

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

Re: **P1.-ASTRAZENECA AB.- Solicitud de Patente de Invención**
para "COMPUESTO Y METODO PARA EL TRATAMIENTO
DEL DOLOR",- Clase A61.-Expediente número 00-097.100.-

1. Me refiero a mi solicitud arriba nombrada de fecha 21 de Diciembre de 2000.
2. Anexos me permito presentar a usted:
 - (1) Copias autenticas (2) de las primeras solicitudes de patente presentadas para el Invento arriba nombrado **respecto de la cual reclamo prioridad**, a saber:
 - (a) Copia autentica debidamente certificada de la solicitud estadinense número 60/171.906 de 23 de Diciembre de 1999, junto con la traducción oficial correspondiente a las legalizaciones y reivindicaciones de dicho documento;
 - (b) Copia autentica debidamente certificada de la solicitud estadinense número 60/236.783 de 29 de Septiembre de 2000, junto con la traducción oficial correspondiente a las legalizaciones y reivindicaciones de dicho documento;
 - (c) Copia debidamente autenticada por el Notario Sexto del Circulo de Bogotá, de los documentos que acreditan la calidad de Traductor Oficial de la señora María Luisa Brigard de Restrepo, quien elaboró la

traducción de las legalizaciones y reivindicaciones de las solicitudes estadinenses número 60/171.906 y 60/236.783 de 23 de Diciembre de 1999 y 29 de Septiembre de 2000 respectivamente.

- (2) Documento que acredita el traspaso que los autores del invento de la referencia hacen a favor de mí representando, de sus derechos sobre el mismo, debidamente legalizado hasta por el Ministerio de Relaciones Exteriores de Colombia bajo el número 214839 de 30 de Enero de 2001;
- (3) Traducción Oficial No. 417, debidamente legalizada hasta por el Ministerio de Relaciones Exteriores de Colombia bajo el número 408341 de 9 de Febrero de 2001, correspondiente a dicho documento de traspaso;
- (4) Documento que acredita el traspaso que los autores del invento de la referencia hacen a favor de mí representando, de sus derechos sobre el mismo, debidamente legalizado hasta por el Ministerio de Relaciones Exteriores de Colombia bajo el número 214840 de 30 de Enero de 2001;
- (5) Traducción Oficial No. 418, debidamente legalizada hasta por el Ministerio de Relaciones Exteriores de Colombia bajo el número 408342 de 9 de Febrero de 2001, correspondiente a dicho documento de traspaso.

3. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor SuperIntendente,


JOSE ANTONIO FLOREDA
T.P. 10.784
Código 231

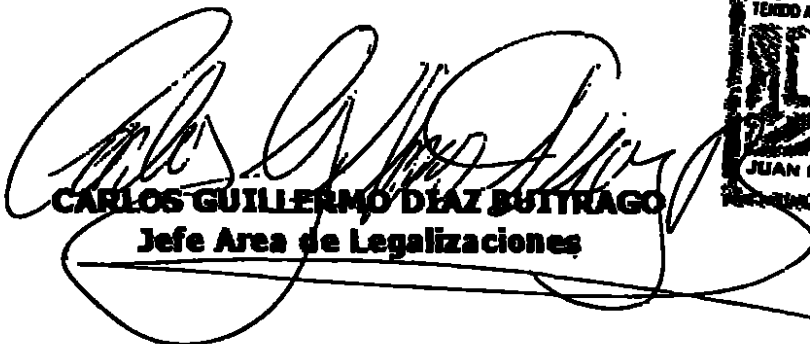
**EL SUSCRITO JEFE DEL AREA DE
LEGALIZACIONES DEL MINISTERIO DE
RELACIONES EXTERIORES**

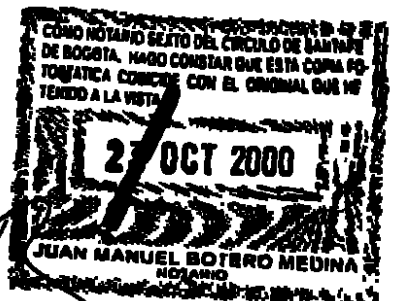
CERTIFICA

Que revisados los registros que se llevan en la oficina de Legalizaciones aparece la firma de la señora **MARIA LUISA DE BRIGARD DE RESTREPO**, como traductora e intérprete oficial para los idiomas **INGLES - ESPAÑOL, ESPAÑOL-INGLES**, según resolución 0793 del 21 de marzo de 1958

La anterior certificación se expide a solicitud de la interesada quien se identificó con cédula de ciudadanía No. 20.024.691 expedida en Santa Fe de Bogotá.

Dada en Santa Fe de Bogotá, D.C. a los veinticinco (25) días de marzo de 1999


CARLOS GUILLERMO DIAZ BUTRAGO
Jefe Area de Legalizaciones



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TRADUCCION OFICIAL No. 5.800

de un documento escrito en INGLÉS, el cual para su identificación se sella con el sello del Traductor al mismo tiempo que la presente. -

ESTADOS UNIDOS DE AMERICA

A CUANTOS LLEGAREN LAS PRESENTES:

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de Fábrica de los Estados Unidos

TRADUCCION OFICIAL No. 5.800
4 de diciembre del 2000
Marta Leticia Rodríguez
Intendente General
del Oficio de Traducción

POR LA PRESENTE SE CERTIFICA QUE ANEXA AQUI SE ENCUENTRA UNA COPIA FIEL, TOMADA DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE FABRICA DE LOS ESTADOS UNIDOS, DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA ABAJO QUE SATISFACEN LOS REQUISITOS PARA QUE SEA OTORGADA UNA FECHA DE REGISTRO SEGUN 35 USC 111.

NUMERO DE SOLICITUD: 60/171,906

FECHA DE REGISTRO: 23 de diciembre de 1999.

Por Autorización del

COMISIONADO DE PATENTES Y MARCAS DE FABRICA

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(Firma)

L. EDELEN

Funcionario Certificador

Sello: OFICINA DE PATENTES Y MARCAS DE FABRICA - ESTADOS UNIDOS DE AMERICA -

Por el presente certifico que los documentos y/o derechos anexos se están depositando en la "Oficina de Correo Expreso para el Destinatario" del servicio Postal de los Estados Unidos en la fecha indicada arriba en un sobre dirigido a la Casilla Provisional, Comisionado Auxiliar de Patentes, Washington, D.C., 20231.

5.800
Maria Luisa Brizuela
Intérprete Oficial
Cm. No. 0702 del MIA de Justicia

23 de diciembre de 1999

(Fecha)

Kenneth F. Mitchel

(Nombre Impreso)

(Firma)

(Firma)

Zeneca Inc.

Expediente No.

EN LA OFICINA DE PATENTES Y MARCAS DE FABRICA
DE LOS ESTADOS UNIDOS

CASILLA PROVISIONAL

Comisionado Auxiliar para Patentes

Washington, DC 20231

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Señor:

TRANSMISION DE SOLICITUD DE PATENTE PROVISIONAL

SEGUN 37 CFR §1.53(c)

Con la presente se transmite para un registro de Solicitud Provisional la solicitud de patente de: Inventor(es): Thomas Michael Bare, 1057 Windy Knoll Road, West Chester, Pennsylvania 19382; Rebecca Urbaneck, 1800 Concord Pike, Wilmington, Delaware 19850-5437; Dean Brown, 1800 Concord Pike, Wilmington, Delaware 19850-5437.

PROSECUTOR. OFFICE
by
Maria Luisa Brizuela de Restrepo
Interprete Oficial
No OTRO del país de Invención

Para: **COMPUESTOS DE PIRIDAZINO-QUINOLINO-DIONA PARA EL DOLOR**

Dirigir toda la correspondencia a:

Número del Cliente 22466

Sírvase dirigir todas las llamadas telefónicas a Kenneth F. Mitchell al teléfono número (302) 866-7466.

1. Anexos están:

- X Descripción (77 páginas)
- X Otros (especificar) Tarjeta de Devolución Postal

2. Los derechos totales por la suma de \$150.00 deben cargarse a la Cuenta de Depósito No. 26-0166. Esta hoja se incluye en duplicado.

2. X El Comisionado Auxiliar es autorizado por la presente para cobrar cualesquiera derechos adicionales requeridos o acreditar cualquier excedente de pago a la Cuenta de Depósito Número 26-0166. Esta hoja se incluye en duplicado.

La invención no fue hecha por una agencia del Gobierno de los Estados Unidos ni bajo un contrato con una agencia del Gobierno de los Estados Unidos.

TRANSACCION. OFICIAL No. 5800
Marta Luisa Briceño de Rodríguez
Interpretada Oficial
del Nivel del MIA de San Juan

Presentado respetuosamente,

ZENECA Inc.

Fecha: 23 de diciembre de 1999

Por: _____ (Firma)

Kenneth F. Mitchell, Ph. D.

Reg. No. 42,007

Apoderado de los Solicitantes

Teléfono: 302/886-7466

Zeneca, Inc.

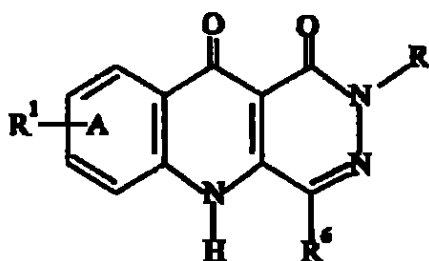
Expediente No.

ANEXO:

KFM.1999004

REIVINDICACIONES

1. Un método para el tratamiento del dolor que consiste en administrar a un individuo necesitado de ello una cantidad efectiva de cualquier compuesto acorde con el diagrama estructural I,



I

PROCESO. OFICIAL No. 5800
Marta Leticia Briceño de Restrepo
Intendente Oficial
No. 1700 del 11 de 1999

o sales farmacéuticamente aceptables de tales compuestos, tautómeros de tales compuestos
o isómeros ópticos de tales compuestos,

en donde:

R^1 es escogido, en cada caso independientemente, entre hidrógeno, halo y NO_2 , y
el anillo A tiene cuatro grupos R^1 ;

R es escogido entre $(CH_2)_nL$, y $C(R^2)(CH_2)_nL$, en donde n es un entero escogido
entre la escala de 0 a 6, y R^2 es escogido, en cada caso independientemente, entre
hidrógeno, halógeno, alquilo C_{1-4} y cicloalquilo C_{3-6} ;

L es fenilo sustituido con uno, dos, tres o cuatro grupos escogidos entre: OR^3 en donde R^3 es escogido entre hidrógeno y piridil-metilo, $N(R^4)_2$ en donde R^4 es escogido, en cada caso independientemente, entre hidrógeno y alquilo C_{1-4} , halógeno, carboxilo, alquilo C_{1-4} , O-alquilo C_{1-4} , alqueno C_{2-4} , alquino C_{2-4} , un grupo heteroarilo escogido entre furanilo, oxazolilo, oxadiazolilo, tiadiazola, tetrazolilo, morfolinilo y bencimidazolilo, morfolino-N-carbonilo, el grupo $OC(O)N(R^5)_2$ en donde R^5 es escogido, en cada caso independientemente, entre hidrógeno, alquilo C_{1-4} y fenilo; o

L es heteroarilo escogido entre piridilo, isooxazolilo, oxadiazolilo, tiofenilo, benzo[b]tiofenilo, benzofuranilo y pirazinilo; en donde dicho heteroarilo está sustituido con uno, dos o tres grupos escogidos entre: hidroxilo, alquilo C_{1-4} , hidroxialquilo C_{1-4} y metoxifenilo; o

TRANSLACION OFICIAL No. 5.820
Luis Brizuela
Escritor de Patentes
Escritor de Patentes

L es tetrahidrofuranilo; y

R^6 es escogido entre OH y $OC(O)N(R^7)_2$ en donde R^7 es escogido, en cada caso independientemente, entre hidrógeno y alquilo C_{1-4} .

2. Un método acorde con la Reivindicación 1 en el que en dicho compuesto acorde con el diagrama estructural I:

R^1 y el anillo A en combinación son 7-halofenilo y L es escogido entre grupos fenilo, piridilo, isooxazolilo, oxadiazolilo, tiofenilo, benzo[b]tiofenilo, benzofuranilo y pirazinilo, en donde, cuando están sustituidos, dichos grupos están sustituidos con una,

dos o tres mitades escogidas independientemente en cada caso entra halo, hidroxilo, oxo, carbonilo, carbonilamino, alquilo C_{1-4} e hidroxialquilo C_{1-4} .

3. Un método acorde con la Reivindicación 2 en el que en dicho compuesto acorde con el diagrama estructural I:

R^1 y el anillo A en combinación son 7-clorofenilo.

4. Un método acorde con la Reivindicación 1 en el que en dicho compuesto acorde con el diagrama estructural I:

PROCESO OFICIAL No. 5.200
Marta Luisa Brizuela de Riquelme
Interpretación Oficial
del 27 de mayo del 2005

R es $(CH_2)_nL$ en donde n es un entero escogido entre la escala de 0 a 6, y L es fenilo sustituido con uno, dos, tres o cuatro grupos escogidos entre: OR^3 en donde R^3 es escogido entre hidrógeno y piridil-metilo, $N(R^4)_2$ en donde R^4 es escogido, en cada caso independientemente, entre hidrógeno y alquilo C_{1-4} , halógeno, carboxilo, alquilo C_{1-4} , O-alquilo C_{1-4} , alquenilo C_{2-4} , alquinilo C_{2-4} , un grupo heteroarilo escogido entre furanilo, oxazolilo, oxadiazolilo, tiadiazola, tetrazolilo, morfolinilo y bencimidazolilo, morfolino-N-carbonilo, el grupo $OC(O)N(R^5)_2$ en donde R^5 es escogido, en cada caso independientemente, entre hidrógeno, alquilo C_{1-4} y fenilo y R^6 es hidroxilo.

