOH. Add 0.2 mL of concentrated aqueous HCl and stir for 1 hr. Concentrate the solvent under reduced pressure to provide the dihydrochloride salt. (0.10 g). Mass spectrum (LCMS) $m/z=329.0$ ($\text{M}+\text{H}^+$).

Example 2

4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol

To {2-[7-(4-methoxy-phenyl)-isoquinoline-5-sulfonyl]-ethyl}-carbamic acid tert-butyl ester (25.00 g, 56.49 mmol) in dichloromethane (1 L) at 0°C add BBr_3 (1.0 M in dichloromethane, 200 mL, 200 mmol) over 40 min with vigorous overhead stirring. Remove the cooling bath and stir at room temperature overnight. Cool to 0°C and add methanol (500 mL) dropwise over 1 h. Filter the resulting slurry, rinse the flask with dichloromethane (100 mL) and wash the solids with the rinse. Wash the solids with additional dichloromethane (150 mL) and dry under vacuum filtration. Slurry the solids in methanol (400 mL) with stirring, then concentrate under vacuum to a thin paste. Add additional methanol (400 mL) and further slurry the solids with stirring. Concentrate the slurry under vacuum to dryness, and dry the solids overnight under vacuum. Pulverize the solids to a powder and slurry in methanol (1 L). Add Amberlyst® A-21 resin (75 g) and stir until the powder is dissolved. Filter the resin, slurry the filtered resin in methanol (220 mL) and filter the resin again, combining the filtrates. Wash the filtered resin with methanol (100 mL) and add this wash to the filtrates. Combine the filtrates with ethanol (200 mL) and concentrate the mixture under vacuum to a volume of about 200 mL to afford a slurry. Add ethanol (400 mL), warm the slurry to 50°C for about 15 min, then concentrate under vacuum to a volume of about 200 mL. Cool the slurry to room temperature, filter and rinse the filter cake with ethanol (2 x 15 mL), then dry under

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vacuum to afford the title compound (10.22 g, 55%) as a tan solid. Mass spectrum (LCMS) $m/z=329.0$ ($M+H^+$).

Example 3

5 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol
Cool a slurry of 2-[7-(4-methoxy-phenyl)-isoquinoline-5-sulfonyl]-ethylamine dihydrochloride (9.5504 g, 22.99 mmol) in anhydrous CH_2Cl_2 (400 mL) to $\sim 5^\circ C$ with an ice/ H_2O bath, then treat the cold slurry with a dropwise addition of a 1.0 M solution of BBr_3 in CH_2Cl_2 (92.00 mL, 92.00 mmol). Upon complete addition of the BBr_3 , remove
10 the cold bath and allow the resulting reaction mixture to stir under N_2 at room temperature overnight. Quench the resulting reaction mixture with MeOH, then concentrate *in vacuo* to give a residue. Dissolve the resulting residue in MeOH (200 mL) and concentrate *in vacuo* to give a residue. Repeat the above 3 times, then slurry the residue in Et_2O (300 mL) and treat with saturated aqueous $NaHCO_3$ until $\sim pH7$ is
15 reached. Recover the resulting solid by vacuum filtration, then dissolve in a 3/1 solution of $CHCl_3/MeOH$. Saturate the filtrate with NaCl, then extract with a 3/1 solution of $CHCl_3/MeOH$ (2 X 400 mL each). Dry the combined organics over $MgSO_4$, filter, then concentrate *in vacuo* to give a solid/residue. Slurry the solid/residue in a small amount of MeOH (20 mL) and excess hexanes, then recover the resulting solid by vacuum filtration,
20 washing with hexanes and drying under vacuum filtration to give (7.60 g) of a light-yellow solid. Slurry the light-yellow solid (7.60 g) in anhydrous CH_2Cl_2 (400 mL) and cool to $\sim 5^\circ C$ with an ice/ H_2O bath, then treat with a dropwise addition of a solution of 1.0 M BBr_3 in CH_2Cl_2 (70.00 mL, 70.00 mmol). Upon complete addition of the BBr_3 , remove the cold bath and allow the resulting reaction mixture to stir at room temperature
25 under N_2 for 4 hours. Add an additional amount of 1.0 M BBr_3 in CH_2Cl_2 (70.00 mL, 70.00 mmol) dropwise to the reaction mixture, then allow the resulting reaction mixture to stir at room temperature under N_2 overnight. Heat the reaction at $\sim 40^\circ C$ for ~ 1 hour, then treat with a dropwise addition of additional 1.0 M BBr_3 in CH_2Cl_2 (23.00 mL, 23.00 mmol). Heat the resulting reaction mixture at $\sim 40^\circ C$ for ~ 2 hours, then quench with
30 MeOH and concentrate *in vacuo* to give a residue. Dissolve the resulting residue in MeOH (200 mL) and concentrate *in vacuo* to give a yellow residue. Repeat the above 3 times. Treat the yellow residue with saturated $NaHCO_3$ to adjust the pH to ~ 7 , then

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recover the resulting solid by vacuum filtration. Pre-absorb the solid on silica gel, then semi-purify by column chromatography (ISCO 330 g silica gel, neat CH_2Cl_2 to 5% of 2N NH_4/MeOH in CH_2Cl_2 to 10% of 2N NH_4/MeOH in CH_2Cl_2) to give a solid. Pre-absorb the solid on silica gel, then re-purify by column chromatography (ISCO 330 g silica gel, neat CH_2Cl_2 to 5% of 2N NH_4/MeOH in CH_2Cl_2 to 10% of 2N NH_4/MeOH in CH_2Cl_2) to give a solid. Pre-absorb the solid on silica gel, then re-purify by column chromatography (ISCO 330 g silica gel, neat CH_2Cl_2 to 5% of 2N NH_4/MeOH in CH_2Cl_2 to 10% of 2N NH_4/MeOH in CH_2Cl_2 to 15% of 2N NH_4/MeOH in CH_2Cl_2) to give a solid. Pre-absorb the solid on silica gel, then re-purify by column chromatography (ISCO 330 g silica gel, 5% MeOH in CHCl_3 to 10% MeOH in CHCl_3 to 20% MeOH in CHCl_3 to 30% MeOH in CHCl_3) to give (6.85 g, 90.8% yield) of the title compound as a yellow solid: MS (ES^+ , m/z): 329.0 ($\text{M}+1$).

Example 4

4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride, unknown hydrate state

Place 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol (171.8 mg) in a vial along with 15 mL of 95% EtOH. Place vial on hot plate set to 80 °C while stirring the solution to dissolve the compound. Since little to no dissolution occurs, add one molar equivalent of HCl (0.52 mL of 1N HCl in water) to dissolve the solid and result in a bright yellow solution. Less than a minute after the HCl addition, solid formation begins and increases with time. After ~ 2 hours at 80 °C, remove the sample from the heat and allow it to stir overnight at RT. Collect solid using vacuum filtration through filter paper. After air drying overnight, recover 107.8 mg of the title compound (63% yield). X-ray powder diffraction: angle, 2θ (% intensity): 11.8 (100.0); 16.6 (52.9); 8.2 (43.4).

Example 5

4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate

A. Preparation of seed material. To a slurry of 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol (0.2509 g, 0.764 mmol) in ethanol (5 mL) add aqueous HCl (1 M, 0.765 mL, 0.765 mmol). Heat the resulting mixture to reflux overnight. Cool to room

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temperature and vacuum filter the slurry. Rinse the solids with ethanol, and dry under vacuum filtration to afford 0.1842 g of a yellow solid.

B. 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate To a slurry of 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol (1.75 g, 5.33 mmol) in ethanol (35 mL) add aqueous HCl (1 M, 5.33 mL, 5.33 mmol). Heat the mixture to reflux and add water (2.5 mL) to obtain a solution. Cool the solution to 60 °C and seed with about 2 mg of the yellow solid obtained from A. above to obtain a slurry. Cool to room temperature and stir overnight. Vacuum filter the slurry and rinse the filter cake with ethanol, then dry under vacuum filtration to afford 1.331 g (67%) of the title compound as a yellow solid. Mass spectrum (LCMS) $m/z=329.0$ ($M+H^+$). Karl Fischer: 3.00%. X-ray powder diffraction: angle, 2 θ (% intensity): 4.9 (47.3); 14.8 (55.8); 10.2 (45.5).

Example 6

4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride salt
Slurry 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol (16.57 g, 50.45 mmol) in methanol (500 mL). Separately, prepare a solution of HCl in methanol by adding acetyl chloride (12.60 mL, 177.05 mmol) to methanol (165 mL). Add the solution of HCl in methanol dropwise at room temperature over 30 min to the slurry of 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol in methanol. Stir for 30 min, then add ethyl acetate (600 mL) dropwise over 1 hour. Stir for 30 min, then filter the solids and wash with ethyl acetate (2 x 100 mL). Dry solids *in vacuo* at 50 °C, then further dry *in vacuo* at room temperature with slow administration of nitrogen to afford the title compound (18.3 g, 90%). Mass spectrum (LCMS) $m/z=329.0$ ($M+H^+$).

Example 7

4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride salt
The 60 L reactor is charged with ethanol (24 L) and (2-{7-[4-(Tetrahydro-pyran-2-yloxy)-phenyl]-isoquinoline-5-sulfonyl}-ethyl)-carbamic acid tert-butyl ester (2.652 kg; 5.173 moles). The (2-{7-[4-(Tetrahydro-pyran-2-yloxy)-phenyl]-isoquinoline-5-sulfonyl}-

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ethyl)-carbamic acid tert-butyl ester is rinsed in with ethanol (2.6 L). Hydrochloric acid solution (31.9%; 1780g; 15.6 moles) is diluted with water (1.42 L). This HCl solution is charged to the reactor and rinsed in with water (0.2 L). The reaction is warmed to 70°C. A yellow solution forms around 30°C and a yellow slurry reforms near 70°C. The reaction is held at 70°C for ½ hr and then is warmed up to reflux (78°C). HPLC indicates a complete reaction after 1.5 hrs at reflux. Water (14.5 L) is added to the reaction over 13 minutes. The temperature drops to 69°C. The reaction is heated back to reflux (80°C) and a solution forms. The reaction is cooled to 68°C and solid begins forming. The reaction is held at 68°C for ½ hr and then cools to 2°C over 1.5 hrs. The slurry is held at 1-2°C for 1 hr. The solid is filtered and washed with ethanol (4.6 L). The wet cake weight is 3.236 kg. The solid is dried in vacuo at 50°C to give 2.106 kg (101.4% of theory) 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride salt. Mass Spectrum (LCMS) $m/z = 402.31$ (M+H).

15

Example 8

4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate
The ethanol and sodium hydroxide solutions used for this example are filtered through a 0.22 micron cartridge filter. The water used is purified water with endotoxin control.

20

Ethanol (8 L) and 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride salt (841.6 g; 2.097 moles) are charged to a 22 L flask equipped with overhead stirring, condenser, nitrogen inlet, heating mantle, thermocouple, and addition funnel. The 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol di-hydrochloride salt is rinsed in with ethanol (3.28 L). The reaction is warmed to 55°C. Sodium hydroxide solution (1100 mL; 1.91 N; 2.10 moles) is added to the reaction over 7 minutes during which time the temperature increases to 62°C. The reaction is heated to reflux (78°C) and held for 1 hr. Water (1.96 L) is added to the reaction at once. The temperature decreases to 68°C. The reaction is heated back to reflux (79°C) and a solution forms. Darco G-60® (438 g) is added to the reaction. The reaction is held at reflux for 1.25 hours. The hot Darco G-60® slurry is filtered through GFF paper into another 22 L flask. A light yellow solution results. The original flask and Darco G-60® filter cake are rinsed with hot ethanol (2.5 L). During the rinse, solid begins to form in the filtrate. The 22 L flask

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containing the filtrate and wash is equipped with overhead stirring, nitrogen inlet, and a thermocouple. The slurry is stirred and allowed to cool to ambient temperature. The solid is filtered and washed with ethanol (2 L). The wet cake weight is 672 g. The solid is dried in vacuo at 50°C to give 540 g (68.9% yield) of 4-[5-(2-amino-ethanesulfonyl)-
5 isoquinolin-7-yl]-phenol monohydrochloride hemihydrate. Mass Spectrum (LCMS) m/z = 329.3) ($M+H^+$).

The exemplified compounds are inhibitors of Akt activity. The inhibitory activity of these compounds may be demonstrated by the methods below.

10

Akt1 Phosphorylation Assay

The assay measures the phosphorylation of PKC alpha pseudo-substrate peptide by a Competitive Fluorescence Polarization Immunoassay (FPIA). The assay indirectly measures the concentration of phospho-peptide product formed in a reaction by
15 measuring the change in the intensity of polarized light emitted from a fluorescently-labeled tracer peptide when displaced from a phospho-specific antibody by the phospho-peptide product. Calibration with a standard curve yields quantitative results.

Enzyme and Substrate

20 Active human recombinant Akt1 (full-length) purified from Sf9 insect cells is obtained from Upstate Biotechnology, Inc. Peptide Substrate (M.W.1561) is purchased.

Standard Assay Solutions

Solution (A): 20% DMSO (dimethylsulphoxide) or Compound in 20% DMSO or 500 mM EDTA; Solution (B): Assay Buffer Mix: 75 μ M Peptide Substrate, 38 mM
25 $MgCl_2$, 70 mM HEPES, pH 7.4, 0.01% Triton X-100®, and 50 μ M ATP (adenosine triphosphate); Solution (C): Akt Kinase Mix: 70 mM HEPES, pH 7.4, 1 mM DTT (dithiothreitol), 0.01% Triton-X-100®, and Akt1 enzyme.

Procedure for FPIA

Five μ L of Solution (A) are first mixed with 10 μ L Solution (B). The enzymatic
30 reaction is initiated by adding 10 μ L Solution (C) to the mixture. (Final concentration or amount in a 25 μ L reaction mix: 4% DMSO (minimal inhibited wells) or various

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compound concentration in 4% DMSO or 100mM EDTA (maximal inhibited wells); 30 μ M Peptide Substrate; 15 mM $MgCl_2$; 70 mM HEPES, pH 7.4; 20 μ M ATP; 0.4 mM DTT; 0.01% Triton X-100®. The reactions are performed in black half-area flat-bottom 96-well microtiter plates.

- 5 After 60 min at room temperature the reaction is terminated by adding 25 μ L of Quench/Detection Mix containing 260 mM EDTA, 3.4 nM Fluorescein Tracer, and 7.5 nM Anti-Phosphoserine Antibody. The plates are incubated in darkness at room temperature for more than 3 hr, and then fluorescence polarization (at λ_{ex} =485 nm, λ_{em} =530 nm) is read using a Tecan Ultra plate reader. The concentration of
- 10 phosphorylated product per well is calculated from millipolarization (mP) units using a prepared peptide competitor dilution series as a standard curve. Exemplified compounds of the invention inhibit Akt1 phosphorylation with an IC_{50} of 1 μ M or less. The compound of Example 1 inhibits Akt1 phosphorylation in this assay with an IC_{50} of 45 +/- 17 nM (n = 3).

15

Cell-Based ELISA (cELISA) for the Detection of Phospho-GSK3b (pGSK3b):

A Target of Akt in Cells

- Exponentially grown U87MG cells derived from a human glioblastoma are seeded into 96-well plates and incubated overnight at 37°C with 5%CO₂. Ten concentrations of
- 20 the compound of Example 1 are prepared from a 4 mM stock (in 100% DMSO) by 1:2 serial dilutions into the culture media. Equal volume of each of the serial dilutions is added directly to the cells. Treatment is stopped 2 hr later. At the end of the treatment, the culture media is removed, and the cells are washed once with ice-cold phosphate-buffered saline (PBS). The cells are fixed in PREFER according to vendor's procedure,
- 25 followed by rinses in PBS/0.1% SDS and PBS/0.1% Triton X-100®, then overnight incubation in SEA Block Blocking buffer. The blocking step is followed by an overnight incubation of the cells with a rabbit antibody against pGSK3b/Ser9, followed by a goat anti-rabbit IgG. The pGSK3b is detected by SuperSignal ELISA Femto following the vendor's procedure. The compound of Example 1 inhibits pGSK3b in this assay with an
- 30 IC_{50} of 2.04 +/- 0.68 (n=8) μ M.

In Vivo Target Inhibition Studies

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In vivo target inhibition by a single IV injection:

Exponentially growing U87MG cells derived from a human glioblastoma are implanted subcutaneously in the rear flank of nude mice. When the tumors reach the size of 200-250 mm³, compounds are administered to the animals by a single IV injection in a dose-response study or in a time-course study. At the end of each treatment, animals are asphyxiated with CO₂. Tumors are harvested by surgical excision, quickly frozen in liquid nitrogen and stored at -80°C until analysis. Sera are prepared from blood harvested from the heart by cardiac puncture and stored at -80°C until analysis.

10 Sample Analysis:

The Akt inhibitor is extracted from serum with acetonitrile/methanol and analyzed alongside an internal standard by LC/MS/MS. Tumors are homogenized in 2 volumes of a lysis buffer containing 25 mM Tris (pH 7.5), Roche complete protease inhibitors, and 1 mM vanadate with Powergen 125 homogenizer, then sequentially passed thorough an 18 gauge needle and a 23 gauge needle. Soluble cytoplasmic extracts are collected from the supernatant fraction after the lysates are centrifuged for 30 minutes at 20,000 x g. Protein concentrations in the cytoplasmic extracts are determined with a BCA kit. Phospho-GSK3b (pGSK3b) in the soluble extracts is analyzed with the ELISA Kit.

For glioblastoma in this assay, the compound of Example 1 inhibits pGSK3b by 76% at 1 hour using single IV injection of 25 mg/kg.

In Vivo Tumor Growth Inhibition Studies

Exponentially growing U87MG cells derived from a human glioblastoma or H1155 cells derived from human non-small cell lung cancer are implanted subcutaneously in the rear flank of nude mice. Three days after tumor cell implants, microsurgery is performed to cannulate catheter for continuous infusion via jugular vein. Example 1, formulated in D5W (5% dextrose in water) at various concentrations, is then infused into mice next day at 40 µL/hr for 4 days. Tumors are measured twice weekly until the study is terminated, usually at 24 days after the initial implants. Tumor growth inhibition is calculated by dividing the average tumor volume of the treated group by that of the vehicle-treated group.

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For glioblastoma, the compound of Example 1 inhibits tumor growth by 50% using continuous infusion of 27 mg/kg/day when measured at the end of the assay.

For non-small cell lung cancer, the compound of Example 1 inhibits tumor growth by 63% using continuous infusion of 24 mg/kg/day when measured at the end of the assay.

Pharmaceutical Composition Studies

A pharmaceutical composition of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate comprises a non-reducing sugar and a pH adjusting agent. Solubility studies show that mannitol does not appear to impact the solubility of 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate. However, the hemihydrate's solubility is pH dependent and is > 19 mg/mL at $\text{pH} \leq 3.91$. Also, the rate at which precipitation takes place is pH dependent.

Table 1: Effect of initial solution pH on rate of precipitation (ppt). All solutions are prepared initially at approximately 15 mg 4-[5-(2-amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate /mL and stored at room temperature.

Media	pH (initial)	Precipitation (ppt)
0.05 N HCl/3% mannitol	1.68	no ppt up to 96 hours
0.01 N HCl/3% mannitol	3.19	no ppt up to 96 hours
0.005 N HCl/3% mannitol	3.58	ppt after 48 hr
0.0025 N HCl/3% mannitol	3.91	ppt after 36 hr
0.001 N HCl/3% mannitol	4.20	ppt after 24 hr

LC/MS analysis of the re-dissolved precipitate indicates that it is a "dimer-like" molecule (Mass spectrum (LCMS) $m/z = 640.2$ ($M+H^+$)) that forms from two 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol molecules (Mass spectrum (LCMS) $m/z = 329.1$ ($M+H^+$) for 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol) after the loss of ammonia. Additionally, precipitation does not appear to be due to a change to a less soluble solid state form.

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We claim:

1. A compound which is 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol
or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt
5 thereof.
2. The compound according to Claim 1 which is 4-[5-(2-Amino-ethanesulfonyl)-
isoquinolin-7-yl]-phenol di-hydrochloride.
- 10 3. The compound according to Claim 1 which is 4-[5-(2-Amino-ethanesulfonyl)-
isoquinolin-7-yl]-phenol monohydrochloride hemihydrate.
4. The compound according to any one of Claims 1 to 3 for use as a medicament.
- 15 5. Use of the compound according to any one of Claims 1 to 3 for the manufacture
of a medicament for treating multiple myeloma, non small cell lung cancer, glioblastoma,
neuroblastoma, melanoma, or neoplasms of the prostate, breast, ovaries, primary stomach,
intestinal-type, endometrium, thyroid, pancreas, lung, or bladder.
- 20 6. Use of the compound according to any one of Claims 1 to 3 for the manufacture
of a medicament for treating multiple myeloma, non small cell lung cancer, glioblastoma,
or neoplasms of the prostate, breast, or ovaries.
7. Use of the compound according to any one of Claims 1 to 3 for the manufacture
25 of a medicament for treating non small cell lung cancer or glioblastoma.
8. Use of the compound according to any one of Claims 1 to 3 for the manufacture
of a medicament for treating hepatitis C, rubella, human immunodeficiency virus (HIV),
hepatitis B, or human cytomegalovirus (HCMV).
- 30 9. A method of treating multiple myeloma, non small cell lung cancer,
glioblastoma, neuroblastoma, melanoma, or neoplasms of the prostate, breast, ovaries,

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primary stomach, intestinal-type, endometrium, thyroid, pancreas, lung, or bladder in a patient in need thereof comprising administering an effective amount of the compound of any one of Claims 1 to 3.

5 10. A method of treating multiple myeloma, non small cell lung cancer, glioblastoma, or neoplasms of the prostate, breast, or ovaries comprising in a patient in need thereof comprising administering an effective amount of the compound of any one of Claims 1 to 3.

10 11. A method of treating non small cell lung cancer or glioblastoma in a patient in need thereof comprising administering an effective amount of the compound of any one of Claims 1 to 3.

15 12. A method of treating hepatitis C, rubella, human immunodeficiency virus (HIV), hepatitis B, or human cytomegalovirus (HCMV) in a patient in need thereof comprising administering an effective amount of the compound of any one of Claims 1 to 3.

20 13. A pharmaceutical composition comprising the compound according to any one of Claims 1 to 3, and a pharmaceutically acceptable carrier, diluent or excipient.

25 14. A lyophilized pharmaceutical composition comprising the compound according to any one of Claims 1 to 3, and a pharmaceutically acceptable carrier, diluent or excipient wherein the pH of said composition when diluted with aqueous diluent is less than 4.2 and greater than 2.0.

15. The lyophilized pharmaceutical composition according to Claim 14 wherein the pH is less than 3.2 and greater than 2.0.

30 16. The lyophilized pharmaceutical composition according to Claim 15 wherein the pH is less than 2.8 and greater than 2.0.

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17. A pharmaceutical composition comprising the compound according to any one of Claims 1 to 3, and a pharmaceutically acceptable carrier, diluent or excipient in solution wherein the pH of said composition is less than 4.2 and greater than 2.0.

5 18. The pharmaceutical composition according to Claim 17 wherein the pH of said composition is less than 3.2 and greater than 2.0.

19. The pharmaceutical composition according to Claim 18 wherein the pH of said composition is less than 2.8 and greater than 2.0.

10

20. 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol monohydrochloride hemihydrate in crystalline form having an X-ray powder diffraction pattern with intense peaks at $2\theta = 4.9$, 14.8 , and 10.2 .

INHIBIDORES DE AKT (PROTEÍNA CINASA B)

CAMPO DE LA INVENCION

La presente invención proporciona compuestos que
5 son inhibidores de Akt, composiciones que comprenden estos
compuestos, y métodos para usar estos compuestos.

ANTECEDENTES DE LA INVENCION

Las proteínas cinasas están involucradas en las
10 trayectorias de transducción de señal que enlazan factores de
crecimiento, hormonas y otras moléculas de regulación celular
al crecimiento celular, supervivencia y metabolismo bajo
condiciones tanto patológicas como normales. Una de tales
proteínas cinasas, la proteína cinasa B (también conocida
15 como Akt), es una serina/treonina cinasa que juega un papel
central en la promoción de proliferación y supervivencia de
un amplio intervalo de tipos celulares, con ello, protegiendo
las células de apoptosis (muerte celular programada).

Un número de proteínas cinasas y fosfatasas regulan
20 la actividad de Akt. Por ejemplo, la activación de Akt es
mediada por 3-cinasa fosfatidilinositol (PI3-K), la cual
inicia el enlace de segundos fosfolípidos mensajeros al
dominio de enlace de homología de pleckstrinas (PH) de Akt.
El enlace ancla Akt a la membrana plasmática y resulta en la
25 fosforilación y activación de la enzima. Las amplificaciones

de la subunidad catalítica de la PI3-K, p110 α , o mutaciones en la subunidad regulatoria PI3-K, p85 α , conducen a la activación de Akt en varios tipos de cáncer humanos. Estudios recientes también han demostrado la función de la trayectoria
5 de PI3-K/AKT en el ciclo de vida de numerosos virus.

El documento WO 01/91754, pertenece a inhibidores de proteína cinasa. El documento WO 2005/011697, involucra inhibidores de proteína cinasa A y B. El documento WO 2005/054202, pertenece a inhibidores de AKT.

10 Debido a su función fundamental en la regulación de la supervivencia celular, la Akt proporciona un nuevo objetivo terapéutico para el tratamiento efectivo de varios trastornos, particularmente cáncer e infecciones virales. Sin embargo, tal tratamiento requiere el desarrollo de
15 inhibidores potentes, selectivos, biodisponibles, de Akt. Existe una necesidad de inhibidores de Akt alternativos. De este modo, la presente invención proporciona nuevos inhibidores de Akt que exhiben potencia, selectividad y/o biodisponibilidad incrementada, composiciones que comprenden
20 estos compuestos, y métodos para usar estos compuestos. Adicionalmente, las composiciones farmacéuticas que contienen nuevos inhibidores de Akt cuando se diluyen o están en solución, necesariamente deben estar libres de precipitación para ser suministradas a un paciente. La presente invención
25 proporciona composiciones farmacéuticas que comprenden nuevos

inhibidores de Akt a intervalos de pH particulares que carecen de tal precipitación.

SUMARIO DE LA INVENCION

5 La presente invención proporciona 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable, o un hidrato del compuesto o la sal del mismo.

 La presente invención también proporciona
10 diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

 La presente invención además, proporciona semihidrato monoclóridrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

15 La presente invención también proporciona 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato
20 monoclóridrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, para uso como un medicamento.

 Adicionalmente, la presente invención proporciona el uso de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable, o un hidrato
25 del compuesto o la sal del mismo, que incluye, diclorhidrato

de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclорhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, para la manufactura de un medicamento para tratar mieloma múltiple, cáncer de pulmón de
5 células no pequeñas, glioblastoma, neuroblastoma, melanoma o neoplasmas de la próstata, mama, ovarios, estómago primario, tipo intestinal, endometrio, tiroides, páncreas, pulmón o vejiga.

La presente invención también proporciona el uso de
10 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclорhidrato de 4-[5-(2-amino-etansulfonil)-
15 isoquinolin-7-il]-fenol, para la manufactura de un medicamento para tratar mieloma múltiple, cáncer de pulmón de células no pequeñas, glioblastoma o neoplasmas de la próstata, mama u ovarios.