5. Un método acorde con la Reivindicación 1 en el que en dicho compuesto acorde con el diagrama estructural I:

R es $(CH_2)_nL$ en donde n es un entero escogido entre la escala de 0 a 6, y L es

heteroarilo escogido entre piridilo, isooxazolilo, oxadiazolilo, tiofenilo, benzo[b]tiofenilo, benzofuranilo y pirazinilo; en donde dicho heteroarilo está sustituido con uno, dos o tres grupos escogidos entre: hidroxilo, alquilo C₁₋₄, hidroxialquilo C₁₋₄ y metoxifenilo y R⁶ es hidroxilo.

- 6. Un método acorde con la Reivindicación 1 en el que en dicho compuesto acorde con el diagrama estructural I:**

R es $(CH_2)_nL$ en donde n es un entero escogido entre la escala de 0 a 6
tetrahidrofuranilo y R^6 es hidroxilo.

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 E. **Carlos Lainez Brizuela de Rodriguez**
 Interrogado Oficial
 Num. Pm. 0193 del 701a de Justicia

- 7. Un método acorde con la Reivindicación 1 en el que en dicho compuesto acorde con el diagrama estructural I:**

R es $C(R^2)(CH_2)_nL$, en donde n es un entero escogido entre la escala de 0 a 6, y R^2 es escogido, en cada caso independientemente, entre hidrógeno, halógeno, alquilo C_{1-4} y cicloalquilo C_{3-6} .

- 8. Un método acorde con la Reivindicación 1 en el que dicho compuesto es escogido entre:**

**7-cloro-4-hidroxi-2-(2,4,6-trimetilfenil)-1,2,5,10-tetrahidropiridazino[4,5 -
b]quinolino-1,10-diona;**

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7,9-dicloro-4-hidroxi-2-(4-metoxi-2-metilfenil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

7-cloro-4-hidroxi-2-(2-cloro-4-metilfenil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

metil-4-[(7-cloro-4-hidroxi-1,10-dioxo-2,5-dihidropiridazino[4,5-b]quinolin-2-il)metil]benzoato;

7-cloro-4-hidroxi-2-(4-[1,3,4]oxadiazol-2-il-bencil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (7);

TRANSCORP. OFFICIAL NO. 5800
Jorge Rodríguez de Rodríguez
Instituto Superior de Investigaciones Científicas
CSIC - CSIC del CSIC

7-cloro-4-hidroxi-2-[4-(morfolino-4-carbonil)bencil]-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (7);

7-cloro-4-hidroxi-2-[3-(1h-tetrazol-5-il)-bencil]-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (7);

4-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-n-furan-2-ilmetil-benzamida;

preparación de metanosulfonato de 7-cloro-4-hidroxi-2-(4-dimetilaminobencil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (4);

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metanosulfonato de 7-cloro-4-hidroxi-2-(bencimidazol-5-ilmetil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

7-cloro-4-hidroxi-2-(4-hidroxi-3-metil-bencil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (2);

7-cloro-4-hidroxi-2-[3-metil-4-(piridin-2-ilmetoxi)-bencil]-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (2);

4-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-2-metilfeniléster de ácido dimetilcarbámico (7) (método a)

REGISTRO ESPECIAL No. 52017
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4-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-2-metilfeniléster de ácido dimetilcarbámico (7) (método b)

3-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-feniléster de ácido dimetilcarbámico;

4-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-feniléster de ácido metilfenilcarbámico;

4-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-feniléster de ácido dietilcarbámico;

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3-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-feniléster de ácido dietilcarbámico;

3-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-feniléster de ácido morfolino-4-carboxílico;

3-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-feniléster de ácido metilfenilcarbámico;

3-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-feniléster de ácido difenilcarbámico;

PROSECUTOR GENERAL
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INVESTIGADOR DE RUMORES

3-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-feniléster de ácido pirrodinilo-1-carboxílico;

4-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-2-metilfeniléster de ácido morfolino-4-carboxílico;

4-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-2-metilfeniléster de ácido metilfenilcarbámico;

5-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-2-metoxifeniléster de ácido dimetilcarbámico;

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5-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-2-metoxifeniléster de ácido dietilcarbámico;

5-(7-cloro-4-hidroxi-1,10-dioxo-5,10-dihidro-1h-piridazino[4,5-b]quinolin-2-ilmetil)-2-metoxifeniléster de ácido morfolino-4-carboxílico;

metanosulfonato de 7-cloro-4-hidroxi-2-(4-piridilmetil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

metanosulfonato de 7-cloro-4-hidroxi-2-(3-piridilmetil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

ANEXO. ORIGINAL NO. 5900
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metanosulfonato de 7-cloro-4-hidroxi-2-(2-piridilmetil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

metanosulfonato de 7-cloro-4-hidroxi-2-[3-hidroxi-5(hidroximetil)-2-metil(4-piridil)]metil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

(7-cloro-1,10-dioxo-2-piridin-4-ilmetil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolin-4-il-éster de ácido dimetilcarbámico (2);

7-cloro-4-hidroxi-2-(4-piridiletil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

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metanosulfonato de 7-cloro-4-hidroxi-2-(pirazin-2-ilmetil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (5);

7-cloro-4-hidroxi-2-(furan-2-ilmetil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

7-cloro-4-hidroxi-2-[2,5-dimetil(3-furanyl)]metil-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (4);

7-cloro-4-hidroxi-2-(tetrahydro-furan-2-ilmetil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

TRANSMISION. SPECIAL NO. 5800
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7-cloro-4-hidroxi-2-(5-isoxazolino)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (3);

7-cloro-4-hidroxi-2-[5-(4-metoxi-fenil)-[1,3,4]oxadiazol-2-ilmetil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona (6);

2-(benzo[b]tiofen-2-ilmetil)-7-cloro-4-hidroxi-2,5-dihidropiridazino[4,5-b]quinolino-1,10-diona;

7-cloro-10-hidroxi-2-[(3-metil(2-tienil)metil]-2,3-dihidropiridazino[4,5-b]quinolino-1,4-diona;

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7-cloro-4-hidroxi-2-[4-(carbometoxi)fenil-2-etil]-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

metanosulfonato de (+/-) 7-cloro-4-hidroxi-2-(3-piridilet-1-il)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

metanosulfonato de (+/-) 7-cloro-4-hidroxi-2-(2-piridilet-1-il)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

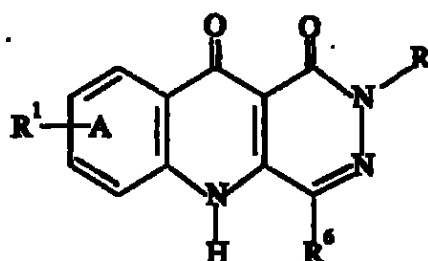
metanosulfonato de (+/-) 7-cloro-4-hidroxi-2-(4-piridilet-1-il)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona;

TRANSCRIPCION OFICIAL No. 5820
Escriba Luis Briceño
Intendente Oficial
San. No. 0722 del Min. de Justicia

metanosulfonato de (+/-) 7-cloro-4-hidroxi-2-(4-piridilpropil)-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona; y

7-cloro-4-hidroxi-2-(2-benzofuranil)metil-1,2,5,10-tetrahidropiridazino[4,5-b]quinolino-1,10-diona.

9. Una composición farmacéutica que tiene como ingrediente activo un compuesto acorde con el diagrama estructural I



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o sales farmacéuticamente aceptables de tales compuestos, tautómeros de tales compuestos
o isómeros ópticos de tales compuestos,

en donde:

TRANSCURRIDO: OPTICAL No. 5.800
 María Luisa Briceño de Rodríguez
 Intendente General
 1999 del año de la Unión

R^1 es escogido, en cada caso independientemente, entre hidrógeno, halo y NO_2 , y
el anillo A tiene cuatro grupos R^1 ;

R es escogido entre $(\text{CH}_2)_n\text{L}$, y $\text{C}(\text{R}^2)(\text{CH}_2)_n\text{L}$, en donde n es un entero escogido
entre la escala de 0 a 6, y R^2 es escogido, en cada caso independientemente, entre
hidrógeno, halógeno, alquilo C_{1-4} y cicloalquilo C_{3-6} ;

L es fenilo sustituido con uno, dos, tres o cuatro grupos escogidos entre: OR^3 en
donde R^3 es escogido entre hidrógeno y piridil-metilo, $\text{N}(\text{R}^4)_2$ en donde R^4 es escogido,
en cada caso independientemente, entre hidrógeno y alquilo C_{1-4} , halógeno, carboxilo,
alquilo C_{1-4} , O-alquilo C_{1-4} , alqueno C_{2-4} , alquino C_{2-4} , un grupo heteroarilo escogido
entre furanilo, oxazolilo, oxadiazolilo, tiadiazola, tetrazolilo, morfolinilo y

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bencimidazolilo, morfolino-N-carbonilo, el grupo $OC(O)N(R^5)_2$ en donde R^5 es escogido, en cada caso independientemente, entre hidrógeno, alquilo C_{1-4} y fenilo; o

L es heteroarilo escogido entre piridilo, isooxazolilo, oxadiazolilo, tiofenilo, benzo[b]tiofenilo, benzofuranilo y pirazinilo; en donde dicho heteroarilo está sustituido con uno, dos o tres grupos escogidos entre: hidroxilo, alquilo C_{1-4} , hidroxialquilo C_{1-4} y metoxifenilo; o

L es tetrahidrofurano; y

R^6 es escogido entre OH y $OC(O)N(R^7)_2$ en donde R^7 es escogido, en cada caso independientemente, entre hidrógeno y alquilo C_{1-4} y

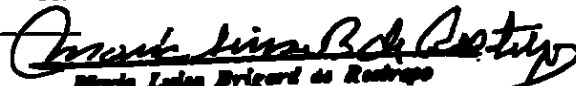
un excipiente o diluyente farmacéuticamente aceptable.

ES TRADUCCION FIEL Y COMPLETA, de la cual queda copia en los archivos de esta oficina de traducciones, para su confrontación y a la que me remito.

Derechos de Traducción: \$144.000,00

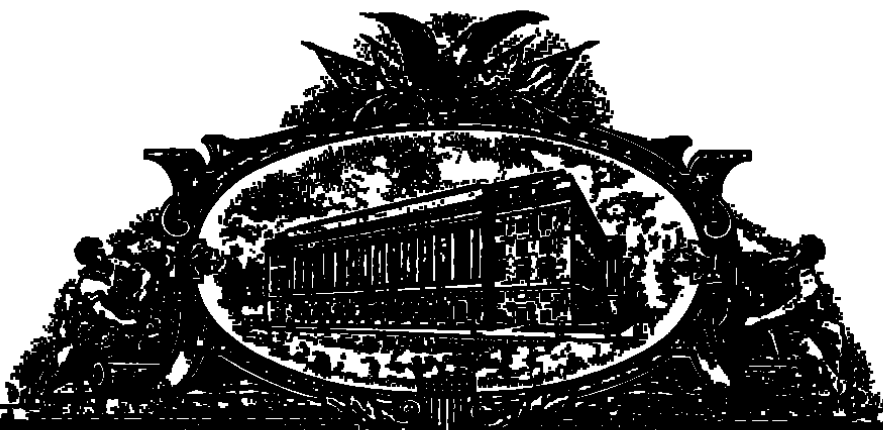
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María Luisa Brigard de Rodríguez
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FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/171,906

FILING DATE: December 23, 1999



By Authority of the
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CERTIFICATE OF EXPRESS MAIL (37 CFR 1.10)

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I hereby certify that the attached papers and/or fee are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service on the date shown below in an envelope addressed to Box Provisional, Assistant Commissioner for Patents, Washington, DC 20231.

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(Date)

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(Print Name)

K.F. Mitchell
(Signature)

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Zeneca Inc.
Docket No.

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

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Sir:

TRANSMISSION OFFICIAL No. 5.800
Maria Luisa Rodriguez de Rodriguez
Intendente Oficial
C/ de la Cruz del Sur de America

TRANSMITTAL OF PROVISIONAL PATENT APPLICATION
UNDER 37 CFR §1.53(c)

Transmitted herewith for a Provisional Application filing is the patent application of:
Inventor(s): Thomas Michael Bare, 1057 Windy Knoll Road, West Chester, Pennsylvania 19382; Rebecca Urbanek, 1800 Concord Pike, Wilmington, Delaware 19850-5437; Dean Brown, 1800 Concord Pike, Wilmington, Delaware 19850-5437.

For: PYRIDAZINO QUINOLINE DIONE COMPOUNDS FOR PAIN

Direct all correspondence to:

Customer Number 22466

Please direct all telephone calls to Kenneth F. Mitchell at telephone number (302) 886-7466

1. Enclosed are:

- ☒ Specification (77 pages).
- ☐ sheets of drawings.
- ☒ Other (specify) Return Postal Card

- 2. ☒ The total fee in the amount of \$150.00 should be charged to Deposit Account No. 26-0166. This sheet is enclosed in duplicate.

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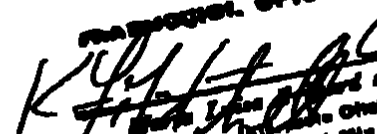
3. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required or to credit any overpayment to Deposit Account No. 26-0166. This sheet is enclosed in duplicate.

The invention was not made by an agency of the United States Government or under a contract with an agency of the United States Government.

Respectfully submitted,

ZENECA, Inc.

Dated: December 13, 1999

By: 
Kenneth F. Mitchell, Ph.D.
Reg. No. 42,007
Attorney for Applicants
Telephone: 302/886-7466

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State of Louisiana
Department of Agriculture
Division of Plant Industry
Bureau of Plant Industry
Bureau of Plant Industry
Bureau of Plant Industry

Zeneca, Inc.
Docket No.

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54**PYRIDAZINO QUINOLINE COMPOUNDS FOR TREATMENT OF PAIN****BACKGROUND****FIELD OF THE INVENTION**

This invention relates to the treatment or prevention of pain or nociception.