La presente invención adicionalmente proporciona el
20 uso de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclорhidrato de 4-[5-(2-amino-etansulfonil)-
25 isoquinolin-7-il]-fenol, para la manufactura de un

medicamento para tratar cáncer de pulmón de células no pequeñas o glioblastoma.

La presente invención además proporciona el uso de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, para la manufactura de un medicamento para tratar hepatitis C, rubéola, virus de inmunodeficiencia humana (VIH), hepatitis B, o citomegalovirus humano (HCMV).

Adicionalmente, la presente invención proporciona 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, para el tratamiento de mieloma múltiple, cáncer de pulmón de células no pequeñas, glioblastoma, neuroblastoma, melanoma o neoplasmas de la próstata, mama, ovarios, estómago primario, tipo intestinal, endometrio, tiroides, páncreas, pulmón o vejiga.

La presente invención también proporciona 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del

mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, para el tratamiento de mieloma múltiple, cáncer de pulmón de células no pequeñas, glioblastoma o neoplasmas de la próstata, mama u ovarios.

La presente invención adicionalmente proporciona 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, para el tratamiento de cáncer de pulmón de células no pequeñas o glioblastoma.

La presente invención además proporciona 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, para el tratamiento de hepatitis C, rubéola, virus de inmunodeficiencia humana (VIH), hepatitis B, o citomegalovirus humano (HCMV).

La presente invención adicionalmente proporciona un

método para tratar mieloma múltiple, cáncer de pulmón de células no pequeñas, glioblastoma, neuroblastoma, melanoma o neoplasmas de la próstata, mama, ovarios, estómago primario, tipo intestinal, endometrio, tiroides, páncreas, pulmón o
5 vejiga, en un paciente en necesidad del mismo, que comprende administrar una cantidad efectiva de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-
10 etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

La presente invención también proporciona un método para tratar mieloma múltiple, cáncer de pulmón de células no
15 pequeñas, glioblastoma o neoplasmas de la próstata, mama u ovarios, en un paciente en necesidad del mismo, que comprende administrar una cantidad efectiva de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la
20 sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

La presente invención adicionalmente proporciona un
25 método para tratar cáncer de pulmón de células no pequeñas o

glioblastoma, en un paciente en necesidad del mismo, que comprende administrar una cantidad efectiva de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la
5 sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

La presente invención además proporciona un método
10 para tratar hepatitis C, rubéola, virus de inmunodeficiencia humana (VIH), hepatitis B o citomegalovirus humano (HCMV), en un paciente en necesidad del mismo, que comprende administrar una cantidad efectiva de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente
15 aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

La presente invención también proporciona una
20 composición farmacéutica que comprende 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato
25 monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-

il]-fenol, y un portador diluyente o excipiente farmacéuticamente aceptable.

La presente invención adicionalmente proporciona una composición farmacéutica liofilizada que comprende 4-[5-
5 (2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-
10 il]-fenol, y un portador, diluyente o excipiente farmacéuticamente aceptable, en donde el pH de dicha composición cuando se diluye con diluyente acuoso, es menos de 4.2 y mayor de 2.0, menos de 3.2 y mayor de 2.0, o menos de 2.8 y mayor de 2.0.

15 La presente invención también proporciona una composición farmacéutica que comprende 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo, que incluye diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-
20 il]-fenol, y un portador, diluyente o excipiente farmacéuticamente aceptable en solución, en donde el pH de dicha composición es menos de 4.2 y mayor de 2.0, menos de 3.2 y mayor de 2.0, o menos de 2.8 y mayor de 2.0.
25

La presente invención además proporciona semihidrato monoclóridato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol en forma cristalina, que tiene un patrón de difracción en polvo de rayos-X, con picos intensos a $2\theta = 4.9, 14.8$ y 10.2 .

La presente invención también proporciona 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo farmacéuticamente aceptable o hidrato del mismo.

10

DESCRIPCIÓN DETALLADA DE LA INVENCION

Los compuestos de la presente invención son inhibidores de Akt y se cree, son empleados en el tratamiento de trastornos relacionados con la actividad de Akt. De este modo, los compuestos de la presente invención son agentes antineoplásicos y/o antivirales.

Los compuestos de la presente invención son empleados para el tratamiento de neoplasmas que exhiben defectos en PTEN, neoplasmas con actividad PI3-cinasa desregulada, o neoplasmas que exhiben actividad Akt elevada. Los inhibidores de Akt se cree, son empleados en el tratamiento de mieloma múltiple (Hsu et al., *Blood* (2001) 98(9) 2853-2855); cáncer de pulmón de células no pequeñas (Balsara, *Carcinogenesis* (2004) 25(11) 2053-2059); glioblastoma (Koul, et al., *Mol. Cancer Ther.* (2006) 5: 637-644); neuroblastoma (Li, et al., *Cancer Res.* (2005) 65(6),

2070-2075); melanoma (Dai, et al., *J. of Clin. Oncology* (2005) 23(7), 1473-1482) y neoplasmas de la próstata (Majumder, et al., *Oncogene* (2005) 24, 7465-7474); mama (Tokunaga, et al., *J. of Clin. Oncology* (Meeting Abstracts) (2005) 23(16S), 9500); ovarios (Cheung, et al., *PNAS* (1992) 89, 9267-9271; Yuan et al. (2000); Hu et al. (2000)); estómago primario o tipo intestinal (Ang, et al., *Cancer Lett.* (2005) 225(1), 53-59); endometrio (Jin, et al., *British J. of Cancer* (2004) 91 1808-1812); tiroides (Ringel, et al., *Cancer Res.* (2001) 61(16), 6105-6111; De Vita, et al., *Cancer Res.* (2000) 60, 3916-3920); páncreas (Schliemen, et al., *Brit. J. of Cancer* (2003) 89, 2110-2115); pulmón (Massion, et al., *Am. J. Resp. Crit. Care Med.* (2004) 170, 1088-1094); o vejiga (Rieger-Christ, et al., *Oncogene* (2004) 23(27), 4745-4753). Los inhibidores de Akt, también se cree, son empleados en el tratamiento de virus tales como hepatitis C y NS4A de la hepatitis C (Mannova, et al., *J. Virol.* (2005) 79(14), 8742-8749; He et al. (2002)); rubéola (Cooray, et al., *Virology J.* (2005) 2(1), 1-12); proteína Tat del virus de inmunodeficiencia humana (VIH) (Borgatti et al. (1997)); Proteína X de la hepatitis B (Lee et al. (2001)), o citomegalovirus humano (HCMV) (Johnson et al. (2001)).

El 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol forma sales de adición de ácido farmacéuticamente aceptables con, por ejemplo, las sales fisiológicamente

aceptables las cuales son a menudo usadas en la química farmacéutica. Tales sales también son parte de esta invención. Las sales farmacéuticamente aceptables y metodología común para prepararlas, son bien conocidas en la técnica. Véase por ejemplo, P. Stahl, *et al.*, Handbook of Pharmaceutical Salts: Properties, Selection and Use, (VCHA/Wiley-VCH, 2002); S.M. Berge, *et al.*, "Pharmaceutical Salts," *Journal of Pharmaceutical Sciences*, Vol. 66, No. 1, Enero 1977.

10 Las sales preferibles de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol incluyen, el monoclóridato y el diclorhidrato.

Los intermediarios descritos en este documento pueden formar sales.

15 Además de las sales, los compuestos de la presente invención y los intermediarios descritos en este documento, pueden formar un hidrato o un hidrato de la sal farmacéuticamente aceptable.

Un compuesto preferible es 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol. Aún otro compuesto preferible es el diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol. Un compuesto más preferible es semihidrato monoclóridato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

25 Como se usa en este documento, el término

"paciente", se refiere a un mamífero que es afligido con uno o más trastornos asociados con actividad Akt elevada. Se entenderá que la mayoría de los pacientes preferidos son un humano. También se entiende que esta invención se refiere
5 específicamente a la inhibición del mamífero Akt/PKB.

Los términos "tratamiento", "tratar", "tratando", y similares, son significados que incluyen efectos tales como disminución y/o inhibición del crecimiento de neoplasmas, amplificación y/o sobreexpresión de Akt1, Akt2 y/o Akt3,
10 proliferación y supervivencia celular, y/o replicación viral.

Como se usa en este documento, el término "cantidad efectiva", se refiere a una cantidad que inhibe Akt a una extensión que proporciona un efecto farmacológico.

La presente invención también proporciona una
15 composición farmacéutica que comprende 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, o una sal del mismo farmacéuticamente aceptable, o hidrato del mismo, y un portador, diluyente o excipiente farmacéuticamente aceptable. Las composiciones farmacéuticas de la presente invención
20 incluyen formas antes de la dilución, tales como una forma liofilizada, y formas subsecuentes a la dilución, tales como una forma lista para administración a un paciente. El pH de estas composiciones farmacéuticas es de menos de aproximadamente 4.2 a más de aproximadamente 2.0. Más
25 preferiblemente, el pH es de menos de aproximadamente 3.2 a

más de aproximadamente 2.0. El pH más preferible es de menos de aproximadamente 2.8 a más de aproximadamente 2.0. Un "portador, diluyente o excipiente farmacéuticamente aceptable", es un medio en general, aceptado en la técnica para el suministro de agentes biológicamente activos a 5 pacientes, por ejemplo, mamíferos, preferiblemente humanos. Tales portadores, diluyentes o excipientes son en general, formulados de conformidad con un número de factores también dentro de la revisión de aquellos de habilidad ordinaria en 10 la técnica, para determinar y considerarlos. Estos incluyen sin limitación: el tipo y naturaleza del agente activo a ser formulado; el sujeto al cual la composición que contiene el agente es administrada; la ruta propuesta de administración de la composición; y la indicación terapéutica a ser 15 dirigida. Los portadores y excipientes farmacéuticamente aceptables incluyen, tanto medio líquido acuoso como no acuoso, así como también una variedad de formas de dosificación sólidas, y semi-sólidas. Tales portadores, diluyentes o excipientes, pueden incluir un número de 20 diferentes ingredientes y aditivos además del agente activo, tales ingredientes adicionales están incluidos en la formulación por una variedad de razones, por ejemplo, estabilización del agente activo, bien conocido por aquellos de habilidad en la técnica. Descripciones de portadores, 25 diluyentes o excipientes farmacéuticamente adecuados, y

factores involucrados en su selección, se encuentran en una variedad de fuentes fácilmente disponibles. Véase por ejemplo, Remington: The Science and Practice of Pharmacy (A. Gennaro, et al., eds., 19th ed., Mack Publishing Co., 1995).

5 Los compuestos de la presente invención pueden ser administrados sistémicamente, tal como intravenosamente (por ejemplo, por bolo o infusión), en formulaciones de unidad de dosificación de composiciones farmacéuticas que contienen portadores, diluyentes o excipientes no tóxicos
10 farmacéuticamente aceptables, convencionales.

 Para composiciones farmacéuticas de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, el ingrediente activo estará ordinariamente presente en una cantidad de aproximadamente 0.5 a 95% en peso, basada en el peso total de
15 la composición. Pueden ser aplicados revestimientos apropiados para incrementar la aceptabilidad o retardar la adsorción.

 Las cantidades terapéuticamente efectivas de los compuestos de la invención para tratar los trastornos
20 descritos en este documento en un paciente, se pueden determinar en una variedad de formas conocidas por aquellos de habilidad en la técnica. Se debe entender, sin embargo, que los niveles de dosis específicos para cualquier paciente particular, dependerán de una variedad de factores que
25 incluyen, la actividad del compuesto específico empleado, la

edad, peso corporal, salud general, sexo, dieta, tiempo de administración, ruta de administración y velocidad de excreción, combinación de fármaco y severidad de la enfermedad particular. La frecuencia de la dosis también se
5 puede variar dependiendo del compuesto usado y la enfermedad particular tratada. Por ejemplo, una dosis diaria típica puede contener desde aproximadamente 1 mg hasta 1 g del ingrediente activo.

Los compuestos de esta invención pueden ser
10 preparados empleando la información proporcionada en este documento, además de otras manipulaciones estándares que se conocen en la literatura o ejemplifican en los procedimientos experimentales.

Los términos y abreviaturas usadas en este
15 documento, tienen sus significados normales a menos que se designe de otro modo. Por ejemplo, "LC", se refiere a cromatografía líquida; "dppb" se refiere a bis(difenilfosfino)butano; $\text{Pd}(\text{OAc})_2$ se refiere a acetato de paladio; "DMF" se refiere a N,N-dimetilformamida; "DMSO" se
20 refiere a dimetilsulfóxido; "Et₂O" se refiere a éster dietílico; "EtOAc" se refiere a acetato de etilo; "TFA" se refiere a ácido trifluoroacético; MeOH se refiere a metanol.

PREPARACIONES

Preparación 1

Diclorhidrato de 2-[7-(4-metoxi-fenil)-isoquinolin-5-sulfonil]-etilamina

5 Se trató una suspensión de éster terc-butílico del ácido {2-[7-(4-metoxi-fenil)-isoquinolin-5-sulfonil]-etil}-carbámico (11.0 g, 24.85 mmol) en MeOH anhidro (150 ml) con HCl 4N en dioxano (350 ml). La mezcla resultante se agitó a temperatura ambiente durante la noche, después se concentró
10 *in vacuo* a un volumen 1/2 y se trató con exceso de EtOAc, ocasionando que precipitara un sólido amarillo. El sólido resultante se recuperó por filtración a vacío bajo una atmósfera de N₂; se lavó con EtOAc y se secó bajo vacío (bajo una atmósfera de N₂), para proporcionar el compuesto del
15 título (10.23 g, 99% de rendimiento) como un sólido amarillo: EM (ER): m/z 343.0 (M⁺+H).

Preparación 2

Ácido 7-bromo-isoquinolin-5-sulfónico

20 Se agregó H₂SO₄ humeante (2,000 ml, 21.33 mol; 25-29.5% de SO₃ libre), a un matraz de fondo redondo de 5-l, equipado con un agitador mecánico, condensador a reflujo, línea de N₂, y termómetro. Se enfrió el H₂SO₄ humeante a ~10°C con un baño de hielo/acetona, después se agregó HCl de 7-
25 bromoisoquinolina (500.00 g, 2.04 mol) en forma de porciones,

manteniendo la temperatura de la mezcla de reacción por debajo de ~15-20°C. Después de la adición completa de HCl de 7-bromoisoquinolina, la mezcla de reacción resultante se calentó a ~100°C durante la noche. La mezcla de reacción se enfrió a temperatura ambiente, después, se vertió lentamente en una solución agitada de H₂O helada. Lo precipitado resultante se recuperó por filtración a vacío, lavando con H₂O, después Et₂O, y se secó bajo filtración a vacío, seguido por secado en un horno de secado bajo presión reducida a ~35°C, para proporcionar el compuesto del título (501.42 g, 85% de rendimiento), como un sólido blancuzco; TOF-EM [ER+; m/z] 287.9331/287.9330.

Preparación 3

15 Ácido 7-(4-(metoxi-fenil)-isoquinolin-5-sulfónico

Se trató una solución de ácido 7-bromo-isoquinolin-5-sulfónico (150.00 g, 0.520 mol) y ácido 4-metoxifenilborónico (90.87 g, 0.598 mol) en DMF (1,400 ml) y MeOH (375 ml) con Na₂CO₃ acuoso 2M (652 ml). La suspensión resultante 3X de desoxigenó con N₂, después se agregó Pd(PAc)₂ (2.33 g, 0.0104 mol) y dppb (5.54 g, 0.0130 mol). La mezcla de reacción resultante se calentó a ~70°C por 3 horas, después se dejó enfriar a temperatura ambiente durante la noche. La mezcla de reacción se diluyó con H₂O (4,000 ml) y

el pH se ajustó a ~pH 2 con HCl acuoso 5N. La suspensión resultante se dejó agitar a temperatura ambiente por 30 minutos, después, el sólido marrón se recuperó por filtración a vacío, se lavó con H₂O, y se secó bajo filtración a vacío.

5 El sólido marrón se disolvió en DMF (1,000 ml) y Na₂CO₃ acuoso 2M (650 ml) y la solución resultante se filtró a través de una almohadilla de Celite®, se lavó con DMF (400 ml)/H₂O (400 ml). Lo filtrado se trató con HCl acuoso 5N para ajustar el pH a ~pH 2. Dejando la suspensión resultante agitar a

10 temperatura ambiente por 30 minutos, entonces el sólido se recuperó por filtración a vacío, lavando con H₂O y secando bajo filtración a vacío durante la noche. El sólido se disolvió en DMF (1,000 ml) y Na₂CO₃ acuoso 2M (1,000 ml) nuevamente, y la solución resultante se trató con Celite®

15 para crear una suspensión. La suspensión resultante se agitó a temperatura ambiente por 30 minutos, después se filtró a través de una almohadilla de Celite®, se lavó con H₂O. Lo filtrado se trató con HCl acuoso 5N para ajustar el pH a ~pH

2. La suspensión resultante se agitó a temperatura ambiente

20 por 30 minutos, después el sólido se recuperó por filtración a vacío, se lavó con H₂O y se secó bajo filtración a vacío durante la noche. El sólido se trituroó, después se lavó con EtOAc, se secó bajo filtración a vacío durante la noche, seguido por secado en un horno de secado bajo presión

reducida a $\sim 35^{\circ}\text{C}$, para proporcionar el compuesto del título (136.79 g, 83% de rendimiento) como un sólido amarillo: TOF-EM [ER+; m/z] 316.0624/316.0643. Anal. Calcd. Para $\text{C}_{16}\text{H}_{13}\text{NO}_4\text{S}$: C 60.94; H 4.15; N 4.44; S 10.16. Encontrado C 60.76; H 4.13; N 4.50; S 9.90.

Preparación 4

Cloruro de 7-(4-metoxi-fenil)-isoquinolin-5-sulfonilo

A una suspensión de ácido 7-(4-metoxi-fenil)-isoquinolin-5-sulfónico (12.13 g, 38.5 mmol) en 1,2-dicloroetanol (200 ml) y DMF (2.99 ml, 38.5 mmol), se agregó cloruro de oxalilo (26.8 ml, 308 mmol) por goteo. La suspensión se agitó mecánicamente bajo atmósfera de nitrógeno mientras se calentaba a $60\text{--}65^{\circ}\text{C}$ por 4 horas. La suspensión se enfrió a -10°C . Se esperó por 30 minutos, después se filtró. El sólido amarillo se lavó con éter/diclorometano al 20% y se secó bajo una atmósfera de nitrógeno, proporcionando el compuesto del título (14.8 g) como un polvo amarillo.

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Preparación 5

Sal sódica de 7-(4-metoxi-fenil)-isoquinolin-5-iol

A. Una suspensión de cloruro de 7-(4-metoxi-fenil)-isoquinolin-5-sulfonilo (4.0 g, 12 mmol) en dioxano (40 ml), se agregó clorhidrato de tricarboxi etil fosfina (13.73 g, 48 mmol) y agua (10 ml). La mezcla se calentó a 100°C y se agitó

por 3 horas. La mezcla se enfrió en un baño helado y se agregó NaOH (5N, 60 ml), lentamente vía una pipeta. Se filtró y secó en aire lo precipitado amarillo pálido para proporcionar el compuesto del título (3.2 g, 95%). Espectro
5 de masas (CLEM) $m/z = 266.2$ ($M-Na^+$).

Alternativamente, el compuesto del título puede ser elaborado como sigue:

B. (i) Se desoxigenó la solución de ácido 7-(4-metoxi-fenil)-isoquinolin-5-sulfónico (100.00 g, 0.317 mol)
10 en tolueno anhidro (2,500 ml) 3X con N_2 , después se trató con Ph_3P (332.58 g, 1.268 mol), I_2 (80.46, 0.317 mol) y Bu_3N (152.00 ml, 0.638 mol). La mezcla de reacción resultante se dejó a reflujo por 1 hora bajo N_2 , después se enfrió a temperatura ambiente durante la noche, mientras se soplaba
15 aire en la mezcla de reacción. La mezcla de reacción se trató con una solución acuosa de NaOH 1N (500 ml), después la mezcla de reacción resultante se agitó a temperatura ambiente durante la noche, mientras se soplaba aire en la mezcla de reacción. Lo precipitado bronceado resultante se recuperó por
20 filtración a vacío. Se lavó con una solución 1/1 de THF/ Et_2O , después se secó bajo filtración a vacío para proporcionar bis-(7-(4-metoxi-fenil)-isoquinolin-5-)-disulfuro (72.50 g, 86% de rendimiento) como un sólido ligeramente bronceado: 1H RMN (400 MHz, DMSO) δ 9.39 (s, 2H), 8.51 (d, 2H), 8.41 (s, 2H), 8.11 (d, 2H), 8.00 (d, 2H), 7.53 (d, 4H), 6.94 (d, 4H),
25

3.78 (s, 6H).

B. (ii) Una suspensión de bis-(7-(4-metoxi-fenil)-isoquinolin-5)-disulfuro, se trató en THF anhidro (850 ml) con NaBH_4 (2.84 g, 75.05 mmol). La mezcla de reacción
5 resultante se calentó a $\sim 35^\circ\text{C}$ bajo N_2 por ~ 1 hora, después se calentó a $\sim 45^\circ\text{C}$ bajo N_2 por 1 hora.

Preparación 6

Sulfinato de 7-(4-metoxi-fenil)-isoquinolin-5-sódico

10 A cloruro de 7-(4-metoxi-fenil)-isoquinolin-5-sulfinilo (1.48 g, 4 mmol) en agua (10 ml), se agregó Na_2SO_3 (1.01 g, 8 mmol) y NaHCO_3 (1.01 g, 12 mmol). La mezcla se calentó a 100°C y se agitó por 1 hora. La mezcla de reacción se enfrió a temperatura ambiente, y el agua se removi6 bajo
15 presión reducida. Se agregó metanol (40 ml) al residuo, se agitó por 10 minutos. El sólido blanco se filtró, lavó con metanol y lo filtrado se combinó. Se concentró para dar el compuesto del título (1.1 g).

20

Preparación 7

(3,5-dicloro-benciliden)-(2,2-dietoxi-etil)amina

La 2,2-dietoxi-etilamina (1852.5 g; 1.00 equivalentes; 13.63 moles), 3,5-dicloro-benzaldehído (2453 g; 1.00 equivalentes; 13.60 moles) y tolueno (12 l), se cargaron
25 en un matraz de 22 l equipado con una trampa Dean Stark,

condensador, entrada de nitrógeno, agitador de cabeza y termopar. La reacción amarillo ligero se calentó a reflujo. El solvente comenzó a destilar a 88°C. Se colectó un total de ~650 ml de destilado (~240 ml de agua). La temperatura se incrementó a 114°C durante la destilación. La RMN después de 2 horas a reflujo, mostró el producto. El calor se excluyó después de 2.5 horas. La solución es filtrada por gravedad en un garrafón a través de un papel filtro acanalado para remover algunos particulados (que incluyen una pequeña sección de tubo de vidrio, la cual es más probable de 3,5-dicloro-benzaldehído). La solución filtrada es concentrada usando un matraz Buchi con el baño de agua fijo a 45°C. Una vez que el solvente se detiene de entrar, la temperatura se incrementa a 70°C con llenado a vacío y se mantiene por ~1.5 horas para remover cualquier tolueno residual. El peso de (3,5-dicloro-bencilideno)-(2,2-dietoxi-etil)-amina es 4059.3 g (102.9% de teoría). Espectro de Masas (CLEM) $m/z = 291.2$ ($M+H^+$).

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Preparación 8

Clorhidrato de 5,7-dicloro-isoquinolina

Se cargó ácido triflico (2.97 l; 33.52 moles; 5.03 kg) a un matraz de 12 l equipado con una trampa Dean Stark, agitador de cabeza, condensador, entrada de nitrógeno, embudo de adición de 3 l (amortiguado del matraz vía un condensador)

25

y termopar. El ácido triflico es calentado a 120°C. Se diluyó (3,5-dicloro-bencilideno)-(2,2-dietoxi-etil)-amina (1350.5; 1.00 equivalentes; 4.65 moles) con diclorometano (1350 ml), y se carga al embudo de adición. Se inicia la adición con la temperatura a 119°C. La adición se completa durante 90 minutos manteniendo la temperatura a 120°C. Aproximadamente, se colectan 1500 ml del destilado durante la adición. El % de área de CLAR 45 minutos después de la adición se completa e indica 94.14% del producto y 4.86% (3,5-dicloro-bencilideno)-(2,2-dietoxi-etil)-amina. El calor se excluyó después de 1.5 horas. La reacción se enfrió a ~80°C. En este punto, el matraz es colocado en un baño de agua helada y se enfría adicionalmente. Se agregó metanol (2.7 l) con la reacción a 9°C. La adición se completó durante 60 minutos. La temperatura máxima es 27°C. Se forma algún sólido durante la adición de metanol. La suspensión es transferida en porciones a un embudo de adición de 2 l y se agrega a una solución de hidróxido de amonio (5.1 l; 36.67 moles; 4.59 kg) y agua (5.1 l) en un matraz de 22 l, enfriado en agua helada a <2°C. El embudo de adición tiene una extensión de tubo de Teflon® para prevenir que el material corra hacia abajo del lado del matraz, lo cual puede conducir a un sólido más aceitoso que se adhiere a la pared del matraz. El material se agrega en pequeños sedimentos para prevenir que el embudo de adición se tapa debido a los sólidos. La adición se completa durante 35

minutos que incluyen, un enjuague de metanol (900 ml) del matraz y embudo de adición. La temperatura máxima es 26°C. Se forma un sólido marrón. La suspensión se enfría a 14°C. El sólido se filtra en una almohadilla de polipropileno. El sólido se lava con 2 x 4 l y 1 x 2 l de agua. El sólido entonces se lava con 2 l de heptano para ayudar al secado. El sólido se seca *in vacuo* a 50°C a un peso de 999.0 g (108.4% de teoría). Se carga la 5,7-dicloro-isoquinolina (997 g; 1.00 equivalentes; 5.03 moles) y metanol (14.97 l) a un matraz de 22 l equipado con un embudo de adición, entrada de nitrógeno, agitador de cabeza y termopar. La suspensión marrón se agita a temperatura ambiente (22°C). El cloruro de acetilo (1180 ml; 16.58 moles; 1.30 kg) se carga al embudo de adición. Esto se agrega al matraz por goteo. La suspensión marrón se oscurece. El sólido parece disolverse principalmente agregando 100-200 ml del cloruro de acetilo. No se forma una solución completa. El sólido reaparece conforme la adición continua. La adición se completa durante 45 minutos. La temperatura máxima es 43°C, la cual se alcanza aproximadamente en forma media en la adición y se mantiene con agua fría en el baño. Se remueve una muestra de la reacción después que se agregan ~3/4 de cloruro de acetilo por RMN. Puesto que la muestra parecer ser la sal, el exceso de cloruro de acetilo probablemente no se necesita. La suspensión se agita y se deja enfriar. Después de 40 minutos,

la temperatura es de 30°C. La suspensión se enfría a <2°C y se mantiene por 1.5 horas en un baño de agua helada. La suspensión se filtra. El sólido se lava con 2 x 1.3 l de metanol enfriado. El peso de la pasta húmeda es 811.4 g. El
5 sólido se seca in vacuo a 50°C. El peso seco es 648.4 g. Esto representa un rendimiento de 61.0%. Espectro de masas (CLEM) $m/z = 199.05$ ($M+H^+$).