RELATED ART

Pain causes a great deal of suffering and is a sensory experience distinct from sensations of touch, pressure, heat and cold. It is often described by sufferers by such terms as bright, dull, aching, pricking, cutting or burning and is generally considered to include both the original sensation and the reaction to that sensation. This range of sensations, as well as the variation in perception of pain by different individuals, renders a precise definition of pain difficult. Where pain is "caused" by the stimulation of nociceptive receptors and transmitted over intact neural pathways, this is termed nociceptive pain. A second form of pain may be caused by damage to neural structures, and often is manifested as neural hypersensitivity; this form of pain is referred to as neuropathic pain.

The level of stimulation at which pain is perceived is referred to as the "pain threshold". Where the pain threshold is raised, for instance, by the administration of an analgesic drug, a greater intensity or more prolonged stimulus is required before pain is experienced. Analgesics are a class of pharmaceutical agent which, following administration to a patient in need of such treatment, relieve pain without loss of consciousness. This is in contrast to other pain-relieving drugs, for example, general anaesthetics which obtund pain by producing a hiatus in consciousness, or local anaesthetics which block transmission in peripheral nerve fibres thereby preventing pain.

Tachykinin antagonists have been reported to induce antinociception in animals, which is believed to be analogous to analgesia in man (for review see Maggi et al, J. Auton. Pharmacol. (1993) 13, 23-93). In particular, non-peptide NK-1 receptor antagonists have been shown to produce such analgesia, thus, for example, in classical tests of chemo-nociception (phenylbenzoquinone-induced writhing and formalin test) the NK-1 receptor antagonist RP 67,580 produced analgesia with potency comparable to that of morphine (Garret et al, Proc. Natl. Acad. Sci. USA (1993) 88, 10208-10212).

The opioid analgesics are a well-established class of analgesic agent. These compounds are sometimes referred to as opiates although this term should be reserved for chemical relatives of morphine. The term opioid is generally accepted to refer in a generic

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sense to all drugs, natural or synthetic, with morphine-like actions. The synthetic and semi-synthetic opioid analgesics are derivatives of five chemical classes of compound: phenanthrenes; phenylheptylamines; phenylpiperidines; morphinans; and benzomorphans. Pharmacologically these compounds have diverse activities, thus some are strong agonists at the opioid receptors (e.g. morphine); others are moderate to mild agonists (e.g. codeine); still others exhibit mixed agonist-antagonist activity (e.g. nalbuphine); and yet others are partial agonists (e.g. nalorphine). Whilst an opioid partial agonist such as nalorphine, (the N-alkyl analogue of morphine) will antagonise the analgesic effects of morphine, when given alone it can be a potent analgesic in its own right.

Of all of the opioid analgesics, morphine remains the most widely used and is a suitable archetype compound. Unfortunately, apart from its useful therapeutic properties, morphine also has a number of drawbacks including respiratory depression, decreased gastrointestinal motility (resulting in constipation) and, in some individuals, nausea and vomiting may occur. Another characteristic is the development of tolerance and physical dependence which may limit the clinical use of such compounds. There is therefore a need to develop methods which enable the clinician to use lower doses of opioid analgesics such as morphine, thereby reducing the likelihood of tolerance and dependence, and thus avoiding the major problem of drug withdrawal associated with cessation of administration.

Morphine-induced emesis may be effectively blocked by the administration of a non-peptide NK-1 receptor antagonist but it is believed that other effects of morphine, notably morphine-induced analgesia, are not blocked by an NK-1 receptor antagonist. Thus, treatment with morphine and an NK-1 receptor antagonist relieves pain without reduction of the morphine analgesic activity, and with the added benefit of enabling lower doses of morphine to be used, as well as reducing the likelihood of nausea and vomiting.

Presently, the primary method of treating the rheumatoid diseases and arthritis is by drug therapy using anti-inflammatory compounds directed at blocking or reducing synovial inflammation thereby improving function and analgesics directed to reducing pain. Aspirin and other salicylate compounds are frequently used in treatment to interrupt amplification of the inflammatory process and temporarily relieve the pain. Other drug compounds used for these purposes include phenylpropionic acid derivatives such as Ibuprofen and Naproxin, Sulindac, phenyl butazone, corticosteroids, antimalarials such as chloroquine and

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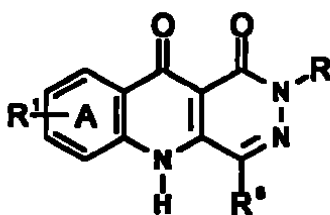
hydroxychloroquine sulfate, and fenemates. For a thorough review of various drugs utilized in treating rheumatic diseases, reference is made to J. Hosp. Pharm., 36:622 (May 1979).

While known drug therapies have utility, there are drawbacks to their use. For instance, it may take up to six months of consistent use of some of these medications in order for the product to have effect in relieving the patient's pain. Consequently if a treatment is without effect in particular subject, up to six months may elapse before the physician is aware of treatment failure. Many of existing drugs also have serious side effects in certain patients, and subject must be carefully monitored to ensure that any side effects are not unduly threatening. Most existing drugs bring only temporary relief to sufferers and must be taken consistently on a daily or weekly basis for continued relief. With disease progression, the amount of medication needed to alleviate the pain may increase thus increasing the potential for adverse side effects.

SUMMARY OF THE INVENTION

The present invention provides a method for the treatment of pain and compounds and compositions for use therein.

The present invention is a method for treating a subject suffering from pain comprising administering to said subject an effective amount of any compound in accord with structural diagram I,



I

or a pharmaceutically-acceptable salt thereof, wherein:

R¹ at each occurrence is independently selected from hydrogen, halo and NO₂, and ring-A has four R¹ groups;

R is selected from (CH₂)_nL, and C(R²)_n(CH₂)_nL wherein n is an integer selected from the range 0 to 6 and R² at each occurrence is independently selected from hydrogen, halogen, C₁-alkyl and C₃-cycloalkyl; and

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L is phenyl substituted with one, two, three or four groups selected from: OR^3 where R^3 is selected from hydrogen and pyridyl-methyl, $N(R^4)_2$ where R^4 at each occurrence is independently selected from hydrogen and C_{1-4} alkyl, halogen, carboxyl, C_{1-4} alkyl, $O-C_{1-4}$ alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, a heteroaryl group selected from furanyl, oxazolyl, oxadiazolyl, thiadiazole, tetrazolyl, morpholynyl and benzimidazolyl, morpholino-N-carbonyl, the group $OC(O)N(R^5)_2$ where R^5 at each occurrence is independently selected from hydrogen, C_{1-4} alkyl and phenyl; or

L is heteroaryl selected from pyridyl, isooxazolyl, oxadiazolyl, thiophenyl, benzo[b]thiophenyl, benzofuranyl and pyrazinyl; where any said heteroaryl is substituted with one, two or three groups selected from: hydroxy, C_{1-4} alkyl, hydroxy C_{1-4} alkyl and methoxyphenyl; or

L is tetrahydrofuranyl;

R^6 is selected from OH or $OC(O)N(R^7)_2$ wherein R^7 at each occurrence is independently selected from hydrogen or C_{1-4} alkyl.

In particular compounds in accord with structural diagram I useful in the method of the present invention, ring-A has a 7-linked halo group and L is selected from substituted or unsubstituted phenyl, pyridyl, isooxazolyl, oxadiazolyl, thiophenyl, benzo[b]thiophenyl, benzofuranyl and pyrazinyl groups, where, when substituted, said groups are substituted with one, two or three moieties independently selected at each occurrence from halo, hydroxy, oxo, carbonyl, carbonylamino, C_{1-4} alkyl and hydroxy C_{1-4} alkyl.

In other particular compounds in accord with structural diagram I useful in the method of the present invention, n is an integer selected from 1, 2 or 3 and L is selected from substituted or unsubstituted furanyl, thiophenyl, pyridyl, pyrrolidinyl, benzimidazolyl, benzothiophenyl and phenyl moieties, R^1 is hydrogen or chloro, and ring-A has a 7-linked chloro group.

In yet other particular compounds in accord with structural diagram I useful in the method of the present invention, R^1 and ring-A in combination are 7-halophenyl. In more particular compounds useful in the method of the invention R^1 and ring-A in combination are 7-chlorophenyl.

In still other particular compounds in accord with structural diagram I useful in the method of the present invention, R is $(CH_2)_nL$, wherein n is an integer selected from the range 0 to 6, and L is phenyl substituted with one, two, three or four groups selected from: OR^3

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where R^1 is selected from hydrogen and pyridyl-methyl, $N(R^4)_2$ where R^4 at each occurrence is independently selected from hydrogen and C_{1-4} alkyl, halogen, carboxyl, C_{1-4} alkyl, $O-C_{1-4}$ alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, a heteroaryl group selected from furanyl, oxazolyl, oxadiazolyl, thiadiazole, tetrazolyl, morpholiny and benzimidazolyl, morpholino-N-carbonyl, the group
5 $OC(O)N(R^5)_2$ where R^5 at each occurrence is independently selected from hydrogen, C_{1-4} alkyl and phenyl, and R^6 is hydroxy.

In further particular compounds in accord with structural diagram I useful in the method of the present invention, R is $(CH_2)_nL$, wherein n is an integer selected from the range 0 to 6, and L is heteroaryl selected from pyridyl, isooxazolyl, oxadiazolyl, thiophenyl,
10 benzo[b]thiophenyl, benzofuranyl and pyrazinyl; where any said heteroaryl is substituted with one, two or three groups selected from: hydroxy, C_{1-4} alkyl, hydroxy C_{1-4} alkyl and methoxyphenyl and R^6 is hydroxy.

In yet further particular compounds in accord with structural diagram I useful in the method of the present invention R is $(CH_2)_nL$, wherein n is an integer selected from the range
15 0 to 6, L is tetrahydrofuranyl and R^6 is hydroxy.

In other compounds in accord with structural diagram I useful in the method of the present invention, R is $C(R^3)_2(CH_2)_nL$ wherein n is an integer selected from the range 0 to 6 and R^3 at each occurrence is independently selected from hydrogen, halogen, C_{1-4} alkyl and C_{3-6} cycloalkyl.

20 Compounds in accord with structural diagram I useful in the method of the present invention, include pharmaceutically-acceptable salts of such compounds, tautomers of such compounds and optical isomers of such compounds.

Particular compounds in accord with structural diagram I useful in the method of the present invention, are those in the Examples described hereinafter.

25 Pharmaceutical compositions having as an active ingredient compounds in accord with structural diagram I are within the scope of the present invention.

The method of the present invention and compounds used therein are useful for the treatment of pain of any etiology, including acute and chronic pain and any pain with an inflammatory component. Examples of acute pain include neuropathic pain and pain
30 associated with inflammatory diseases, such as irritable bowel syndrome and rheumatoid-arthritis, other include post-operative pain, migraine, headache and trigeminal neuralgia. Examples of chronic pain include, in particular, pain associated with musculo-skeletal

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disorders such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, sero-negative, that is, non-rheumatoid, arthropathies, non-articular rheumatism and peri-articular disorders, and pain associated with cancer, peripheral neuropathy and post-herpetic neuralgia. In addition to some of those described above, examples of pain with an inflammatory component include rheumatic pain, dental pain and dysmenorrhoea.

DETAILED DESCRIPTION OF THE INVENTION

The invention comprises a method of treating a subject suffering from pain with an effective amount of a compound of formula I, as described heretofore.

Compounds of the invention are active in a number of preclinical pain models in rodents. For example, such compounds are active at 15 mg/kg/d x 5 days (po) in the chronic constriction injury (CCI) model of neuropathic pain. Further, in the rat formalin test, compounds have ED₅₀'s of less than 100 mg/kg (po) and have a duration of action of greater than 6 hrs.

Compounds of the present invention are potent, selective and novel antagonists at the glycine site of the NMDA receptor complex. They inhibit the binding of [³H-Glycine] to rat or human brain NMDA receptors in the 100 nM range. Compounds of the invention are more potent in inhibiting NMDA-induced calcium flux through the NR2A and NR2C subtype than the NR2B and NR2D subtypes. When tested at 10 μM, compounds of the invention do not show any significant binding activity in *in vitro* binding assays to 85 different receptors, including kainate, glutamate and strychnine-sensitive glycine binding sites.

Compounds of the invention show little toxicity after administration of single doses up to 1000 mg/kg (po), and no effects on cardiovascular parameters (hr, bp) are seen after single doses of 30 and 300 mg/kg (po).

As used herein the term "halo" means chloro, bromo, iodo or fluoro.

As used herein the term "range 0 to 6," or the like, means the integral values in the stated range, in this example the values 0, 1, 2, 3, 4, 5 and 6.

Compounds useful in the method of the invention are illustrated by the particular compounds of the Examples.

Example 1: 7-Chloro-4-hydroxy-2-(2,4,6-trimethylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione.

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(tert-Butoxy)-N-[(2,4,6-trimethylphenyl)amino]carboxamide (2).

A suspension of 2,4,6-trimethylphenylhydrazine hydrochloride (15.02 g, 80.46 mmol) in saturated aqueous NaHCO₃ was stirred for 10 minutes and then treated with solid K₂CO₃ (18.97 g, 137.25 mmol). The resulting fine light yellow suspension was stirred for 10 minutes. A solution of di-*t*-butyldicarbonate (25.03 g, 89.00 mmol) in 375 mL tetrahydrofuran was added over 5 minutes and the resulting biphasic mixture was vigorously stirred for 3 hours. The reaction mixture was partitioned in water and the aqueous layer was extracted with ether (3 x 75 mL). The combined organic layers were washed with saturated NaCl (2 x 100 mL) and distilled water (2 x 100 mL), dried over MgSO₄, and concentrated under reduced pressure. Drying under high vacuum afforded an orange oil which crystallized upon standing. The material was triturated in 5% ether in hexanes overnight to afford a beige solid which was isolated by filtration (13.82 g). The solid was recrystallized from ether/hexanes to afford 11.85 g (59%) of the desired product (2) as a cream colored solid. The filtrates from the trituration and crystallization were combined and concentrated to afford 13.50 g of material which was purified by flash chromatography on silica gel using 25:75 ether:hexanes as eluent. This afforded an additional 4.69 g (23%) of the title compound. ¹H NMR (300 MHz, CDCl₃) δ 6.79 (s, 2 H), 6.25 (bs, 1 H), 5.65 (bs, 1 H), 2.34 (s, 6 H), 2.22 (s, 3 H), 1.40 (s, 9 H); MS (CI) *m/z* 249.

N-[(tert-Butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(2,4,6-trimethylphenyl)carboxamide (4).

To a stirred suspension of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (3, 15.25 g, 47.55 mmol) in anhydrous tetrahydrofuran (730 mL) under nitrogen was added 1,3-dicyclohexylcarbodiimide (11.66 g, 56.51 mmol). The (tert-butoxy)-N-[(2,4,6-trimethyl phenyl)amino]carboxamide (2, 16.54 g, 66.07 mmol) was dissolved in 270 mL anhydrous tetrahydrofuran and added to the bright yellow reaction mixture. The reaction mixture was stirred at room temperature for 5 hours and then heated at 60 °C for 20 hours. After cooling to room temperature, the mixture was filtered and solids (dicyclohexyl urea, DCU) were washed with ether. The filtrate was concentrated and then dried under high vacuum to afford a brown oil. The material was purified by flash chromatography on silica gel using a gradient of 99:1 to 95:5 methylene chloride:methanol as eluent. Concentration of the appropriate fractions provided a tan solid (21.40 g) which was sonicated in methanol and filtered to remove the remaining DCU from the desired solid product. This purification

procedure was repeated until all of the DCU was removed. A total of 18.04 g (69%) of N-
[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-
(2,4,6-trimethyl phenyl)carboxamide (4) was obtained. ¹H NMR (300 MHz, DMSO, d₆) δ
13.14 (s, 1 H), 9.08 (bs, 1 H), 8.28 (d, 1 H, *J*_o=8.7 Hz), 7.69 (d, 1 H, *J*_m=1.6 Hz), 7.56 (dd, 1
5 H, *J*_o=8.7 Hz, *J*_m=1.6 Hz), 6.88 (s, 2 H), 3.44 (m, 4 H), 2.32 (s, 6 H), 2.24 (s, 3 H), 1.86 (m, 4
H), 1.09 (bs, 9 H); MS (-CI) *m/z* 552.

7-Chloro-4-hydroxy-2-(2,4,6-trimethylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-
b]quinoline-1,10-dione (5).

To a stirred suspension of N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-
10 (pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(2,4,6-trimethyl phenyl)carboxamide (4, 3.72 g,
6.65 mmol) in 180 mL anhydrous tetrahydrofuran under nitrogen was added methanesulfonic
acid (21.0 mL, 32.40 mmol). The resulting bright yellow solution was stirred at room
temperature for 18 hours. The solution was poured into 1700 mL water, stirred 1 hour, and
filtered to afford a yellow solid. After sonication in methanol, pure 7-chloro-4-hydroxy-2-
15 (2,4,6-trimethylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (5) was
obtained by filtration as a beige solid (1.21 g, 47%), m.p. 187-189 °C. ¹H NMR (300 MHz,
DMSO, d₆) δ 12.79 (bs, 1 H), 12.06 (bs, 1 H), 8.16 (d, 1 H, *J*_o=8.7 Hz), 8.07 (d, 1 H, *J*_m=1.3
Hz), 7.46 (dd, 1 H, *J*_o=8.7 Hz, *J*_m=1.3 Hz), 2.29 (s, 3 H), 2.02 (s, 6 H); MS (CI) *m/z* 382/384.

Calc'd. for C₂₈H₁₈ClN₃O₃•0.3H₂O: C, 62.04; H, 4.32; N, 10.85. Found C, 62.01; H,
20 4.22; N, 10.71.

Example 2: 7,9-Dichloro-4-hydroxy-2-(4-methoxy-2-methylphenyl)-1,2,5,10-
tetrahydropyridazino[4,5-b]quinoline-1,10-dione.

2-Methyl-4-methoxyphenylhydrazine hydrochloride (7).

To a suspension of 4-methoxy-2-methyl aniline (67.0 mL, 71.36 g, 0.52 mole) in 400
25 mL water at 0 °C was added 378 mL 12N HCl over 30 minutes. The temperature was kept
below 10 °C during the addition and then lowered to -10 °C. A solution of sodium nitrite
(35.46 g, 0.51 mole) in 350 mL water was added over 25 minutes while maintaining the
reaction mixture temperature at -10 to -5 °C. The dark purple solution was stirred at -10 to -5
30 °C for 1 hour and then cooled to -15 to -20 °C. A solution of tin(II) chloride dihydrate
(341.54 g, 1.51 mole) in 227 mL HCl was added dropwise to the stirred reaction mixture over
45 minutes. The resulting dark lavender mixture was stirred at -10 to -20 °C for 2.5 hours.

The reaction mixture was quenched with 158 mL ethanol (added dropwise over 15 minutes), stirred at -20 °C for 45 minutes, and filtered through a pre-chilled fritted glass funnel. The solids were washed with cold 1:1 ethanol:ether (4 x 350 mL) and cold ether (2 L) and air dried for 30 minutes. After drying under high vacuum, the 2-methyl-4-methoxyphenylhydrazine hydrochloride (7) was obtained as a white solid (51.90 g, 53%). ¹H NMR (300 MHz, CDCl₃) δ 10.12 (bs, 2 H), 7.14 (m, 1 H), 6.72 (m, 1 H), 6.70 (s, 1 H), 3.76 (s, 3 H), 2.30 (s, 3 H); MS (CI) *m/z* 153.

Methyl 5,7-dichloro-2-(N-[(4-methoxy-2-methylphenyl)amino]carbamoyl)-4-oxohydroquinoline-3-carboxylate (9).

The 2-methyl-4-methoxyphenylhydrazine hydrochloride (7, 49.25 g, 0.26 mole) was partitioned between 1 L 1.5N sodium hydroxide and 500 mL ether. The aqueous layer was extracted with ether until all the solids were taken into ether (9 x 500 mL). The organic layers were dried over Na₂SO₄ and concentrated to a white slurry. The solid was isolated by filtration and dried under high vacuum to afford 33.19 g (218.10 mmol) of the free base. This was dissolved in 1 L anhydrous tetrahydrofuran and cooled to 0 °C. A solution of methyl 5,7-dichloro-2-(chlorocarbonyl)-4-oxohydroquinoline-3-carboxylate 8 (26.69 g, 80.02 mmol) in 450 mL anhydrous tetrahydrofuran was added dropwise over two hours. The reaction mixture was allowed to warm to room temperature and stirred overnight (18 hours). The resulting mixture was poured into 2 L of ice water and 3 L of 1N HCl was added. After 6 hours of stirring, the solids were isolated by filtration, washed with water and ether (2 L each), and dried under high vacuum to afford methyl 5,7-dichloro-2-(N-[(4-methoxy-2-methylphenyl)amino]carbamoyl)-4-oxohydroquinoline-3-carboxylate (9) as a pale yellow solid (28.42 g, 79%). ¹H NMR (300 MHz, CDCl₃) δ 12.22 (s, 1 H), 10.38 (d, 1 H, *J*=4.5 Hz), 7.62 (d, 1 H, *J*_{HH}=1.9 Hz), 7.23 (d, 1 H, *J*_{HH}=1.9 Hz), 7.08 (d, 1 H, *J*_{OH}=8.1 Hz), 6.71 (d, 1 H, *J*_{OH}=8.1 Hz), 6.93 (s, 1 H), 6.28 (d, 1 H, *J*=4.5 Hz), 3.85 (s, 3 H), 3.76 (s, 3 H), 2.32 (s, 3 H).

7,9-Dichloro-4-hydroxy-2-(4-methoxy-2-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (10).

To a suspension of compound (9, 14.30 g, 31.76 mmol) in 1200 mL anhydrous tetrahydrofuran under nitrogen was added methanesulfonic acid (150 mL, 2.31 mole). The solution was heated at reflux for 12 hours, cooled to room temperature, and concentrated to half of its original volume. Methanol (500 mL) was added and the solution was poured into 3 L ice water. After 30 minutes of stirring, the pale yellow solid which formed was isolated by

filtration. HPLC indicated that a 26:1 ratio of the N-2:N-3 isomers had been obtained. The solid was sonicated and then refluxed in methanol, isolated by filtration, and dried under high vacuum to afford 7,9-dichloro-4-hydroxy-2-(4-methoxy-2-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (10) (9.70 g, 71%), m.p. >250 °C. ¹H NMR (300 MHz, DMSO, d₆) δ 12.74 (s, 1 H), 11.94 (s, 1 H), 8.04 (d, 1 H, *J*_{HH}=1.7 Hz), 7.52 (d, 1 H, *J*_{HH}=1.7 Hz), 7.19 (d, 1 H, *J*_{OH}=8.7 Hz), 6.91 (d, 1 H, *J*_{HH}=2.7 Hz), 6.85 (dd, 1 H, *J*_{OH}=8.7 Hz, *J*_{HH}=2.7 Hz), 3.79 (s, 3 H), 2.08 (s, 3 H); MS (CI) *m/z* 418/420/422.

Calc'd. for C₁₉H₁₃Cl₂N₃O₄: C, 54.56; H, 3.13; N, 10.05. Found C, 54.32; H, 3.16; N, 9.96.

Example 3: 7-Chloro-4-hydroxy-2-(2-chloro-4-methyl phenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione.

2-Chloro-4-methyl phenylhydrazine hydrochloride (1).

A suspension of 2-chloro-4-methyl aniline (10.1 mL, 11.63 g, 82.1 mmol) in 64 mL water and 60 mL 12N HCl was cooled to -5 °C (internal temperature) and stirred with a mechanical stirrer. A solution of sodium nitrite (8.26 g, 119.7 mmol) in 56 mL water was added over 30 minutes. The solution became more clear but some solid remained. The mixture was stirred at -5 °C for 20 minutes and then cooled to -10 °C. A solution of tin(II) chloride dihydrate (53.60 g, 237.6 mmol) in 36 mL 12N HCl was added dropwise over 30 minutes while maintaining an internal temperature of -5 to -10 °C. The resulting pinkish brown mixture was stirred at -5 to -10 °C for 2 hours and then filtered cold through a pre-chilled fritted glass funnel. The collected solids were washed with cold 1% ethanol in ether (100mL) followed by cold ether (500 mL) and air dried for 30 minutes. After drying under high vacuum, the desired product (1) was obtained as a very pale yellow crystalline solid (7.76 g, 49%). ¹H NMR (300 MHz, CDCl₃) δ 10.09 (bs, 2 H), 7.89 (s, 1 H), 7.25 (d, 1 H, *J*_{HH}=1.2 Hz), 7.13 (dd, 1 H, *J*_{OH}=8.4 Hz, *J*_{HH}=1.2 Hz), 7.02 (d, 1 H, *J*_{OH}=8.4 Hz), 2.24 (s, 3 H); MS (CI) *m/z* 157/159.

(tert-Butoxy)-N-[(2-chloro-4-methyl phenyl)amino]carboxamide (2).

A suspension of 2-chloro-4-methylphenylhydrazine hydrochloride (7.74 g, 40.09 mmol) in 95 mL saturated aqueous NaHCO₃ was stirred for 10 minutes and then treated with solid K₂CO₃ (9.45 g, 68.37 mmol). The resulting fine light yellow suspension was stirred for 10 minutes. A solution of di-*t*-butyldicarbonate (12.97 g, 46.12 mmol) in 195 mL

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tetrahydrofuran was added over 5 minutes and the resulting biphasic mixture was vigorously stirred for 3 hours. The reaction mixture was partitioned and the aqueous layer was extracted with ether (5 x 25 mL). The combined organic layers were washed with distilled water (2 x 75 mL), dried over MgSO_4 , and concentrated under reduced pressure. Drying under high vacuum afforded a light orange oil (14.07 g). The material was purified by flash chromatography on silica gel using 10:90 ether:hexanes as the eluent. The product (2) was obtained as a light yellow oil which solidified upon standing (9.92 g, 96%). ^1H NMR (300 MHz, CDCl_3) δ 8.88 (s, 1 H), 7.15 (s, 1 H), 7.09 (d, 1 H, $J_{\text{H}}=1.2$ Hz), 6.97 (d, 1 H, $J_{\text{H}}=8.1$ Hz), 6.64 (d, 1 H, $J_{\text{H}}=8.1$ Hz), 2.18 (s, 3 H), 1.41 (s, 9 H); MS (CI) m/z 279/281.

N-[(*tert*-butoxy)carboxylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(2-chloro-4-methyl phenyl)carboxamide (4).