Preparación 9

10 Éster terc-butílico del ácido [2-(7-cloro-isoquinolin-5-
ilsulfanil)-etil]carbámico

Se cargó carbonato de potasio (1952 g; 14.12 moles), con un matraz de 22 l equipado con agitador de cabeza, embudo de adición, entrada de nitrógeno y termopar.
15 Se agrega dimetilformamida (4 l). Se agrega clorhidrato de 5,7-dicloro-isoquinolina (646.1 g; 1.00 equivalentes; 2.76 moles) en porciones, y junto con DMF (330 ml). Se disuelve Boc-Cisteamina (514 g; 1.05 equivalentes; 2.90 moles) en dimetilformamida (1520 ml) y se carga al embudo de adición.
20 La mezcla se calienta a 60°C. Se purga nitrógeno a través del espacio de cabeza por 15 minutos mientras la mezcla se calienta. La solución de Boc-Cisteamina se agrega durante 2 horas y 15 minutos a 60°C. La CLAR 1 hora y 20 minutos después de adición, indica 28.1% de material de partida,
25 67.26% de producto, 4.22% de isómero y 0.25% de bis. La

reacción se calienta a 75°C. Los datos de CLAR en la Tabla A se colectan a 75°C.

5	Tiempo a 75°C	% de HCl de 5,7-dicloro-isoquinolina	% de éster terc-butílico del ácido [2-(7-cloro-isoquinolin-5-ilsulfanil)-etil]-carbámico	% de isómero	% bis-sustituido
10	30 minutos	2.75	90.27	5.54	1.44
	1 hora	0.93	91.39	5.52	2.16
	1.5 horas	0.20	91.25	5.32	3.23

Tabla A. Datos de CLAR de la reacción, para la preparación de éster terc-butílico del ácido [2-(7-cloro-isoquinolin-5-ilsulfanil)-etil]-carbámico.

Después de 1.5 horas, el manto se reemplazó con un baño enfriante. La reacción se enfrió a 20-25°C. El sólido se filtró y se lavó con DMF (2 x 810 ml). La solución DMF se diluyó con MTBE (5.15 l) y LiCl al 5% (5.15 l). Después de la agitación en un matraz de salida en el fondo de 50 l, las capas se separaron. La capa acuosa se extrajo con MTBE (2.9 l). Las capas de MTBE se combinaron y lavaron con LiCl al 5% (2 x 2.9 l). La capa de MTBE se filtró por gravedad a través

de un papel acanalado y se concentró usando un matraz Buchi con el baño de agua puesto a 35°C para dar 892.7 g (95.6% del teórico, no corregido) de éster terc-butílico del ácido [2-(7-cloro-isoquinolin-5-ilsulfanil)-etil]-carbámico. Espectro
5 de Masa (CLEM) m/z 339.85) ($M+H^+$).

Preparación 10

Éster terc-butílico del ácido [2-(7-cloro-isoquinolin-5-sulfonil)-etil]-carbámico

10 Se disolvió el éster terc-butílico del ácido [2-(7-cloro-isoquinolin-5-ilsulfanil)-etil]-carbámico (700 g; 1.00 equiv; 2.07 moles) en alcohol isopropílico (10.53 l) por rotación en matraz Buchi con el baño de agua puesto a 50°C. Se cargaron mequinol (12.29 g; 98.01 mmoles; 12.29 g),
15 tungstato dihidrato de sodio (31.4 g; 95.20 mmoles), la solución de éster terc-butílico del ácido [2-(7-cloro-isoquinolin-5-ilsulfanil)-etil]-carbámico, alcohol isopropílico (2.77 l), agua (3.38 l) y ácido acético (123 ml; 2.15 moles; 128.90 g) en un matraz de 22 l equipado con un
20 condensador, entrada de nitrógeno, agitador de cabeza, embudo de adición y termopar. La adición de agua causa que la solución marrón llegue a ser ligeramente lechosa espesa. La reacción se calentó a 50°C usando un baño de agua caliente. Se agregó peróxido de hidrógeno (607 ml; 5.94 moles; 673.77
25 g) durante 1 hora. Durante la adición, la temperatura se

mantiene a 50-55°C para la evaluación de adición de peróxido de hidrógeno y se agrega agua fría o caliente al baño como se necesite. La reacción es aún marrón lechosa. La temperatura de reacción es 54°C al final de la adición y se mantiene a 53-57°C usando el baño de agua caliente. La CLAR 10 minutos después de la adición indicó pico de 24.69% a 2.89 minutos, 65.57% del producto, 5.03% de isómero, 4.33% de bis, y 0.18% de pico en 3.05 minutos. La CLAR 2 horas después de la adición indicó 0.06% de sulfóxido, 1.27% de pico en 3.046 minutos, 88.10% del producto, 4.61% de isómero y 5.79% de bis. El baño se drenó y se reemplazó con agua helada para enfriar la reacción. Cuando la temperatura de reacción alcanzó 72°C, el peróxido se apagó agregando solución de bisulfito de sodio al 9% (250 ml). No se observó incremento en temperatura. Tiras de prueba de peróxido y papel de yoduro de almidón no indicaron restos de peróxido. Se agregó agua (5.64 l) durante 15-20 minutos. La temperatura se incrementó de 18 a 24°C durante la adición. La mezcla se agitó a temperatura ambiente durante la noche. La suspensión se enfrió en un baño de agua fría a <5°C y mantenida. La CLAR cuantitativa del sobrenadante durante un tiempo, indicó que la cristalización se completó después de 1.5 horas a <5°C. El sólido se filtró y se lavó con agua (2 x 2 l). El peso de la pasta húmeda fue 831 g. El sólido se secó in vacuo a 50°C. El peso del éster terc-butílico del ácido [2-(7-cloro-

isoquinolin-5-sulfonyl)-etil]-carbámico es 531 g (69.31% de teoría, no corregida). Espectro de Masa (EXCL.) m/z = 371.85) ($M+H^+$).

5

Preparación 11

Éster terc-butílico del ácido [2-{7-[4-(tetrahidro-piran-2-iloxi)-fenil]-isoquinolin-5-sulfonyl}-etil]-carbámico

El reactor de 60 l se cargó con PEPPSI™ (Estabilización e Iniciación de la Preparación de
10 Precatalizador Mejorado con Piridina; 51.0 g; 74.9 mmoles), éster terc-butílico del ácido [2-(7-cloro-isoquinolin-5-sulfonyl)-etil]-carbámico (1.383 kg; 3.729 moles), ácido fenil borónico (895 g; 4.03 moles), K_2CO_3 (1.039 kg; 7.518 moles) y etanol (15 l) bajo una purga de nitrógeno.
15 Inicialmente se agregaron 12 l de etanol al reactor y los 3 l restantes se usaron para enjuagar las otras cargas. La reacción se calentó a reflujo (78°C). La CLAR indicó una reacción completa después de 1/2 hora. La reacción se enfrió a 59°C usando el circulador Huber®. Se agregó agua (8.7 l) a
20 la reacción durante 18 minutos. La temperatura disminuyó de 59°C a 42°C durante la adición de agua. La reacción se enfrió usando el circulador Huber® de 42°C a 0°C durante 1 hora. La suspensión se mantuvo a 0°C por 1 hora. El sólido se filtró usando una almohadilla de polipropileno y se lavó con 1:1
25 etanol:agua (3.7 l, helada). El sólido se secó en el filtro

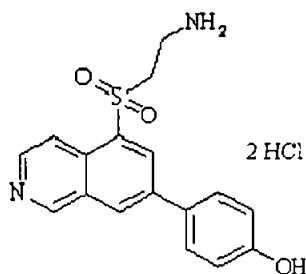
durante la noche. El sólido después se secó in vacuo a 60°C por 22 horas. El peso seco del sólido es 2.171 kg. El nivel de Pd en el éster terc-butílico del ácido (2-{7-[4-(tetrahydro-piran-2-iloxi)-fenil]-isoquinolin-5-sulfonil}-etil)-carbámico es 2196 mcg/g y el sólido contiene 6.9% de agua por KF. Se cargó un matraz de salida en el fondo de 50 L con diclorometano (4 l). El éster terc-butílico del ácido (2-{7-[4-(tetrahydro-piran-2-iloxi)-fenil]-isoquinolin-5-sulfonil}-etil)-carbámico crudo, se cargó al matraz de 50 l y se enjuagó en diclorometano (1 l). Se cargó Na₂SO₄ (1.9 kg) y se enjuagó con diclorometano (0.1 l). Se cargó Darco G-60® (3.8 kg) a un matraz Büchi de 20 l y se formó en suspensión en diclorometano (12 l). Esta suspensión es transferida al matraz de 50 l y enjuagada con diclorometano (4.9 l). La mezcla se agitó por 2 horas a temperatura ambiental. Se ajustó un filtro de acero inoxidable de 50 cm con una tapa de polipropileno y se cargó con Hyflo Super Cel® (1.7 kg). La almohadilla de Hyflo Super Cel® es enjuagada con diclorometano (2 l). Los contenidos del matraz de 50 l, son filtrados sobre la almohadilla Hyflo Super Cel®. El matraz y la pasta de filtro son enjuagados con diclorometano (22 l). Lo filtrado se carga al reactor de 60 l, a través de un filtro de cartucho de 0.45 micrones. Los contenidos del reactor son calentados y concentrados por destilación bajo ligero vacío (~640 mm de Hg). Aproximadamente 33 l del

destilado se colectan durante 2 horas a 35-37°C. Se agrega etanol (10 l). El vacío se ajusta lentamente descendientemente a 280 mm de Hg, mientras se continua la destilación. Una vez que se alcanza el vacío, la temperatura se incrementa para
5 mantener la destilación. Una vez que se alcanza el volumen en el reactor ~10 l, se agrega etanol (6.5 l) a tal relación, para mantener el volumen en un reactor mientras se destila el solvente. La destilación se detiene una vez que todo el etanol se agrega. El volumen final es ~10 l. La temperatura
10 al final de la destilación es 56°C. La suspensión que se forma durante el intercambio de solvente, se enfría a 20°C y se mantiene durante la noche. La suspensión se filtra. El sólido se lava con etanol:agua 1:1 (1.8 l). El peso de la pasta húmeda es 1.878 kg. El sólido se seca in vacuo a 50°C
15 para dar 1.2306 kg (64.36% de rendimiento) del éster terc-butílico del ácido (2-{7-[4-(tetrahidro-piran-2-iloxi)-fenil]-isoquinolina-5-sulfonil}-etil)-carbámico. Espectro de masas (CLEM) $m/z = 513.62$ (M+H).

EJEMPLOS

Ejemplo 1

Sal de diclorhidrato de 4-[5-(2-amino-etansulfonil)-
isoquinolin-7-il]-fenil



10 A. Éster terc-butílico del ácido {2-[7-(4-metoxi-fenil)-isoquinolin-5-ilsulfanil]-etil}-carbámico

A sal sódica de 7-(4-metoxi-fenil)-isoquinolin-5-
iol (0.29 g, 1 mmol) en acetona (10 ml), se agregó éster
terc-butílico del ácido (2-bromo-etil)-carbámico (0.22 g, 1
15 mmol) y carbonato de potasio (0.14 g, 1 mmol). La mezcla se
agitó a temperatura ambiente por 4 horas, después se cargó en
una columna de gel de sílice y se eluyó con acetato de etilo
al 65% en hexanos, para proporcionar el compuesto del título
(0.35 g, 86%). Espectro de masas (CLEM) $m/z = 355.2$ ($M+H^+$).

20

B. Éster terc-butílico del ácido {2-[7-(4-metoxi-fenil)-isoquinolin-5-sulfonil]-etil}-carbámico

A una solución de éster terc-butílico del ácido {2-
[7-(4-metoxi-fenil)-isoquinolin-5-ilsulfanil]-etil}-carbámico
25 (0.35 g, 0.85 mmol) en ácido acético (8 ml), se agregó agua

(2 ml). La mezcla se enfrió a 0°C en un baño helado. Se agregó una solución de KMnO₄ (0.27 g, 1.7 mmol) en agua (2 ml) por goteo, durante un periodo de 5 minutos. Después de la adición, la mezcla de reacción se agitó a 0°C por 30 minutos.

5 Se agregó H₂O₂ (30% en agua) por goteo, hasta que desaparece el color marrón. La mezcla se dividió entre acetato de etilo (50 ml) y solución saturada de bicarbonato (40 ml). La capa orgánica se separó, se secó sobre sulfato de sodio, filtró y concentró. El residuo se cromatografió sobre una columna de

10 gel de sílice con acetato de etilo al 66% en hexanos, para proporcionar el compuesto del título (0.245 g) como un sólido blanco. Espectro de masas (CLEM) m/z = 433.2 (M+H⁺).

C. Diclorhidrato de 4-[5-(2-amino-etansulfonil)-

15 isoquinolin-7-il]-fenol

A éster terc-butílico del ácido {2-[7-(4-metoxi-fenil)-isoquinolin-5-sulfonil]-etil}-carbámico (0.22 g, 0.51 mmol) en diclorometano (10 ml), se agregó BBr₃ (1.0 M en diclorometano, 3.0 ml) a -20°C. Después de la adición, la

20 mezcla de reacción se agitó a -20°C por 2 horas, se calentó lentamente a temperatura ambiente y se agitó a temperatura ambiente por 2 horas. Se agregó metanol (5 ml) para apagar el exceso de BBr₃. El solvente se evaporó bajo presión reducida, y se purificó el residuo vía CLAR de fase inversa (0-100% de

25 acetonitrilo:agua con TFA al 0.01%), para proporcionar 4-[5-

(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, como la sal de TFA (0.18 g). Después, se convirtió 0.12 g de la sal de TFA a la sal de diclorhidrato agregando 2 ml de bicarbonato de sodio acuoso saturado a la sal de TFA y se agitó durante 5 la noche para convertir la sal de TFA a la base libre. La base libre se filtró. La base libre se lavó, primero con agua, después con éter dietílico. El sólido se secó. Después, el sólido se suspendió en MeOH. Se agregó 0.2 ml de HCl acuoso concentrado y se agitó por 1 hora. El solvente se 10 concentró bajo presión reducida para proporcionar la sal de diclorhidrato (0.10 g). Espectro de masas (CLEM) $m/z = 329.0$ ($M+H^+$).