To a stirred suspension of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (3, 14.57 g, 45.43 mmol) in anhydrous tetrahydrofuran (300 mL) under nitrogen was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC, 34.89 g, 82.37 mmol). The white suspension immediately became bright yellow. The (*tert*-butoxy)-N-[(2-chloro-4-methyl phenyl)amino]carboxamide (2, 13.89 g, 54.10 mmol) was added as a solid followed by 50 mL anhydrous tetrahydrofuran. The bright yellow reaction mixture was stirred at room temperature for 22 hours. A second portion of CMC (16.77 g, 39.59 mmol) was added to the reaction mixture. After 2.5 hours at room temperature, the reaction was heated at 60 °C for 5.5 hours. After cooling to room temperature, the reaction mixture was filtered and the collected solids were washed with THF. The filtrate and washes were concentrated and dried under high vacuum to afford a light yellow foam. The material was dissolved in methylene chloride (400 mL), washed with distilled water (2 x 150 mL), and extracted with 10% NaHCO_3 (2 x 500 mL). The organic layer was dried over Na_2SO_4 , concentrated and dried under high vacuum to afford a light tan foam. The material was purified by flash chromatography on silica gel using a gradient of 95:5 to 85:15 chloroform:methanol as eluent to afford 15.42 g (61%) of the desired product (4) as a white solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 13.03 (bs, 1 H), 9.19 (bs, 1 H), 8.25 (d, 1 H, $J_{\text{H}}=8.7$ Hz), 7.68 (d, 1 H, $J_{\text{H}}=1.8$ Hz), 7.54 (dd, 1 H, $J_{\text{H}}=8.7$ Hz, $J_{\text{H}}=1.8$ Hz), 7.50 (d, 1 H, $J_{\text{H}}=1.8$ Hz), 7.45 (d, 1 H, $J_{\text{H}}=7.8$ Hz), 6.81 (d, 1 H, $J_{\text{H}}=7.8$ Hz), 3.47 (m, 4 H), 2.34 (s, 3 H), 1.90 (m, 4 H), 1.40 (s, 9 H); MS (-CI) m/z 559/561.

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7-Chloro-4-hydroxy-2-(2-chloro-4-methyl phenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (5).

To a stirred suspension of N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(2-chloro-4-methyl phenyl)carboxamide (4, 21.16 g, 37.82 mmol) in 900 mL anhydrous tetrahydrofuran under nitrogen was slowly added methanesulfonic acid (120.0 mL, 184.9 mmol). The resulting dark yellow solution was stirred at room temperature for 18 hours. The solution was poured into 7 L water, stirred 3 hours, and filtered to afford a light yellow solid. The solid was sonicated in methanol, isolated by filtration, and dried under high vacuum (30 mm) at 40 °C to afford the product (5) as a white solid (12.93 g, 88 %). ¹H NMR (300 MHz, DMSO, d₆) δ 12.90 (bs, 1 H), 12.10 (bs, 1 H), 8.16 (d, 1 H, J_a=8.7 Hz), 8.07 (d, 1 H, J_a=1.8 Hz), 7.47 (dd, 1 H, J_a=8.7 Hz, J_a=1.8 Hz), 7.47 (d, 1 H, J_a=1.2 Hz), 7.42 (d, 1 H, J_a=8.1 Hz), 7.29 (dd, 1 H, J_a=8.1 Hz, J_a=1.2 Hz), 2.38 (s, 3 H); MS (CI) m/z 388/390/392.

Calc'd. for C₁₈H₁₁Cl₂N₃O₃: C, 55.69; H, 2.86; N, 10.82. Found C, 55.78; H, 2.89; N, 10.79.

Example 4: Methyl 4-[(7-chloro-4-hydroxy-1,10-dioxo-2,5-dihydropyridazino[4,5-b]quinolin-2-yl)methyl]benzoate.

Methyl 4-[(*tert*-butoxy)carbonylamino]methyl]benzoate (2)

A solution of methyl 4-bromomethylbenzoate (25.0 g, 0.11 moles) and *t*-butylcarbazate (68.65 g, 0.52 moles) in N,N-dimethylformamide (170 mL) was stirred and heated to 50°C under nitrogen. Triethylamine (21.1 g, 0.21 moles) was added and the reaction mixture was heated at 50°C for an additional 10 minutes. The reaction mixture was poured into water (1 L) and the resulting mixture extracted with dichloromethane (4 x 350 mL). The combined organic layers were washed with water (4 x 200 mL) and saline (1 x 300 mL) and then dried (MgSO₄). Concentration of the filtered organic layer under vacuum provided a yellow oil. This material was purified by silica gel column chromatography using diethylether/hexane (1/1) as the eluant to obtain the title compound (2) as a clear colorless oil (26.50 g, 87% yield). ¹H NMR (300 MHz, DMSO-d₆): δ 1.38 (s, 9H); 3.84 (s, 3H); 3.92 (d, 2H, J = 4.2 Hz); 4.95 (d, 1H, J = 4.2 Hz); 7.47 (d, 2H, J = 8.4 Hz); 7.90 (d, 2H, J = 8.1 Hz); 8.29 (s, 1H).

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N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(4-carbomethoxybenzyl)carboxamide. (4)

To a stirred mixture of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (3, 18.38 g, 57 mmole) and dry tetrahydrofuran (800 mL) under nitrogen was added 1,3-dicyclohexylcarbodiimide (15.25 g, 74 mmole) in one portion. After stirring the reaction mixture for 5 minutes, a solution of methyl 4-([(tert-butoxy)-carbonylamino]amino)methyl)benzoate (2, 24.1 g, 86 mmole) and tetrahydrofuran (200 mL) was rapidly added and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to a solid-oil mixture. Using methanol the material was preabsorbed onto silica gel. Silica gel column chromatography using methanol/chloroform (5/95) as eluant gave the title compound (4) as a tan solid (33.43g, 100 %) contaminated with dicyclohexylurea. This material was used in the subsequent reaction.

Methyl 4-[(7-chloro-4-hydroxy-1,10-dioxo-2,5-dihydropyridazino[4,5-b]quinolin-2-yl)methyl]benzoate (5)

To a stirred mixture of N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(4-carbomethoxybenzyl)carboxamide (4, 33.6 g, 57.3 mmole) and dry tetrahydrofuran (1156 mL) under nitrogen was added methanesulfonic acid (140 mL, 207.3 g, 2.16 moles) all at once. The mixture was stirred overnight at room temperature. The reaction mixture was poured into ice water (3 L) and the resulting solid filtered. The collected solids were suspended in methanol (300 mL), sonicated for 30 minutes and filtered. The collected solids were submitted to the identical sonication process two more times and the collected solids were air dried to give the pure title compound (5) as a white solid (13.38 g, 40 % yield), m.p. > 290°C. MS: 412 (M+1, small parent ion), 410 (M-1, large parent ion) ¹H NMR (300 MHz, DMSO-d₆): δ 3.83 (s, 3H), 5.18 (s, 2H); 7.43 (m, 3H,); 7.92 (d, 2H, J = 8.1 Hz); 8.02 (s, 1H); 8.14 (d, 1H, J = 8.4 Hz)

Calc'd. for C₂₈H₁₄ClN₃O₅: C, 58.27; H, 3.56; N, 10.00 Found: C, 58.21; H, 3.51; N, 10.00.

Example 5: 7-Chloro-4-hydroxy-2-(4-[1,3,4]oxadiazol-2-yl-benzyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (7)

2-p-Tolyl-[1,3,4]oxadiazole (2)

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Triethyl orthoformate (5 mL) was added to *p*-toluic hydrazide (1, 2.0 g, 13.3 mmol) and the solution was heated to reflux for 6 hours. The volatiles were removed under reduced pressure, and the organic materials dissolved in ethyl acetate (100 mL). The organic layer was extracted with 10% aqueous potassium carbonate, washed with aqueous sodium chloride (saturated, 1 x 10 mL), and dried over Na₂SO₄. The organic layer was filtered and removed under reduced pressure to give the title compound as a tan solid (2.1 g, 99%). ¹H NMR (300 MHz, DMSO-d₆): δ 2.40 (s, 3H); 7.42 (d, 2H, J = 8.1 Hz); 7.88 (d, 2H, J = 8.1 Hz); 9.30 (s, 1H).

2-(4-Bromomethyl-phenyl)-[1,3,4]oxadiazole (3)

2-*p*-Tolyl-[1,3,4]oxadiazole (2, 1.44 g, 9.03 mmol) was slurried in carbon tetrachloride (40 mL) and to this was added *N*-bromosuccinimide (1.68 g, 9.45 mmol), and benzoyl peroxide (0.21 g, 0.90 mmol). The mixture was heated to reflux for 6 hours, and then cooled to room temperature. The solids were filtered off and discarded. The remaining material was dissolved into methylene chloride (100 mL) and extracted with aqueous sodium bicarbonate (saturated, 2 x 50 mL). The organic layer was washed with aqueous sodium chloride (saturated), and dried over Na₂SO₄. The organic layer was filtered and removed under reduced pressure to give the title compound as a tan solid (1.70 g, 79%). (Contains roughly 5-10 % of the *a,a*-dibrominated material). ¹H NMR (300 MHz, DMSO-d₆): δ 4.79 (s, 2H), 7.67 (d, 2H, J = 8.4 Hz); 8.02 (d, 2H, J = 8.4 Hz); 9.32 (s, 1H).

N'-(4-[1,3,4]Oxadiazol-2-yl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (4)

2-(4-Bromomethyl-phenyl)-[1,3,4]oxadiazole (3, 0.56 g, 2.35 mmol) was dissolved in *N,N*-dimethylformamide (8 mL) and to this was added *tert*-butyl carbazate (0.28 g, 2.11 mmol) followed by *N,N*-diisopropylethylamine (0.33 g, 0.45 mL, 2.62 mmol). The solution was heated to 90 °C for 12 hours and then cooled to room temperature. The material was dissolved into ethyl acetate (150 mL) and extracted with water (3 x 40 mL) and aqueous sodium chloride (saturated, 1 x 15 mL). The organic layer was dried over Na₂SO₄, and evaporated to a thick oil. This residue was chromatographed (SiO₂, 1:1 hexanes:ethyl acetate) to give the title compound as a white solid (0.12 g, 20%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.37 (s, 9H); 3.96 (d, 2H, J = 3.9 Hz); 4.93 (m, 1H); 7.55 (d, 2H, J = 8.4 Hz); 7.93 (d, 2H, J = 8.4 Hz); 9.31 (s, 1H).

N'-[7-Chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N'*-(4-[1,3,4]oxadiazol-2-yl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (6)

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To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (5, 0.12 g, 0.39 mmol) in tetrahydrofuran (8 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (0.19 g, 0.45 mmol) and the reaction was stirred for five minutes. To this mixture was added, via dropwise addition, a solution of *N'*-(4-[1,3,4]oxadiazol-2-yl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (4, 0.11 g, 0.45 mmol) and 4-dimethylaminopyridine (ca. 0.003 g) in tetrahydrofuran (2 mL). The mixture was then refluxed for 16 hours. The solution was filtered, washed with methylene chloride (5 mL) and the insolubles collected. This insoluble material was then washed with water (50 mL) and then diethyl ether (20 mL). The remaining insoluble material was dried under vacuum to give the title compound as a white solid. (0.20 g, 89%). The material was used in the following reaction without further purification.

7-Chloro-4-hydroxy-2-(4-[1,3,4]oxadiazol-2-yl-benzyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (7)

To a stirred solution of *N'*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydroquinoline-3-carbonyl]-*N'*-(4-[1,3,4]oxadiazol-2-yl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (6, 0.20 g, 0.33 mmol) in tetrahydrofuran (5 mL) was added methanesulfonic acid (0.80 mL) and the reaction was heated to 70 °C for 1 hour. The mixture was cooled to room temperature and stirred for 16 hours. The resultant orange solid was collected by filtration and washed with tetrahydrofuran. The material was sonicated for 20 minutes in diethyl ether/methyl alcohol (1:1, 5 mL) and then filtered. The material was dried to give the title compound as an off-white solid (0.56 g, 17%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.31 (s, 3H); 5.18 (s, 2H); 7.44 (m, 3H); 7.83 (d, 2H, *J* = 8.1 Hz); 8.04 (s, 1H); 8.14 (d, 1H, *J* = 8.7 Hz); 10.38 (br s, 1H); 11.30 (s, 1H); 11.95 (br s, 1H).

Example 6: 7-Chloro-4-hydroxy-2-[4-(morpholine-4-carbonyl)-benzyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (7)

4-(*tert*-Butoxycarbonyl-hydrazonomethyl)-benzoic acid (2)

4-Carboxybenzaldehyde (1, 2.0 g, 13.3 mmol) was added to tetrahydrofuran (60 mL) and to this was added *tert*-butylcarbazate (1.76 g, 13.3 mmol) followed by aqueous hydrochloric acid (2N, 2 drops from a Pasteur pipette). The reaction mixture was stirred for 16 hours and then the solvent was removed under reduced pressure. The residual solid was triturated with hexanes and collected to give a white solid (3.1 g, 89%). ¹H NMR (300 MHz,

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DMSO- d_6): δ 1.47 (s, 9H); 7.70 (d, 2H, $J = 8.4$ Hz); 7.94 (d, 2H, $J = 8.4$ Hz); 8.05 (s, 1H); 11.03 (br s, 1H); 12.98 (br s, 1H).

N-[4-(Morpholine-4-carbonyl)-benzylidene]-hydrazinecarboxylic acid *tert*-butyl ester (3)

4-(*tert*-Butoxycarbonyl-hydrazonomethyl)-benzoic acid (2, 1.0 g, 3.78 mmol) was slurried in tetrahydrofuran (10 mL) and to this was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (1.67 g, 3.96 mmol). The mixture was stirred overnight and a precipitate formed. The precipitate was collected and washed with tetrahydrofuran (20 mL) to give the title compound as a white solid (0.80 g, 64%). This material was used in the following reaction without further purification. MS (+CI) m/z 334.

N-[4-(Morpholine-4-carbonyl)-benzyl]-hydrazinecarboxylic acid *tert*-butyl ester (4)

N-[4-(Morpholine-4-carbonyl)-benzylidene]-hydrazinecarboxylic acid *tert*-butyl ester (3, 0.80 g, 2.4 mmol) was added to a Parr shaker bottle and slurried in methyl alcohol (30 mL). To this was added 10% palladium-on-carbon (~100 mg) and the reaction was hydrogenated at 40 psi for 4 hours. The catalyst was filtered on Celite[®], washed with methyl alcohol (2 x 100 mL), and the solvents were removed *in vacuo* to give a oil. The oil was subjected to flash chromatography (SiO₂, ethyl acetate/hexanes as eluent) to give the title compound as an oil (0.46 g, 58%). ¹H NMR (300 MHz, DMSO- d_6): δ 1.37 (s, 9H); 3.22 (t, 2H, $J = 5.1$ Hz); 3.50 (t, 2H, $J = 5.1$ Hz); 3.52 (m, 4H); 3.88 (d, 2H, $J = 4.5$ Hz); 4.82 (m, 1H); 7.33 (d, 2H, $J = 8.4$ Hz); 7.38 (d, 2H, $J = 8.4$ Hz); 8.21 (br s, 1H).