Ejemplo 2

15 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol

A éster terc-butilico del ácido {2-[7-(4-metoxifenil)-isoquinolin-5-sulfonil]-etil}-carbámico (25.00 g, 56.49 mmol) en diclorometano (1 l) a 0°C, se agregó BBr₃. (1.0 M en diclorometano, 200 ml, 200 mmol) durante 40 minutos 20 con agitación de cabeza vigorosa. El baño enfriante se removió y se agitó a temperatura ambiente durante la noche. Se enfrió a 0°C y se agregó metanol (500 ml) por goteo durante 1 hora. La suspensión resultante se filtró, el matraz se enjuagó con diclorometano (100 ml) y los sólidos se 25 lavaron con el enjuague. Los sólidos se lavaron con

diclorometano adicional (150 ml) y se secaron bajo filtración a vacío. Los sólidos se formaron en suspensión en metanol (400 ml) con agitación, después se concentraron bajo vacío a una pasta delgada. Se agregó metanol adicional (400 ml) y
5 además, se formaron en suspensión adicional con agitación. La suspensión se concentró bajo vacío a sequedad y los sólidos se secaron durante la noche bajo vacío. Los sólidos se pulverizaron a un polvo y formaron en suspensión en metanol (1 l). Se agregó resina Amberlyst® A-21 (75 g) y se agitó
10 hasta que el polvo se disolvió. La resina se filtró, la resina filtrada se formó en suspensión en metanol (220 ml) y se filtró nuevamente la resina, combinando los filtrados. La resina filtrada se lavó con metanol (100 ml) y se agregó este lavado a los filtrados. Los filtrados se combinaron con
15 etanol (200 ml) y la mezcla se concentró bajo vacío a un volumen de aproximadamente 200 ml para proporcionar una suspensión. Se agregó etanol caliente (400 ml) a la suspensión a 50°C por aproximadamente 15 minutos, después se concentró bajo vacío a un volumen de aproximadamente 200 ml.
20 La suspensión se enfrió a temperatura ambiente, se filtró y la pasta de filtro se enjuagó con etanol (2 x 15 ml), después se secó bajo vacío para proporcionar el compuesto del título (10.22 g, 55%) como un sólido marrón. Espectro de masas (CLEM) $m/z = 329.0$ ($M+H^+$).

Ejemplo 3

4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenil

Se enfrió una suspensión de diclorhidrato de 2-[7-(4-metoxi-fenil)-isoquinolin-5-sulfonil]-etilamina (9.5504 g, 22.99 mmol) en CH_2Cl_2 anhidro (400 ml) a $\sim 5^\circ\text{C}$ con un baño de hielo/ H_2O , después la suspensión fría se trató con una adición por goteo de una solución 1.0 M de BBr_3 en CH_2Cl_2 (92.00 ml, 92.00 mmol). Después de la adición completa de BBr_3 , el baño frío se removió y la mezcla de reacción resultante se dejó agitar bajo N_2 a temperatura ambiente durante la noche. La mezcla de reacción resultante se apagó con MeOH, después se concentró *in vacuo* para dar un residuo. El residuo resultante se disolvió en MeOH (200 ml) y se concentró *in vacuo* para dar un residuo. Lo anterior se repitió 3 veces, después el residuo se formó en suspensión en Et_2O (300 ml) y se trató con NaHCO_3 acuoso saturado hasta que se alcanzó el $\sim\text{pH}$ 7. El sólido resultante se recuperó por filtración a vacío, después se disolvió en una solución 3/1 de $\text{CHCl}_3/\text{MeOH}$. Lo filtrado se saturó con NaCl, después se extrajo con una solución 3/1 de $\text{CHCl}_3/\text{MeOH}$ (2 x 400 ml cada una). Los orgánicos combinados se secaron sobre MgSO_4 , filtraron, después concentraron *in vacuo* para dar un sólido/residuo. El sólido/residuo se formó en suspensión en una pequeña cantidad de MeOH (20 ml) y exceso de hexanos, después el sólido resultante se recuperó por filtración a

vacío, lavando con hexanos y secando bajo filtración a vacío para dar (7.60 g) de un sólido ligeramente amarillo. El sólido ligeramente amarillo (7.60 g) se formó en suspensión en CH_2Cl_2 anhidro (400 ml) y se enfrió a $\sim 5^\circ\text{C}$ con un baño de hielo/ H_2O , después se trató con una adición por goteo de una solución de BBr_3 1.0 M en CH_2Cl_2 (70.00 ml, 70.00 mmol). Después de la adición completa de BBr_3 , el baño frío se removió y la mezcla de reacción resultante, se dejó agitar a temperatura ambiente bajo N_2 por 4 horas. Se agregó una cantidad adicional de BBr_3 1.0 M en CH_2Cl_2 (70.00 ml, 70 mmol) por goteo a la mezcla de reacción, después la mezcla de reacción resultante se dejó agitar a temperatura ambiente bajo N_2 durante la noche. La reacción se calentó a $\sim 40^\circ\text{C}$ por ~ 1 hora, después se trató con una adición por goteo de BBr_3 1.0 M adicional en CH_2Cl_2 (23.00 ml, 23.00 mmol). La mezcla de reacción resultante se calentó a $\sim 40^\circ\text{C}$ por ~ 2 horas, después se apagó con MeOH y se concentró *in vacuo* para dar un residuo. El residuo resultante se disolvió en MeOH (200 ml) y se concentró *in vacuo* para dar un residuo amarillo. Lo anterior se repitió 3 veces. El residuo amarillo se trató con NaHCO_3 saturado para ajustar el pH a ~ 7 , después el sólido resultante se recuperó por filtración a vacío. El sólido pre-absorbido en gel de sílice, entonces es semi-purificado por cromatografía en columna (ISCO 330 g de gel de sílice, CH_2Cl_2

puro a 5% de NH_4/MeOH 2N en CH_2Cl_2 a 10% de NH_4/MeOH 2N en CH_2Cl_2) para dar un sólido. El sólido pre-absorbido en gel de sílice, entonces es purificado nuevamente por cromatografía en columna (ISCO 330 g de gel de sílice, CH_2Cl_2 puro a 5% de NH_4/MeOH 2N en CH_2Cl_2 a 10% de NH_4/MeOH 2N en CH_2Cl_2) para dar un sólido. El sólido pre-absorbido en gel de sílice, entonces es purificado nuevamente por cromatografía en columna (ISCO 330 g de gel de sílice, CH_2Cl_2 puro a 5% de NH_4/MeOH 2N en CH_2Cl_2 a 10% de NH_4/MeOH 2N en CH_2Cl_2 a 15% de NH_4/MeOH 2N en CH_2Cl_2) para dar un sólido. El sólido pre-absorbido en gel de sílice, entonces es purificado nuevamente por cromatografía en columna (ISCO 330 g de gel de sílice, CH_2Cl_2 puro a 5% de MeOH en CHCl_3 a 10% de MeOH en CHCl_3 a 20% de MeOH en CHCl_3 a 30% de MeOH en CHCl_3) para dar (6.85 g, 90.8% de rendimiento) del compuesto del título como un sólido amarillo. EM (ES^+ , m/z): 329.0 ($\text{M}+1$).

Ejemplo 4

Estado hidrato desconocido, monoclórhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol

Se colocó 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol (171.8 mg) en un vial, junto con 15 ml de EtOH al 95%. El vial se colocó en una placa caliente ajustada a 80°C , mientras la solución se agita para disolver el compuesto. Puesto que ocurre poca o ninguna disolución, se agrega un

equivalente molar de HCl (0.52 ml de HCl 1N en agua), para disolver el sólido y resultar en una solución amarillo brillante. Menos de un minuto después de la adición de HCl, comienza la formación de sólidos y se incrementa con el tiempo. Después de ~2 horas a 80°C, la muestra se removió del calor y se dejó agitar durante la noche a TA. El sólido se colectó usando filtración a vacío a través de un papel filtro. Después del secado al aire durante la noche, se recuperaron 107.8 mg del compuesto del título (63% de rendimiento). Difracción en polvo de rayos X: ángulo, 2θ (% de intensidad): 11.8 (100.0); 16.6 (52.9); 8.2 (43.4).

Ejemplo 5

Semi-hidrato monoclóridato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol

A. Preparación de material de siembra.

A una suspensión de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol (0.2509 g, 0.764 mmol) en etanol (5 ml), se agregó HCl acuoso (1M, 0.765 ml, 0.765 mmol). La mezcla resultante se calentó a reflujo durante la noche. Se enfrió a temperatura ambiente y se filtró la suspensión a vacío. Los sólidos se enjuagaron con etanol, y secaron bajo filtración a vacío para proporcionar 0.1842 g de un sólido amarillo.

B. Semi-hidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol

A una suspensión de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol (1.75 g, 5.33 mmol) en etanol (35 ml), se agregó HCl acuoso (1 M, 5.33 ml, 5.33 mmol). La mezcla se calentó a reflujo y se agregó agua (2.5 ml) para obtener una solución. La solución se enfrió a 60°C y se sembró con aproximadamente 2 mg del sólido amarillo obtenido de A anterior, para obtener una suspensión. Se enfrió a temperatura ambiente y se agitó durante la noche. La suspensión se filtró a vacío y la pasta de filtro se enjuagó con etanol, después se secó bajo filtración a vacío para proporcionar 1.331 g (67%) del compuesto del título como un sólido amarillo. Espectro de masas (CLEM) $m/z = 329.0$ ($M+H^+$). Karl Fischer: 3.00%. difracción en polvo de rayos-X: ángulo, 20 (% intensidad): 4.9 (47.3); 14.8 (55.8); 10.2 (45.5).

Ejemplo 6

Sal de diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol

Se formó en suspensión 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol (16.57 g, 50.45 mmol) en metanol (500 ml). Separadamente, se preparó una solución de HCl en metanol agregando cloruro de acetilo (12.60 ml, 177.05 mmol) a metanol (165 ml). La solución se agregó de HCl en metanol por

goteo a temperatura ambiente durante 30 minutos a la suspensión 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol en metanol. Se agitó por 30 minutos, después se agregó acetato de etilo (600 ml) por goteo durante 1 hora. Se agitó
5 por 30 minutos, después los sólidos se filtraron y lavaron con acetato de etilo (2 x 100 ml). Los sólidos se secaron *in vacuo* a 50°C, después se secaron adicionalmente *in vacuo* a temperatura ambiente con administración lenta de nitrógeno, para proporcionar el compuesto del título (18.3 g, 90%).
10 Espectro de masas (CLEM) $m/z = 329.0$ ($M+H^+$).

Ejemplo 7

Sal de diclorhidrato de 4-[5-(2-amino-etansulfonil)-
isoquinolin-7-il]-fenol

15 El reactor de 60 l se cargó con etanol (24 l) y éster terc-butílico de (2-{7-[4-(tetrahydro-piran-2-iloxi)-fenil]-isoquinolin-5-sulfonil}-etil)-carbámico (2.652 kg; 5.173 moles). El éster terc-butílico de (2-{7-[4-(tetrahydro-piran-2-iloxi)-fenil]-isoquinolin-5-sulfonil}-etil)-
20 carbámico, se enjuagó con etanol (2.6 l). Se diluyó solución de ácido clorhídrico (31.9%; 1780 g; 15.6 moles) con agua (1.42 l). Esta solución de HCl se cargó al reactor y se enjuagó con agua (0.2 l). La reacción se calentó a 70°C. Se formó una solución amarilla aproximadamente a 30°C y una
25 suspensión amarilla se formó nuevamente cerca de 70°C. La

reacción se mantiene a 70°C por 1/2 hora y después se calienta hasta reflujo (78°C). La CLAR indica una reacción completa después de 1.5 horas a reflujo. Se agrega agua (14.5 l) a la reacción durante 13 minutos. La temperatura cae a 5 69°C. La reacción se calienta nuevamente a reflujo (80°C) y se forma una solución. La reacción se enfría a 68°C y comienza a formarse el sólido. La reacción se mantiene a 68°C por 1/2 hora y después se enfría a 2°C durante 1.5 horas. La suspensión se mantiene a 1-2°C por 1 hora. El sólido se 10 filtra y se lava con etanol (4.6 l). El peso de la pasta húmeda es 3.236 kg. El sólido se seca in vacuo a 50°C para dar 2.106 kg (101.4% de teoría) de la sal de diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol. Espectro de masas (CLEM) $m/z = 402.31$ (M+H).

15

Ejemplo 8

Semi-hidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-
isoquinolin-7-il]-fenol

Las soluciones de hidróxido de sodio y etanol 20 usadas para este ejemplo, son filtradas a través de un filtro de cartucho de 0.22 micrones. El agua usada es agua purificada con control de endotoxina.

Se carga etanol (8 l) y sal de diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol (841.6 g; 25 2.097 mmoles) en un matraz de 22 l, equipado con un agitador

de cabeza, condensador, entrada de nitrógeno, manto de calentamiento, termopar y embudo de adición. La sal de diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, se enjuaga con etanol (3.28 l). La reacción se
5 calienta a 55°C. Se agrega solución de hidróxido de sodio (1100 ml; 1.91 N; 2.10 moles) a la reacción durante 7 minutos, tiempo en el cual la temperatura se incrementa a 62°C. La reacción se calienta a reflujo (78°C) y se mantiene por 1 hora. Se agrega agua (1.96 l) a la reacción una vez. La
10 temperatura disminuye a 68°C. La reacción se calienta nuevamente a reflujo (79°C) y se forma una solución. Se agrega Darco G-60® (438 g) a la reacción. La reacción se mantiene a reflujo por 1.25 horas. La suspensión Darco G-60® caliente, se filtra a través de papel GFF en otro matraz de
15 22 l. Resulta una solución ligeramente amarilla. El matraz original y la pasta de filtro Darco G-60®, son enjuagadas con etanol caliente (2.5 l). Durante el enjuague, el sólido comienza a formarse en lo filtrado. El matraz de 22 l que contiene el filtrado y se lava, es equipado con un agitador
20 de cabeza, entrada de nitrógeno y un termopar. La suspensión se agita y se deja enfriar a temperatura ambiente. El sólido es filtrado y lavado con etanol (2 l). El peso de la pasta húmeda es 672 g. El sólido se seca in vacuo a 50°C para dar 540 g (68.9% de rendimiento) de semi-hidrato monoclorhidrato
25 de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

Espectro de masas (CLEM) $m/z = 329.3$ ($M+H^+$).

Los compuestos ejemplificados son inhibidores de la actividad de Akt. La actividad inhibidora de estos compuestos se puede demostrar por los métodos siguientes.

5

Ensayo de Fosforilación de Akt1

El ensayo mide la fosforilación de péptido pseudo-sustrato PKC alfa por un Inmunoensayo de Polarización de Fluorescencia Competitiva (FPIA). El ensayo mide directamente
10 la concentración de producto fosfo-péptido formado en una reacción midiendo el cambio en la intensidad de luz polarizada emitida a partir de un péptido indicador fluorescentemente etiquetado cuando se desplaza de un anticuerpo fosfo-específico por el producto de fosfo-péptido.
15 La calibración con una curva estándar, proporciona resultados de rendimientos cuantitativos.

Enzima y Sustrato

La Akt1 recombinante humana activa (de longitud
20 completa), purificada de células de insecto Sf9, se obtiene de Upstate Biotechnology, Inc. Se adquiere el sustrato peptídico (PM 1561).

Soluciones de Ensayo Estándares

25 Solución (A): DMSO al 20% (dimetilsulfóxido) o

Compuesto en DMSO al 20% ó EDTA 500 mM; Solución (B): Mezcla de amortiguador de ensayo: 75 μ M de Sustrato peptídico, 38 mM de $MgCl_2$, 70 mM de HEPES, pH 7.4, Triton X-100® al 0.01%, y 50 μ M de ATP (trifosfato adenosina); Solución (C): Mezcla de 5 cinasa de Akt: 70 mM de HEPES, pH 7.4, DTT 1 mM (ditiotreitól), Triton-X-100® al 0.01% y enzima Akt1.

Procedimiento para FPIA

Cinco μ l de solución (A) son primero mezclados con 10 10 μ l de solución (B). La reacción enzimática es iniciada agregando 10 μ l de solución (C) a la mezcla. (Concentración final o cantidad en una mezcla de reacción de 25 μ l; DMSO al 4% (cavidades mínimas inhibidas) o varias concentraciones de compuesto en DMSO al 4% o EDTA 100 mM (cavidades máximas 15 inhibidas); 30 μ M de sustrato peptídico; 15 mM de $MgCl_2$; 70 mM de HEPES, pH 7.4; 20 μ M de ATP; 0.4 mM de DTT; Triton X-100® al 0.01%). Las reacciones se realizan en placas microtituladoras de 96 cavidades de fondo plano de área media negra.

20 Después de 60 minutos a temperatura ambiente, la reacción se termina agregando 25 μ l de Mezcla de Detección/Apagado, que contiene 260 mM de EDTA, 3.4 nM de Indicador de Fluoresceína, y 7.5 nM de anticuerpo anti-fosfoserina. Las placas son incubadas en la oscuridad a

temperatura ambiente por más de 3 horas, y después se lee la polarización de fluorescencia (a $\lambda_{ex} = 485 \text{ nm}$, $\lambda_{ex} = 530 \text{ nm}$), usando un lector de placa Tecan Ultra. La concentración del producto fosforilado por placa es calculada de unidades de milipolarización (mP) usando una serie de dilución 5 competidora de péptido preparada como una curva estándar. Los compuestos ejemplificados de la invención, inhiben la fosforilación de Akt1 con un IC_{50} de $1 \mu\text{M}$ o menos. El compuesto del Ejemplo 1, inhibe la fosforilación de Akt1 en 10 este ensayo con un IC_{50} de $45 \pm 17 \text{ nM}$ ($n = 3$).

ELISA a base de Célula (ELISAc) para la Detección de Fosfo-
GSK3b (pGSK3b):

Un objetivo de Akt en Células

15 Células U87MG que crecen exponencialmente derivadas de glioblastoma humano, son sembradas en placas de 96 cavidades y se incubaron durante la noche a 37°C con CO_2 al 5%. Diez concentraciones del compuesto del Ejemplo 1, se prepararon de una solución base de 4 mM (en DMSO al 100%) por 20 diluciones seriales 1:2 en el medio de cultivo. Se agrega un volumen igual de cada una de las diluciones seriales directamente en las células. El tratamiento se detiene 2 horas después. Al final del tratamiento, se remueve el medio de cultivo, y las células son lavadas una vez con salina 25 amortiguada de fosfato enfriada en hielo (PBS). Las células

se fijan en PREFER de conformidad con el procedimiento del
vendedor, seguida por enjuagues en PB/SDS al 0.1%/Triton X-
100® al 0.1%, después se incubó durante la noche en
amortiguador de bloqueo de bloque SEA. La etapa de bloque se
5 sigue por una incubación de las células durante la noche con
un anticuerpo de conejo contra pGSK3b/Ser9, seguida por un
IgG anti-conejo de cabra. El pGSK3b se detecta por
SuperSignal ELISA Femto, siguiendo el procedimiento del
vendedor. El compuesto del Ejemplo 1 inhibe a pGSK3b en este
10 ensayo con un IC₅₀ de 2.04 +/- 0.68 (n=8) µm.

Estudios de Inhibición de Objetivo *in vivo*

Inhibición de objetivo *in vivo* por una inyección IV única
Células U87MG que crecen exponencialmente derivadas
15 de un glioblastoma humano, son subcutáneamente implantadas en
el flanco trasero de ratones desnudos. Cuando los tumores
alcanzan el tamaño de 200-250 mm³, los compuestos son
administrados a los animales por una inyección IV única en un
estudio de respuesta de dosis o en un estudio en el curso de
20 tiempo. Al final de cada tratamiento, los animales son
asfixiados con CO₂. Los tumores son colectados por escisión
quirúrgica, congelados rápidamente en nitrógeno líquido, y
almacenados a -80°C hasta los análisis. El suero se prepara
de la sangre colectada del corazón por punción cardiaca y se
25 almacena a -80°C hasta el análisis.

Análisis de muestra:

Se extrae el inhibidor de Akt del suero con acetonitrilo/metanol y se analiza junto con un estándar interno por CL/EM/EM. Los tumores son homogeneizados en 2 volúmenes de un amortiguador de lisis que contiene 25 mM de Tris (pH 7.5). Los inhibidores de proteasa completos de Roche y 1 mM de vanadato con un homogenizador Powergen 125, son entonces pasados secuencialmente a través de una aguja de calibre 18 y una aguja de calibre 23. Los extractos citoplásmicos solubles, son colectados de la fracción sobrenadante, después que los lisados son centrifugados por 30 minutos a 20,000 x g. Las concentraciones de proteínas en los extractos citoplásmicos, se determinan con un kit BCA. El Fosfo-GSK3b (pGSK3b) en los extractos solubles, es analizado con el kit de ELISA.

Para determinar glioblastoma en este ensayo, el compuesto del Ejemplo 1 inhibe a pGSK3b por 76% a 1 hora usando inyección IV única de 25 mg/kg.

20 Estudios de Inhibición de Crecimiento de Tumor *in vivo*

Células U87MG que crecen exponencialmente derivadas de un glioblastoma humano o células H1155 derivadas de cáncer de pulmón de células no pequeñas humanas, son subcutáneamente implantadas en el flanco trasero de ratones desnudos. Tres días después de los implantes de células tumorales, se

realiza microcirugía para canular el catéter para infusión continua vía la vena yugular. El Ejemplo 1, formulado en D5W (5% de dextrosa en agua) a varias concentraciones, es entonces infundido en ratones al siguiente día a 40 µl/hr por 4 días. Los tumores son medidos dos veces semanalmente hasta que se termina el estudio, usualmente a 24 días después de los implantes iniciales. La inhibición del crecimiento del tumor se calcula dividiendo el volumen de tumor promedio del grupo tratado, por aquel del grupo tratado con vehículo.

10 Para glioblastoma, el compuesto del Ejemplo 1 inhibe el crecimiento del tumor por 50%, usando infusión continua de 27 mg/kg/día cuando se mide al final del ensayo.

 Para cáncer de pulmón de células no pequeñas, el compuesto del Ejemplo 1 inhibe el crecimiento del tumor por 15 63% usando infusión continua de 24 mg/kg/día cuando se mide al final del ensayo.

Estudios de Composición Farmacéutica

 Una composición farmacéutica de semi-hidrato 20 monoclóridato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, comprende un azúcar no reductor y un agente que ajusta el pH. Los estudios de solubilidad muestran que el manitol no parece impactar la solubilidad del semi-hidrato monoclóridato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol. Sin embargo, la solubilidad del semihidrato es 25

dependiente del pH y es >19 mg/ml a $\text{pH} \leq 3.91$. También, la relación en la cual toma lugar la precipitación, es dependiente del pH.

- 5 Tabla 1: Efecto de pH de solución inicial en la velocidad de precipitación (ppt). Todas las soluciones son preparadas inicialmente a aproximadamente 15 mg de semihidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol/ml y almacenadas a temperatura ambiente.

10

Medio	pH (inicial)	Precipitación (ppt)
HCl 0.05		Sin ppt hasta
N/manitol al 3%	1.68	96 horas
HCl 0.01		sin ppt hasta
N/manitol al 3%	3.19	96 horas
HCl 0.005		ppt después
N/manitol al 3%	3.58	de 48 hr
HCl 0.0025		ppt después
N/manitol al 3%	3.91	de 36 hr
HCl 0.001		ppt después
N/manitol al 3%	4.20	de 24 hr

15

Los análisis de CL/EM del precipitado disuelto nuevamente, indican que es una molécula "similar a dímero" (Espectro de masas (CLEM) m/z) 640.2 ($M+H^+$), que forma dos

20 moléculas de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol (Espectro de masas (CLEM) m/z = 329.1 ($M+H^+$), para 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol), después de la pérdida de amoníaco. Adicionalmente, la precipitación no parece ser debido a un cambio en una forma de estado

25 sólido menos soluble.

REIVINDICACIONES

Habiéndose descrito la invención como antecede, se reclama como propiedad lo contenido en las siguientes reivindicaciones.

5

1. Un compuesto, caracterizado porque es 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, o una sal del mismo farmacéuticamente aceptable o un hidrato del compuesto o la sal del mismo.

10

2. El compuesto de conformidad con la reivindicación 1, caracterizado porque es diclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

3. El compuesto de conformidad con la reivindicación 1, caracterizado porque es semi-hidrato monoclorhidrato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol.

15

4. El compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3, para uso como un medicamento.

5. Uso del compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3, para la manufactura de un medicamento para tratar mieloma múltiple, cáncer de pulmón de células no pequeñas, glioblastoma, neuroblastoma, melanoma o neoplasmas de la próstata, mama, ovarios, estómago primario, tipo intestinal, endometrio, tiroides, páncreas, pulmón o vejiga.

25

6. Uso del compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3, para la manufactura de un medicamento para tratar mieloma múltiple, cáncer de pulmón de células no pequeñas, glioblastoma, o neoplasmas de la próstata, mama u ovarios.

7. Uso del compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3, para la manufactura de un medicamento para tratar cáncer de pulmón de células no pequeñas o glioblastoma.

8. Uso del compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3, para la manufactura de un medicamento para tratar hepatitis C, rubéola, virus de inmunodeficiencia humana (VIH), hepatitis V, o citomegalovirus humano (HCMV).

9. Un método para tratar mieloma múltiple, cáncer de pulmón de células no pequeñas, glioblastoma, neuroblastoma, melanoma o neoplasmas de la próstata, mama, ovarios, estómago primario, tipo intestinal, endometrio, tiroides, páncreas, pulmón o vejiga, en un paciente en necesidad el mismo, caracterizado porque comprende administrar una cantidad efectiva del compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3.

10. Un método para tratar para la manufactura de un medicamento para tratar mieloma múltiple, cáncer de pulmón de células no pequeñas, glioblastoma, o neoplasmas de la

próstata, mama u ovarios, que comprende en un paciente en necesidad del mismo, caracterizado porque comprende administrar una cantidad efectiva del compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3.

5 11. Un método para tratar cáncer de pulmón de células no pequeñas o glioblastoma en un paciente en necesidad del mismo, caracterizado porque comprende administrar una cantidad efectiva del compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3.

10 12. Un método para tratar la hepatitis C, rubéola, virus de inmunodeficiencia humana (VIH), hepatitis B, o citomegalovirus humano (HCMV) en un paciente en necesidad del mismo, caracterizado porque comprende administrar una cantidad efectiva del compuesto de conformidad con cualquiera
15 de las reivindicaciones 1 a 3.

 13. Una composición farmacéutica, caracterizada porque comprende el compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3, y un portador, diluyente o excipiente farmacéuticamente aceptable.

20 14. Una composición farmacéutica liofilizada, caracterizada porque comprende el compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3, y un portador, diluyente o excipiente farmacéuticamente aceptable, en donde el pH de dicha composición cuando se diluye con el diluyente
25 acuoso, es menos de 4.2 y mayor de 2.0.