N-[7-Chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N*-[4-(morpholine-4-carbonyl)-benzyl]-hydrazinecarboxylic acid *tert*-butyl ester (6)

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (5, 0.47 g, 1.47 mmol) in tetrahydrofuran (10 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (0.71 g, 1.68 mmol) and the reaction was stirred for five minutes. To this mixture was added, via dropwise addition, a solution of *N*-[4-(morpholine-4-carbonyl)-benzyl]-hydrazinecarboxylic acid *tert*-butyl ester (4, 0.46 g, 1.40 mmol) and 4-dimethylaminopyridine (0.005 g, 0.04 mmol) in tetrahydrofuran (5 mL), and the mixture was then refluxed for 16 hours. The solution was filtered and the insolubles washed with tetrahydrofuran (2 x 20 mL), water (2 x 20 mL) and diethyl ether (2 x 20 mL). The solids were collected to give the title compound as a white solid (0.42 g, 47%).

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This material was used in the following reaction without further purification. MS (-CI) *m/z* 637/638

7-Chloro-4-hydroxy-2-[4-(morpholine-4-carbonyl)-benzyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (7)

5 To a stirred solution of *N*'-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydroquinoline-3-carbonyl]-*N*'-[4-(morpholine-4-carbonyl)-benzyl]-hydrazinecarboxylic acid *tert*-butyl ester (6, 0.42 g, 0.65 mmol) in tetrahydrofuran (20 mL) was added methanesulfonic acid (1.2 mL) and the reaction was heated to 70 °C for 1 hour. The reaction was then cooled to room temperature and stirred for 3 hours. The tetrahydrofuran was removed under reduced pressure to yield an orange oil. To this was added diethyl ether (50 mL) and the reaction stirred for 10 minutes. The diethyl ether was carefully decanted to leave a thick orange oil. To this was added water (5 mL) and a fluffy yellow precipitate formed. The mixture was stirred for 10 minutes and then filtered. The solids were washed with water (10 mL) followed by diethyl ether (20 mL). The solids were collected and sonicated in methyl alcohol/diethyl ether (1:1, 5 mL) for 10 minutes. The solids were collected and dried under reduced pressure to give the title compound as a cream-colored solid (0.147 g, 49%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.33 (m, 4H); 3.51 (m, 4H); 5.14 (s, 2H); 7.36 (m, 4H); 7.43 (d, 1H, *J* = 8.7 Hz); 8.02 (s, 1H); 8.14 (d, 1H, *J* = 8.7 Hz); 11.94 (br s, 1H); 12.67 (br s, 1H). MS (+CI) *m/z* 467/469

Example 7: 7-Chloro-4-hydroxy-2-[3-(1H-tetrazol-5-yl)-benzyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (7)

3-(1H-Tetrazol-5-yl)-benzaldehyde (2)

25 3-Cyanobenzaldehyde (1, 2.0 g, 15.2 mmol) was dissolved in 2-methoxyethanol (8 mL) and to this was added sodium azide (0.91 g, 15.2 mmol) and lithium chloride (0.96 g, 22.8 mmol). The mixture was stirred and heated to reflux for 16 hours and then poured into a mixture of concentrated hydrochloric acid (3 mL) and crushed ice (~30 g). The thick suspension was stirred until a fine pink solid had formed. The solids were filtered, washed with water and diethyl ether to give the title compound (1.21 g, 46%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.86 (t, 1H, *J* = 7.8 Hz); 8.13 (dd, 1H, *J* = 1.5, 7.8 Hz); 8.37 (ddd, 1H, *J* = 1.5, 1.5, 7.8 Hz); 8.56 (d, 1H, *J* = 1.5 Hz); 10.13 (s, 1H).

N'-[3-(1H-Tetrazol-5-yl)-benzylidene]-hydrazinecarboxylic acid *tert*-butyl ester (3)

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3-(1H-Tetrazol-5-yl)-benzaldehyde (2, 1.21 g, 6.95 mmol) was dissolved in tetrahydrofuran (40 mL) and to this was added *tert*-butyl carbazate (0.96 g, 7.30 mmol). The reaction was stirred at room temperature for 16 hours and the tetrahydrofuran removed under reduced pressure. The residual solid was triturated with hexanes to give the title compound as an off-white solid (2.0 g, 100%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.48 (s, 9H); 7.69 (t, 1H, *J* = 7.8 Hz); 7.78 (d, 1H, *J* = 7.3 Hz); 8.03 (d, 1H, *J* = 7.8 Hz); 8.09 (s, 1H); 8.31 (s, 1H); 11.08 (br s, 1H).

N-[3-(1H-Tetrazol-5-yl)-benzyl]-hydrazinecarboxylic acid *tert*-butyl ester (4)

N-[3-(1H-Tetrazol-5-yl)-benzylidene]-hydrazinecarboxylic acid *tert*-butyl ester (3, 0.50 g, 1.7 mmol) was added to a Parr shaker bottle and slurried in methyl alcohol (20 mL). To this was added 10% palladium-on-carbon (~100 mg) and the reaction was hydrogenated at 42 psi for 5 hours. The catalyst was filtered on Celite®, washed with methyl alcohol (2 x 100 mL), and the solvents were removed *in vacuo* to give a oil. The oil was dissolved in methylene chloride (20 mL) and this was removed under reduced pressure to azeotrope away any residual methyl alcohol. The title compound was isolated as an oil (0.40 g, 60%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.96 (br s, 2H); 4.94 (br s, 1H); 7.50 (m, 2H); 7.89 (m, 1H); 8.01 (s, 1H); 8.27 (br s, 1H). MS (-CI) *m/z* 289, 290.

N-[7-Chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N*-[3-(1H-tetrazol-5-yl)-benzyl]-hydrazinecarboxylic acid *tert*-butyl ester (6)

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (5, 0.43 g, 1.34 mmol) in tetrahydrofuran (20 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (0.68 g, 1.60 mmol) and the reaction was stirred for five minutes. To this mixture was added, via dropwise addition, a solution of *N*-[3-(1H-tetrazol-5-yl)-benzyl]-hydrazinecarboxylic acid *tert*-butyl ester (0.42 g, 1.47 mmol) and 4-dimethylaminopyridine (4, 0.05 g, 0.41 mmol) in tetrahydrofuran (5 mL), and the mixture was then refluxed for 16 hours. The solution was filtered and the insolubles washed with tetrahydrofuran. The tetrahydrofuran was removed under reduced pressure to give a sticky yellow oil. This material was chromatographed (SiO₂, 90/10) to give the title compound as a yellow solid (0.48 g, 60%). The material was used without further purification in the following reaction.

7-Chloro-4-hydroxy-2-[3-(1H-tetrazol-5-yl)-benzyl]-1,2,5,10-tetrahydropyridazine[4,5-*b*]quinoline-1,10-dione (7)

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To a stirred solution of *N*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydroquinoline-3-carbonyl]-*N*-[3-(1H-tetrazol-5-yl)-benzyl]-hydrazinecarboxylic acid *tert*-butyl ester (6, 0.48 g, 0.81 mmol) in tetrahydrofuran (15 mL) was added methanesulfonic acid (1.9 mL) and the reaction was stirred at room temperature for 16 hours. The tetrahydrofuran was removed under reduced pressure to yield an orange oil. To this was added diethyl ether (50 mL) and the reaction stirred for 10 minutes. The diethyl ether was carefully decanted to leave a thick orange oil. To this was added water (5 mL) and a fluffy yellow precipitate formed. The mixture was stirred for 10 minutes and then filtered. The solids were washed with water (10 mL) followed by diethyl ether (20 mL). The solids were collected and sonicated in methyl alcohol/diethyl ether (1:5, 5 mL) for 10 minutes. The solids were collected and dried under reduced pressure to give the title compound as a cream-colored solid (0.14 g, 41%, m.p. > 275 °C). ¹H NMR (300 MHz, DMSO-*d*₆): δ 5.22 (s, 2H); 7.44 (d, 1H, *J* = 8.1 Hz); 7.56 (m, 2H); 7.95 (m, 2H); 8.03 (s, 1H); 8.15 (d, 1H, *J* = 8.7 Hz); 11.97 (br s, 1H); 12.69 (br s, 1H).

Example 8: 4-(7-Chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1*H*-pyridazino[4,5-*b*]quinolin-2-ylmethyl)-*N*-furan-2-ylmethyl-benzamide:

7-Chloro-4-hydroxy-2-(4-carboxy-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione (2)

To a stirred suspension of 7-Chloro-4-hydroxy-2-(4-carbomethoxy-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione (4.24 g, 10.2 mmol) in water (500 mL) at room temperature sodium hydroxide was added (1.22 g, 30.5 mmol). The slurry was stirred at 50 °C for 1.5 hours then the reaction acidified to pH ~ 1 with aqueous hydrochloric acid (12 N). The slurry was filtered and the solid dried *in vacuo* at 55 °C. Analysis by ¹H NMR showed the solid to be a mixture of starting ester and desired product (2) of the ratio 33/66% respectively. The reaction material was resubjected to the above conditions for 18 hours then acidified to pH ~ 1 with aqueous hydrochloric acid (12 N). The slurry was filtered to give the title compound as a tan solid (1.60 g, 39%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 5.18 (s, 2H); 7.46 (m, 3H); 7.92 (d, 1H, *J* = 8.1 Hz); 8.03 (d, 1H, *J* = 1.8 Hz); 8.15 (d, 1H, *J* = 8.7 Hz)

4-(7-Chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1*H*-pyridazino[4,5-*b*]quinolin-2-ylmethyl)-*N*-furan-2-ylmethyl-benzamide (3)

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To a stirred suspension of PS-carbodiimide (77 mg, 0.75 mmol, 0.98 mmol/g, Argonaut Technologies Inc., lot no. 113-78) in *N,N*-dimethylformamide (3 mL) at room temperature was added 7-chloro-4-hydroxy-2-(4-carboxy-phenyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione (2, 100 mg, 0.25 mmol). The reaction was stirred at 50 °C for 4 hours. The slurry was filtered and the resin washed with *N,N*-dimethylformamide (4 x 10 mL). The resin was added to *N,N*-dimethylformamide (3 mL) treated with furfurylamine (97 mg, 1.0 mmol) and heated at 50 °C for 1 hour. The reaction was filtered, washed with *N,N*-dimethylformamide (2 x 3 mL), and the solvents were evaporated to dryness. The residual oil was treated with aqueous hydrochloric acid (1 mL, 1N) to give the title compound as a tan solid (5 mg, 4 %). MS (-CI) *m/z* 474 ¹H NMR (300 MHz, DMSO-*d*₆): δ 4.45 (d, 2H, *J* = 5.4 Hz); 5.16 (s, 2H); 6.26 (d, 1H, *J* = 3 Hz); 6.39 (dd, 1H, *J* = 1.8, 3.0 Hz); 7.37 (d, 2H, *J* = 8.1 Hz); 7.45 (dd, 1H, *J* = 7.2, 9.0 Hz); 7.57 (s, 1H); 7.84 (d, 2H, *J* = 8.4 Hz); 8.03 (d, 1H, *J* = 1.8 Hz); 8.14 (d, 1H, *J* = 8.7 Hz); 8.94 (m, 1H); 11.95 (br s, 1H); 12.66 (br s, 1H).

Example 9: 7-Chloro-4-hydroxy-2-(4-dimethylaminobenzyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione methanesulfonate (4).

(*tert*-Butoxy)-*N*-[(4-dimethylaminobenzyl)amino]carboxamide (1).

To a stirred solution of *t*-butylcarbazate (3.14 g, 23.8 mmol) in ethanol (40 mL) at reflux was added 4-dimethylaminobenzaldehyde (2.50 g, 16.8 mmol) over 5 minutes and the mixture stirred at reflux for 2 days. The reaction mixture was cooled to room temperature, then added to 5% palladium-on-carbon (425 mg) and ethanol (20 mL) in a Parr shaker bottle. The reaction was hydrogenated at 50 psi for 24 h. The mixture was filtered through celite, which was washed with methyl alcohol. The combined filtrate and washes were concentrated *in vacuo*. The material was subjected to flash chromatography (silica gel, 25% ethyl acetate/toluene) to yield the title compound as an oil (1, 3.5 g, 78%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.39 (s, 9H); 2.86 (s, 6H); 3.71 (d, 2H, *J* = 5.1 Hz); 4.40 (dt, 1H, *J* = 4.8, 5.1 Hz); 6.67 (d, 2H, *J* = 8.7 Hz); 7.11 (d, 2H, *J* = 8.7 Hz); 8.18 (s, 1H, br).

N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-*N*-(4-dimethylaminobenzyl)carboxamide (3).

To a stirred mixture of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (2, 2.8 g, 8.7 mmol) and dry tetrahydrofuran (130 mL) under nitrogen was

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added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (5.6 g, 13.1 mmol). The yellow reaction mixture was treated rapidly with (*tert*-butoxy)-N-[(4-dimethylaminobenzyl)amino]carboxamide (1, 1.6 g, 7.1 mmol) in tetrahydrofuran (40 mL). After stirring overnight, the reaction mixture was filtered, and the filtrate was concentrated to give a crusty yellow foam (6.85 g). The material was subjected to flash chromatography (silica gel, 5% MeOH/methylene chloride) to give the title compound (3, 4.11 g, 83%).

7-Chloro-4-hydroxy-2-(4-dimethylaminobenzyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (4).

To a stirred mixture of N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinoly)]-N-(4-dimethylaminobenzyl)carboxamide (3, 3.5 g, 6.2 mmol) and dry tetrahydrofuran (120 mL) under nitrogen was added methanesulfonic acid (15.3 mL, 0.23 mol). The clear yellow solution was stirred for 2 days at room temperature. The precipitate that formed was collected by suction filtration and washed with tetrahydrofuran and ether. The solid was treated with methanol and sonicated for 30 minutes. The solid was again collected by filtration, washed with methanol and ether, and dried at 90 °C under vacuum to give the title compound (4, 2.3 g, 72%) as a white powder, m.p. softens 218-225 °C, decomposes 270-280 °C. ¹H NMR (300 MHz, DMSO-*d*₆, TFA shake): δ 2.54 (CH₃CO₂H, s, 3H) 3.23 (s, 6H), 5.18 (s, 2H); 7.44 (dd, 1H, J = 1.8, 8.7 Hz); 7.51 (d, 2H, J = 8.4 Hz); 7.67 (d, 2H, J = 8.4 Hz); 8.08 (d, 1H, J = 1.8 Hz); 8.19 (d, 1H, J = 8.7 Hz).

Calc'd. for C₂₈H₁₇ClN₄O₅•CH₃SO₃H•H₂O: C, 49.36; H, 4.54; N, 10.96. Found: C, 49.22; H, 4.51; N, 10.93

Example 10: 7-Chloro-4-hydroxy-2-(benzimidazol-5-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate.

N-((1-Aza-2-benzimidazole-5-yl)vinyl)(*tert*-butoxy) carboxamide (1).

To a stirred solution of *tert*-butylcarbazate (2.49 g, 18.9 mmol) in tetrahydrofuran (40 mL) was added benzimidazole-5-carboxaldehyde (2.77 g, 18.9 mmol) in tetrahydrofuran (20 mL). The reaction was stirred overnight and the solvent evaporated to give the title compound as a white solid (3.88 g, 79%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.48 (s, 9H); 7.57 (d, 1H, J = 7.2 Hz); 7.62 (d, 1H, J = 7.2 Hz); 7.79 (s, 1H); 8.12 (s, 1H); 8.23 (s, 1H); 10.83 (s, 1H)

(*tert*-Butoxy)-N-[(benzimidazol-5-ylmethyl)]carboxamide (2).

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N-((1-Aza-2-benzimidazole-5-yl)vinyl)(tert-butoxy) carboxamide (0.500 g, was dissolved in ethyl alcohol (80 mL) and placed in a Parr shaker bottle. Hydrochloric acid (1.92 mL of 1M solution in ethyl alcohol) was added, followed by 10% palladium-on-carbon (750 mg). The reaction was hydrogenated at 40 psi for 2.5 hr and then filtered through celite to remove the catalyst. The catalyst/celite was washed with ethyl alcohol (3 x 100 mL) and the combined filtrate and washes were concentrated *in vacuo*. The residual oil (ca. 0.54 g) was dissolved in water (3 mL) to which a small portion of saturated sodium bicarbonate was then added (ca. 2 mL) followed by saturated sodium chloride (ca. 2 mL). The resulting aqueous layer was then extracted with ethyl acetate (200 mL). The organic extract was dried over MgSO₄, filtered and evaporated to dryness to give the title compound (2) as a white solid (0.27 g, 47%). ¹H NMR (300 MHz, DMSO-d₆): δ (TFA shake) 1.36 (s, 9H); 4.51 (s, 2H); 7.68 (dd, 1H, J = 1.5, 8.5 Hz); 7.95 (d, 1H, J = 8.5 Hz); 8.00 (s, 1H); 9.68 (s, 1H).

(+/-)-N-[(tert-Butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)-N-(benzimidazol-5-ylmethyl) carboxamide (3).

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (0.95 g, 3.00 mmol) in tetrahydrofuran (40 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (1.88 g, 4.50 mmol) and the reaction was stirred for five minutes. To this mixture was added dropwise a solution of (tert-butoxy)-N-[(benzimidazol-5-ylmethyl)]carboxamide (0.77 g, 3.00 mmol) and 4-dimethylaminopyridine (ca. 0.020 g) in tetrahydrofuran (20 mL). The mixture was stirred overnight at room temperature and then filtered. The collected solids were washed with methylene chloride (2 x 150 mL). The combined filtrate and washes were washed with water (100 mL) and brine and then dried over MgSO₄, filtered and concentrated. The residual yellow foam was subjected to chromatography (silica gel, gradient of chloroform/methyl alcohol beginning from 95/5 and ending at 90/10 respectively) to give the title compound (3) as a yellow foam (0.300 g, 19%).

7-Chloro-4-hydroxy-2-(benzimidazol-5-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (4).

To a stirred solution of (+/-)-N-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)-N-(benzimidazol-5-ylmethyl) carboxamide (0.300 g, 0.53 mmol) in tetrahydrofuran (15 mL) was added methanesulfonic acid (1.4 mL) and the reaction was stirred overnight. The reaction mixture was filtered to separate a yellow solid.

This material was washed with tetrahydrofuran and the resultant solid dried under vacuum. The material was then sonicated in 5 mL of 1/1 diethyl ether/methyl alcohol for fifteen minutes, filtered and washed with the same solvent system (40 mL) to give the title compound (4) as a yellow solid (0.07 g, 25%; m.p. > 280 °C). ¹H NMR (300 MHz, DMSO-d₆): δ 2.32 (CH₃SO₃H); 5.29 (s, 2H); 7.45 (dd, 1H, J = 1.8, 8.7 Hz); 7.56 (d, 1H, J = 7.2 Hz); 7.81 (m, 2H); 8.03 (d, 1H, J = 2.0 Hz); 8.16 (d, 1H, J = 8.7 Hz); 9.53 (s, 1H); 12.00 (s, 1H); 12.75 (s, 1H).

Example 11: 7-Chloro-4-hydroxy-2-(4-hydroxy-3-methyl-benzyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (2)

To a stirred solution of *N*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydroquinoline-3-carbonyl]-*N*-(4-hydroxy-3-methyl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (1, 0.30 g, 0.54 mmol) in tetrahydrofuran (10 mL) was added methanesulfonic acid (1.2 mL) and the reaction was stirred at room temperature for 16 hours. The tetrahydrofuran was removed under reduced pressure to yield an orange oil. To this was added diethyl ether (50 mL) and the reaction stirred for 10 minutes. The diethyl ether was carefully decanted to leave a thick orange oil. To this was added water (5 mL) and a fluffy yellow precipitate formed. The mixture was stirred for 10 minutes and then filtered. The solids were washed with water (10 mL) followed by diethyl ether (20 mL). The solids were collected and sonicated in methyl alcohol/diethyl ether (1:10, 5 mL) for 10 minutes. The solids were collected and dried under reduced pressure to give the title compound as a cream-colored solid (0.06 g, 30%). ¹H NMR (300 MHz, DMSO-d₆): δ 2.07 (s, 3H); 4.94 (s, 2H); 6.69 (d, 1H, J = 8.1 Hz); 6.96 (d, 1H, J = 8.1 Hz); 7.03 (s, 1H); 7.42 (d, 1H, J = 8.4 Hz); 8.01 (s, 1H); 8.14 (d, 1H, J = 8.4 Hz); 9.23 (s, 1H); 11.89 (s, 1H); 12.54 (s, 1H). MS (-CI): *m/z* 382/383.

Example 12: 7-Chloro-4-hydroxy-2-[3-methyl-4-(pyridin-2-ylmethoxy)-benzyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (2)

To a stirred solution of *N*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydroquinoline-3-carbonyl]-*N*-(4-hydroxy-3-methyl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (1, 0.30 g, 0.53 mmol) in *N,N*-dimethylformamide (5 mL) was added 2-picoly chloride hydrochloride (0.11 g, 0.66 mmol) followed by potassium carbonate (0.500 g, 3.61 mmol). The mixture was heated to 100 °C for 8 hours. The solution was cooled to room temperature

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and diluted with methylene chloride (50 mL). The organic layer was extracted with water (2 x 30 mL), brine (1 x 30 mL) and dried over Na₂SO₄. The volatiles were removed under reduced pressure to give a dark yellow oil. MS (-CI): *m/z* 644/647. The oil was subsequently dissolved in tetrahydrofuran (5 mL) and to this was added methanesulfonic acid (1 mL). The mixture was stirred for 16 hours and the volatiles removed under reduced pressure. The residual oil was triturated with diethyl ether (10 mL) for ten minutes and then the ether layer was carefully decanted away from the oil. The oil was then treated with water (5 mL) which induced precipitation to occur. The solids were collected by filtration, and washed with diethyl ether. The material was then sonicated in diethyl ether/methyl alcohol (4:1, 5 mL, 5 minutes) and filtered to give a brown solid (0.006 g, 3% yield for two steps). ¹H NMR (300 MHz, DMSO-d₆): δ 2.21 (s, 3H); 2.30 (s, 3H); 5.00 (s, 2H); 5.21 (s, 2H); 6.93 (d, 1H, *J* = 8.7 Hz); 7.09 (d, 1H, *J* = 8.7 Hz); 7.14 (s, 1H); 7.43 (m, 2H); 7.59 (d, 1H, *J* = 7.8 Hz); 7.95 (app t, 1H, *J* = 7.8 Hz); 8.01 (s, 1H); 8.14 (d, 1H, *J* = 8.7 Hz); 8.61 (d, 1H, *J* = 4.8 Hz); 11.95 (br s, 1H); 12.53 (br s). MS (-CI): *m/z* 473/474.

Example 13: Dimethyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methyl-phenyl ester (7) (Method A)

***N*-(4-Hydroxy-benzylidene)-hydrazinecarboxylic acid *tert*-butyl ester (2)**

To a stirred solution of 4-hydroxybenzaldehyde (1, 2.5 g, 20.0 mmol) in tetrahydrofuran (80 mL) was added *tert*-butyl carbazate (2.72 g, 20.5 mmol) and hydrochloric acid (conc., approximately 2 drops from a Pastuer pipette). The solution was stirred overnight at room temperature and the volatiles removed under reduced pressure. The resultant solid was triturated with hexanes and collected to give the title compound as a tan solid (4.7 g, 100%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.45 (s, 9H); 6.77 (d, 2H, *J* = 8.7 Hz); 7.40 (d, 2H, *J* = 8.7 Hz); 7.88 (br s, 1H); 9.78 (s, 1H); 10.66 (br s, 1H).

***N*-(4-Dimethylcarbamoyloxy-benzylidene)-hydrazinecarboxylic acid *tert*-butyl ester (3)**

To a slurry of *N*-(4-hydroxy-benzylidene)-hydrazinecarboxylic acid *tert*-butyl ester (2, 1.0 g, 4.23 mmol) in anhydrous pyridine (5 mL) was slowly added *N,N*-dimethylcarbonyl chloride (0.45 g, 0.39 mL, 4.23 mmol). The mixture was refluxed for 5 hours and then cooled to room temperature. Over time, a solid fell out of solution. The solid was collected, washed with water (20 mL) and then diethyl ether (20 mL). This material was then dried under

reduced pressure to give the title compound as a cream colored solid (1.01 g, 76%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.46 (s, 9H); 2.91 (s, 3H); 3.04 (s, 3H); 7.15 (d, 2H, J = 8.7 Hz); 7.55 (d, 2H, J = 8.7 Hz); 7.98 (br s, 1H); 10.89 (br s, 1H).

N-(4-Dimethylcarbamoyloxy-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (4)

N-(4-Dimethylcarbamoyloxy-benzylidene)-hydrazinecarboxylic acid *tert*-butyl ester (3, 1.0 g, 3.25 mmol) was dissolved in methyl alcohol (60 mL) and placed in a Parr shaker bottle. To this was added 10% palladium-on-carbon (250 mg) and the reaction was hydrogenated at 40 psi for 6 hours. The catalyst was filtered on CELITE, washed with methyl alcohol (2 x 100 mL), and the solvents were removed *in vacuo* to give a white solid (0.62 g, 62%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.38 (s, 9H); 2.84 (s, 3H); 3.03 (s, 3H); 3.84 (d, 2H, J = 3.9 Hz); 4.75 (m, 1H); 7.06 (d, 2H, J = 8.7 Hz); 7.26 (d, 2H, J = 8.7 Hz); 8.25 (br s, 1H).

N-[7-Chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N*-(4-dimethylcarbamoyloxy-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (6)

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (5, 0.53 g, 1.67 mmol) in tetrahydrofuran (30 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (0.92 g, 2.17 mmol) and the reaction was stirred for five minutes. To this mixture was added, via dropwise addition, a solution of *N*-(4-dimethylcarbamoyloxy-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (4, 0.62 g, 2.00 mmol) and 4-dimethylaminopyridine (ca. .005 g) in tetrahydrofuran (10 mL). The mixture was then refluxed overnight. The solution was filtered, washed with tetrahydrofuran (15 mL) and the insolubles collected. The insoluble material was then washed with water (100 mL) and tetrahydrofuran (20 mL). The remaining insoluble material was dried under vacuum to give the title compound as a white solid (0.785 g, 76%). The material was used in the following reaction without further purification.

Dimethyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-yl)methyl-phenyl ester (7)

To a stirred solution of *N*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N*-(4-dimethylcarbamoyloxy-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (6, 0.78 g, 0.33 mmol) in tetrahydrofuran (10 mL) was added methanesulfonic acid (2.88 mL) and the reaction was heated to 70 °C for 15 minutes. The mixture was cooled to room temperature and stirred for 4 hours. The tetrahydrofuran was removed under reduced

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pressure to yield an orange oil. To this was added diethyl ether (50 mL) and the reaction stirred for 10 minutes. The diethyl ether was carefully decanted to leave a thick orange oil. To this was added water (15 mL) and a fluffy yellow precipitate formed. The mixture was stirred for 10 minutes and then filtered. The solids were washed with water (10 mL) followed by diethyl ether (20 mL). The solids were collected and sonicated in methyl alcohol/diethyl ether (1:1, 20 mL) for 15 minutes. The solids were collected and dried under reduced pressure to give the title compound as a cream-colored solid (0.30 g, 55%). ¹H NMR (300 MHz, DMSO-d₆): δ 2.89 (s, 3H); 3.02 (s, 3H); 5.10 (s, 2H); 7.03 (d, 2H, J = 8.7 Hz); 7.29 (d, 2H, J = 8.7 Hz); 7.42 (d, 1H, J = 8.7 Hz); 8.01 (s, 1H); 8.14 (d, 1H, J = 8.7 Hz); 11.92 (s, 1H); 12.65 (s, 1H).