15. La composición farmacéutica liofilizada de conformidad con la reivindicación 14, caracterizada porque el pH es menos de 3.2 y mayor de 2.0.

16. La composición farmacéutica liofilizada de conformidad con la reivindicación 15, caracterizada porque el pH es menos de 2.8 y mayor de 2.0.

17. Una composición farmacéutica, caracterizada porque comprende el compuesto de conformidad con cualquiera de las reivindicaciones 1 a 3, y un portador, diluyente o excipiente farmacéuticamente aceptable en solución, en donde el pH de dicha composición es menos de 4.2 y mayor de 2.0.

18. La composición farmacéutica de conformidad con la reivindicación 17, caracterizada porque el pH de dicha composición es menos de 3.2 y mayor de 2.0.

19. La composición farmacéutica de conformidad con la reivindicación 18, caracterizada porque el pH de dicha composición es menos de 2.8 y mayor de 2.0.

20. Semi-hidrato monoclóridato de 4-[5-(2-amino-etansulfonil)-isoquinolin-7-il]-fenol, caracterizado porque está en la forma cristalina que tiene un patrón de difracción en polvo de rayos X con picos intensos a $2\theta = 4.9, 14.8$ y 10.2

RESUMEN DE LA INVENCION

La presente invención se refiere a 4-[5-(2-amino-
etansulfonil)-isoquinolin-7-il]-fenol o una sal del mismo
5 farmacéuticamente aceptable o un hidrato del compuesto o la
sal del mismo, como inhibidores de AKt, que son agentes
antineoplásicos y/o antivirales, así como también a
composiciones que comprende estos compuestos y a métodos para
usar estos compuestos.

10

15

20

PATENT COOPERATION TREATY

RECEIVED

JAN 29 2008

From the INTERNATIONAL SEARCHING AUTHORITY

PCT
ELI LILLY AND COMPANY
Patent DivisionNOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

To.

ELI LILLY AND COMPANY
Attn. Hostettler, Danica ✓
Patent Division,
P.O. Box 6288
Indianapolis, IN 46206-6288
ETATS-UNIS D'AMERIQUE

(PCT Rule 44.1)

Date of mailing
(day/month/year)

22/01/2008

Applicant's or agent's file reference

X17330 ✓

FOR FURTHER ACTION

See paragraphs 1 and 4 below

International application No.

PCT/US2007/070945 ✓

International filing date
(day/month/year)

12/06/2007 ✓

Applicant

ELI LILLY AND COMPANY

1. ☒ 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 two months from the date of transmittal of the International Search Report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 338.82.70

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 90bis.1 and 90bis.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

Emmanuel Cherqui

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 and the written opinion of the International Searching Authority, 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 (see *PCT Applicant's Guide*, Volume I/A, Annexes B1 and B2).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, Volume I/A, paragraph 296).

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 46.1).

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 (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.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference X17330	FOR FURTHER ACTION		see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2007/070945	International filing date (day/month/year) 12/06/2007	(Earliest) Priority Date (day/month/year) 20/06/2006	
Applicant ELI LILLY AND COMPANY			

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

This international search report consists of a total of 4 sheets.

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

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed
☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (See Box No. II)

3. ☐ **Unity of invention is lacking** (see Box No III)

4. With regard to the **title**,

- ☒ the text is approved as submitted by the applicant
☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- ☒ the text is approved as submitted by the applicant
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box 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 regard 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

102

International application No
PCT/US2007/070945

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D217/02 A61K31/435 A61P35/00 A61P31/18

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)

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, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/054202 A (LILLY CO ELI [US]; BARDA DAVID ANTHONY [US]; HENRY KENNETH JAMES JUNIO) 16 June 2005 (2005-06-16) pages 1-2,81; example 98; compound 105 -----	1-20



Further documents are listed in the continuation of Box C



See patent family 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
- *G* document member of the same patent family

Date of the actual completion of the international search

15 January 2008

Date of mailing of the international search report

22/01/2008

Name and mailing address of the ISA/

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

BOURGHIDA, E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/070945

Box No. 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. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 9-12 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 No. 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 allsearchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
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 and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

PATENT COOPERATION TREATY

105

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

22 MAR 2008

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

X 17330

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No
PCT/US2007/070945

International filing date (day/month/year)
12.06.2007

Priority date (day/month/year)
20.06.2006

International Patent Classification (IPC) or both national classification and IPC
INV. C07D217/02 A61K31/435 A61P35/00 A61P31/18

Applicant
ELI LILLY AND COMPANY

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 43bis.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") except that 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 66.1bis(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 3 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



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Date of completion of
this opinion

see form
PCT/ISA/210

Authorized Officer

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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2007/070945

Box No. 1 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 filed
 - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))
3. 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:
 - ☐ on paper
 - ☐ in electronic form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed.
 - ☐ filed together with the international application in electronic form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
4. ☐ 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.
5. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**International application No.
PCT/US2007/070945**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. 9-12

because:

☒ the said international application, or the said claims Nos. 9-12 (with respect to industrial applicability) relate to the following subject matter which does not require an international search (*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 (*specify*):

☐ no international search report has been established for the whole application or for said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2007/070945

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	<u>2-12,20</u>
	No: Claims	<u>1,13-19</u>
Inventive step (IS)	Yes: Claims	
	No: Claims	<u>1-20</u>
Industrial applicability (IA)	Yes: Claims	<u>1-8,13-20</u>
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43bis.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43bis.1 and 70.9)

see form 210

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2007/070945

Re Item III

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

- 1.1 Claims 9-12 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).
- 1.2 For the assessment of the present claims 9-12 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 treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- 2.1 Reference is made to the following document:

D1: WO 2005/054202 A (LILLY CO ELI [US]; BARDA DAVID ANTHONY [US];
HENRY KENNETH JAMES JUNIO) 16 June 2005 (2005-06-16)

Novelty:

- 3.1 D1 discloses the compound (4-hydroxy-phenyl)- N-aminoethyl- isoquinoline-5-sulfonic acid only as a synthetic intermediate (see D1: preparation 105 page 42). Thus, claims 1,13-19 are not novel and claims 2-12,20 are novel in the sense of Article 33(2) PCT.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2007/070945

Inventive Step:

- 4.1 D1 is regarded as the closest prior art for the inventive step assessment of the subject-matter of claims 2-12,20 and discloses
(4-hydroxy-phenyl)-isoquinoline-5-sulfonic acid {2- [3- (4-nitro-phenyl)-propylamino]-ethyl}-amide, dihydrochloride salt inhibitor of Akt and used for cancer and viral infections (see D1: example 98 page 81; description pages 1-2).
- 4.2 The compounds of claims 2,3 and 20 differ from the example of D1 in that the aminoethanesulfonyl moiety is unsubstituted whereas in the example of D1 the said aminoethanesulfonyl moiety is substituted by phenyl-propyl chain.
- 4.3 The problem to be solved can be regarded as the provision of further compounds to be used for cancer and viral infections.
- 4.4 The solution proposed by the present application is not regarded as involving an inventive step in the sense of Article 33(3) PCT because the moiety
4-hydroxy-phenyl-N-aminoethyl- isoquinoline-5-sulfonic acid is already known in the prior art for its uses as medicament to treat cancer and viral diseases, and the compounds of claims 2,3,20 only consist of variations of substituents on the said moiety.
Such variations can only be regarded as inventive if they present unexpected effects or properties in relation to the compound disclosed in D1. However, no such effects or properties are indicated in the application.

Hence, an inventive step cannot be acknowledged for the subject-matter of claims 1-20.

Industrial Applicability:

- 5.1 Claims 1-8,13-20 relate to a subject-matter which is considered to have an industrial applicability, and therefore the subject-matter of said claims meets the criteria of Article 33(4) PCT.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2007/070945

Re Item VIII

Certain observations on the international application

- 6.1 The relative term "intense" used in claim 20 has no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claim unclear, Article 6 PCT.

Possible steps after receipt of the international search report (ISR) and written opinion of the International Searching Authority (WO-ISA)

General information	For all international applications filed on or after 01/01/2004 the competent ISA will establish an ISR. It is accompanied by the WO-ISA. Unlike the former written opinion of the IPEA (Rule 66.2 PCT), the WO-ISA is not meant to be responded to, but to be taken into consideration for further procedural steps. This document explains about the possibilities.
Amending claims under Art. 19 PCT	Within 2 months after the date of mailing of the ISR and the WO-ISA the applicant may file amended claims under Art. 19 PCT directly with the International Bureau of WIPO. The PCT reform of 2004 did not change this procedure. For further information please see Rule 46 PCT as well as form PCT/ISA/220 and the corresponding Notes to form PCT/ISA/220.
Filing a demand for international preliminary examination	In principle, the WO-ISA will be considered as the written opinion of the IPEA. This should, in many cases, make it unnecessary to file a demand for international preliminary examination. If the applicant nevertheless wishes to file a demand this must be done before expiry of 3 months after the date of mailing of the ISR/ WO-ISA or 22 months after priority date, whichever expires later (Rule 54bis PCT). Amendments under Art. 34 PCT can be filed with the IPEA as before, normally at the same time as filing the demand (Rule 66.1 (b) PCT). If a demand for international preliminary examination is filed and no comments/amendments have been received the WO-ISA will be transformed by the IPEA into an IPRP (International Preliminary Report on Patentability) which would merely reflect the content of the WO-ISA. The demand can still be withdrawn (Art. 37 PCT).
Filing informal comments	After receipt of the ISR/WO-ISA the applicant may file informal comments on the WO-ISA directly with the International Bureau of WIPO. These will be communicated to the designated Offices together with the IPRP (International Preliminary Report on Patentability) at 30 months from the priority date. Please also refer to the next box.
End of the international phase	At the end of the international phase the International Bureau of WIPO will transform the WO-ISA or, if a demand was filed, the written opinion of the IPEA into the IPRP, which will then be transmitted together with possible informal comments to the designated Offices. The IPRP replaces the former IPER (international preliminary examination report).
Relevant PCT Rules and more information	Rule 43 PCT, Rule 43bis PCT, Rule 44 PCT, Rule 44bis PCT, PCT Newsletter 12/2003, OJ 11/2003, OJ 12/2003

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

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
27 December 2007 (27.12.2007)

PCT

(10) International Publication Number
WO 2007/149730 A3(51) International Patent Classification:
C07D 217/02 (2006.01) A61P 35/00 (2006.01)
A61K 31/435 (2006.01) A61P 31/18 (2006.01)(74) Agents: HOSTETTLER, Danica et al.; Eli Lilly And
Company, Patent Division, P. O. Box 6288, Indianapolis,
Indiana 46206-6288 (US).(21) International Application Number:
PCT/US2007/070945 ✓(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH,
CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG,
ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL,
IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK,
LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW,
MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL,
PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA,
ZM, ZW.

(22) International Filing Date: 12 June 2007 (12.06.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/805,260 ✓ 20 June 2006 (20.06.2006) ✓ US
60/885,765 ✓ 19 January 2007 (19.01.2007) ✓ US(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL,
PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).(71) Applicant (for all designated States except US): ELI
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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii))

Published:

- with international search report

(88) Date of publication of the international search report:
24 April 2008

(54) Title: INHIBITORS OF AKT (PROTEIN KINASE B)

(57) Abstract: 4-[5-(2-Amino-ethanesulfonyl)-isoquinolin-7-yl]-phenol or a pharmaceutically acceptable salt thereof or a hydrate
of the compound or the salt thereof as Akt inhibitors that are antineoplastic and/or antiviral agents as well as compositions comprising
these compounds and methods of using these compounds.

WO 2007/149730 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/070945

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D217/02 A61K31/435 A61P35/00 A61P31/18

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)

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, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/054202 A (LILLY CO ELI [US]; BARDA DAVID ANTHONY [US]; HENRY KENNETH JAMES JUNIO) 16 June 2005 (2005-06-16) pages 1-2,81; example 98; compound 105 -----	1-20



Further documents are listed in the continuation of Box C.



See patent family 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.

A document member of the same patent family

Date of the actual completion of the international search

15 January 2008

Date of mailing of the international search report

22/01/2008

Name and mailing address of the ISA/

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Authorized officer

BOURGHIDA, E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/070945

Box No. 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. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 9-12 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 dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

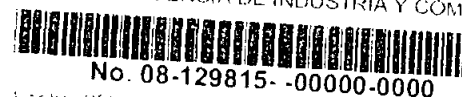
Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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 allsearchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
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 and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.



No. 08-129815-00000-0000

Fecha: 2008-12-05 16:16:04
Ley: 11 PATENTE Y
Act. 411 PRESENTACION

Dep: 2020 NULCREACIONE
Lvs: 378 FASE NACIONAL
Folios: 116

ercio
NCIA

REQUISITOS MINIMOS PARA ADMISION A TRAMITE PCT

PATENTE DE INVENCION

[X]

MODELO DE UTILIDAD

[]

- ◆ Indicación de que se solicita la concesión de una patente [X]
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud [X]
- ◆ Descripción de la invención [X]
- ◆ Dibujos de ser estos pertinentes [X]
- ◆ Comprobante de pago de las tasas establecidas [X]

COMPLETA []

INCOMPLETA []

DISEÑO INDUSTRIAL []

- ◆ Indicación de que se solicita el registro de Diseño Industrial []
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ◆ Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño []
- ◆ Comprobante de pago de las tasas establecidas []

COMPLETA []

INCOMPLETA []

ESQUEMA DE TRAZADO []

- ◆ Indicación de que se solicita el registro de un Esquema de Trazado []
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ◆ Representación gráfica del esquema trazado []
- ◆ Comprobante de pago de las tasas establecidas []

COMPLETA []

INCOMPLETA []

Sandra Borrero
Firma Responsable

Fecha 21-05-08

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