Calc'd. for C₂₁H₁₇ClN₄O₃•0.4H₂O: C, 56.29; H, 4.00; N, 12.50. Found: C, 56.46; H, 3.84; N, 12.38.

Example 14: Dimethyl-carbamate 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methyl-phenyl ester (7) (Method B)
N-(4-Hydroxy-3-methyl-benzylidene)-hydrazinecarboxylic acid tert-butyl ester (2)

To a stirred solution of 4-hydroxy-3-methylbenzaldehyde (1, 2.0 g, 14.6 mmol) in tetrahydrofuran (80 mL) was added *tert*-butyl carbazate (2.03 g, 15.4 mmol). The solution was stirred for 16 hours at room temperature and the volatiles removed under reduced pressure. The resultant solid was triturated with hexanes and collected to give the title compound as a tan solid (3.5 g, 95%). The material was used without further purification in the following reaction.

N-(4-Hydroxy-3-methyl-benzyl)-hydrazinecarboxylic acid tert-butyl ester (3)

N-(4-Hydroxy-3-methyl-benzylidene)-hydrazinecarboxylic acid *tert*-butyl ester (2, 2.0 g, 7.99 mmol) was dissolved in methyl alcohol (60 mL) and placed in a Parr shaker bottle. To this was added 10% palladium-on-carbon (250 mg) and the reaction was hydrogenated at 40 psi for 4 hours. The catalyst was filtered on Celite®, washed with methyl alcohol (2 x 100 mL), and the solvents were removed *in vacuo* to give a white solid (1.89 g, 94%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.38 (s, 9H); 2.08 (s, 3H); 3.62 (br s, 2H); 4.40 (br s, 1H); 6.65 (s, 1H, J = 8.1 Hz); 6.89 (dd, 1H J = 1.8 Hz, 8.1 Hz); 6.98 (s, 1H); 8.17 (br s, 1H).

N-[7-Chloro-4-oxo-2-(pyrrolidin-1-yl)-1,4-dihydro-quinoline-3-carbonyl]-N-(4-hydroxy-3-methyl-benzyl)-hydrazinecarboxylic acid tert-butyl ester (5)

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To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (4, 1.89 g, 5.91 mmol) in tetrahydrofuran (50 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (2.99 g, 7.09 mmol) and the reaction was stirred for five minutes. To this mixture was added, via dropwise addition, a solution of *N*-(4-hydroxy-3-methyl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (3, 1.61 g, 6.38 mmol) and 4-dimethylaminopyridine (0.08 g, 0.65 mmol) in tetrahydrofuran (5 mL), and the mixture was then refluxed for 8 hours. The solution was filtered and the insolubles washed with methylene chloride (2 x 150 mL). The mother liquor was collected and concentrated to dryness. The resultant yellow foam was subjected to chromatography (SiO₂, 95/5 chloroform/methyl alcohol) to give the title compound as a yellow foam (2.10 g, 65%). MS (-CI) *m/z* 551/552

N-[7-Chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N*-(4-dimethylcarbamoyloxy-3-methyl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (6)

To a stirred solution of methylene chloride and *N*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N*-(4-hydroxy-3-methyl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (5, 0.35 g, 0.63 mmol) was added triethylamine (0.10 mL, 0.72 mmol) and 4-dimethylaminopyridine (0.03 g, 0.24 mmol) and *N,N*-dimethylcarbamyl chloride (0.07 mL, 0.78 mmol). The solution was stirred for 16 hours and then diluted with methylene chloride (50 mL). The organic layer was extracted with aqueous ammonium chloride (saturated, 1 x 15 mL), aqueous ammonium hydroxide (15 %, 1 x 15 mL), and aqueous sodium chloride (saturated, 1 x 15 mL). The organic layer was then dried over Na₂SO₄ and concentrated to dryness. The resultant oil was used without further purification in the following reaction. MS (-CI) *m/z* 624/625

Dimethyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1*H*-pyridazino[4,5-*b*]quinolin-2-ylmethyl)-2-methyl-phenyl ester (7)

To a stirred solution of *N*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N*-(4-dimethylcarbamoyloxy-3-methyl-benzyl)-hydrazinecarboxylic acid *tert*-butyl ester (6, 0.25 g, 0.40 mmol) in tetrahydrofuran (10 mL) was added methanesulfonic acid (1.2 mL) and the reaction was stirred at room temperature for 16 hours. The tetrahydrofuran was removed under reduced pressure to yield an orange oil. To this was added diethyl ether (50 mL) and the reaction stirred for 10 minutes. The diethyl ether was carefully decanted to leave a thick orange oil. To this was added water (5 mL) and a fluffy

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yellow precipitate formed. The mixture was stirred for 10 minutes and then filtered. The solids were washed with water (10 mL) followed by diethyl ether (20 mL). The solids were collected and sonicated in methyl alcohol/diethyl ether (1:10, 5 mL) for 10 minutes. The solids were collected and dried under reduced pressure to give the title compound as a cream-colored solid (0.06 g, 30%). ¹H NMR (300 MHz, DMSO-d₆): δ 2.09 (s, 3H); 2.89 (s, 3H); 3.05 (s, 3H); 5.07 (s, 2H); 6.98 (d, 1H, J = 8.1 Hz); 7.13 (d, 1H, J = 8.1 Hz); 7.18 (s, 1H); 7.42 (d, 1H, J = 8.4 Hz); 8.02 (s, 1H); 8.14 (d, 1H, J = 8.4 Hz); 11.93 (br s, 1H); 12.64 (br s). MS (-CI): m/z 453/454.

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Examples 15 - 27:

Table 1 shows carbamate-containing pyridazino quinoline diones of the present invention and the physical characteristics thereof.

Table 1:

Ex.	Compound Name	Y ¹	NMR (300 MHz, DMSO-d ₆): δ	MS	M ²
15	Dimethyl-carbamic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester.	48%	2.88 (s, 3H); 3.01 (s, 3H); 5.11 (s, 2H); 7.02 (m, 2H); 7.13 (d, 1H, J = 7.8 Hz); 7.33 (t, 1H, J = 7.8 Hz); 7.43 (d, 1H, J = 8.7 Hz); 8.02 (s, 1H); 8.15 (d, 1H, J = 8.7 Hz); 11.94 (br s, 1H); 12.70 (br s, 1H).	(-CI) m/z 439/ 441	A
16	Methyl-phenyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester.	40%	3.39 (s, 3H); 5.10 (s, 2H); 7.09 (d, 2H, J = 8.4 Hz); 7.24 (m, 3H); 7.43 (m, 5H); 8.01 (s, 1H); 8.14 (d, 1H, J = 8.7 Hz); 11.92 (br s, 1H); 12.66 (br s, 1H).	(-CI) m/z 501/ 503	A

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17	Diethyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1 <i>H</i> -pyridazino[4,5- <i>b</i>]quinolin-2-ylmethyl)-phenyl ester.	38%	1.10 (m, 3 H); 1.16 (m, 3H); 3.30 (m, 4H); 5.10 (s, 2H); 7.06 (d, 2H, <i>J</i> = 8.4 Hz); 7.33 (d, 1H, <i>J</i> = 8.4 Hz); 7.42 (d, 1H, <i>J</i> = 8.7 Hz); 8.02 (s, 1H); 8.14 (d, 1H, <i>J</i> = 8.7 Hz); 11.92 (br s, 1H); 12.66 (br s, 1H).	(-Cl) <i>m/z</i> 467/ 468/ 469	A
18	Diethyl-carbamic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1 <i>H</i> -pyridazino[4,5- <i>b</i>]quinolin-2-ylmethyl)-phenyl ester.	38%	1.09 (m, 3H); 1.17 (m, 3H); 3.27 (m, 2H); 3.37 (m, 2H); 5.11 (s, 2H); 7.01 (d, 1H, <i>J</i> = 8.1 Hz); 7.05 (s, 1H); 7.13 (d, 1H, <i>J</i> = 7.8 Hz); 7.33 (t, 1H, <i>J</i> = 7.8 Hz); 7.43 (d, 1H, <i>J</i> = 8.7 Hz); 8.02 (d, 1H, <i>J</i> = 1.8 Hz); 8.15 (d, 1H, <i>J</i> = 8.7 Hz); 11.94 (br s, 1H); 12.68 (br s, 1H).	(-Cl) <i>m/z</i> 468/ 469/ 470	A
19	Morpholine-4-carboxylic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1 <i>H</i> -pyridazino[4,5- <i>b</i>]quinolin-2-ylmethyl)-phenyl ester.	18%	2.31 (MeOH adduct); 3.39 (br m, 2H); 3.55 (br m, 2H); 3.82 (br m, 4H); 5.11 (s, 2H); 7.04 (m, 2H); 7.15 (d, 1H, <i>J</i> = 7.8 Hz); 7.35 (t, 1H, <i>J</i> = 7.8 Hz); 7.44 (d, 1H, <i>J</i> = 8.7 Hz); 8.03 (s, 1H); 8.15 (d, 1H, <i>J</i> = 8.7 Hz).	(-Cl) <i>m/z</i> 481/ 482/ 483	B

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20	Methyl-phenyl-carbamic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester.	31%	3.32 (s, 3H); 5.10 (s, 2H); 7.06 (m, 3H); 7.14 (d, 1H, J = 7.8 Hz); 7.30 (m, 2H); 7.43 (m, 4H); 8.02 (s, 1H); 8.14 (d, 1H, J = 8.7 Hz); 11.95 (br s, 1H); 12.68 (br s, 1H).	(-Cl) m/z 501/ 502	B
21	Diphenyl-carbamic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester.	16% ²	5.10 (s, 2H); 7.13 (m, 3H); 7.27 (m, 2H); 7.40 (m, 10H); 8.02 (s, 1H); 8.14 (d, 1H, J = 8.7 Hz); 11.93 (br s, 1H); 12.66 (br s, 1H).	(-Cl) m/z 563/ 564	B
22	Pyrrolidine-1-carboxylic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester.	23%	1.85 (m, 4H); 3.30 (d, 2H, J = 6.3 Hz); 3.46 (d, 2H, J = 6.3 Hz); 5.11 (s, 2H); 7.04 (m, 1H); 7.12 (m, 1H); 7.36 (t, 1H, J = 7.8 Hz); 7.44 (d, 1H, J = 8.7 Hz); 8.04 (s, 1H); 8.13 (d, 1H, J = 8.7 Hz); 11.92 (br s, 1H); 12.68 (br s, 1H).	(-Cl) m/z 466/ 467	B
23	Morpholine-4-carboxylic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methyl-phenyl ester.	26%	2.10 (s, 3H); 3.42 (m, 4H); 3.62 (m, 2H); 7.02 (d, 1H, J = 8.1 Hz); 7.14 (d, 1H, J = 8.1 Hz); 7.19 (s, 1H); 7.44 (d, 1H, J = 8.7 Hz); 8.03 (s, 1H); 8.14 (d, 1H, J = 8.7 Hz); 11.94 (br s, 1H); 12.64 (br s, 1H).	(-Cl) m/z 495/ 496	B

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24	Methyl-phenyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methyl-phenyl ester.	18%	2.08 (s, 3H); 3.33 (s, 3H); 7.04 (m, 2H); 7.13 (m, 2H); 7.17 (s, 1H); 7.29 (m, 1H); 7.44 (m, 5H); 8.02 (s, 1H); 8.15 (d, 1H, J = 8.7 Hz); 11.91 (br s, 1H); 12.63 (br s, 1H).	(-Cl) m/z 514/ 515	B
25	Dimethyl-carbamic acid 5-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methoxy-phenyl ester.	41%	2.86 (s, 3H); 3.00 (s, 3H); 3.73 (s, 3H); 5.03 (s, 2H); 7.02 (m, 2H); 7.15 (d, 1H, J = 8.1 Hz); 7.42 (d, 1H, J = 8.7 Hz); 8.03 (s, 1H); 8.14 (s, 1H); 11.90 (br s, 1H); 12.64 (br s, 1H).	(-Cl) m/z 469/ 470/ 471	B
26	Diethyl-carbamic acid 5-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methoxy-phenyl ester.	3%	1.09 (m, 3H); 1.17 (m, 3H); 3.26 (m, 2H); 3.37 (m, 2H); 3.73 (s, 3H); 5.03 (s, 2H); 7.04 (m, 2H); 7.13 (m, 1H); 7.43 (d, 1H, J = 8.7 Hz); 8.03 (s, 1H); 8.14 (d, 1H, J = 8.7 Hz); 11.90 (br s, 1H); 12.63 (br s, 1H).	(-Cl) m/z 497/ 498/ 499	B
27	Morpholine-4-carboxylic acid 5-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1H-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methoxy-phenyl ester.	10%	3.37 (m, 4H); 3.62 (m, 2H); 5.03 (s, 2H); 7.05 (m, 2H); 7.15 (m, 1H); 7.43 (d, 1H, J = 8.7 Hz); 8.02 (s, 1H); 8.14 (d, 1H, J = 8.7 Hz); 11.89 (br s, 1H); 12.61 (br s, 1H).	(-Cl) m/z 511/ 512	B

¹ is Yield (given is for final step). ² (given for final two steps). ³ Method of Example 13 (A) or 14 (B).

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Example 28: 7-Chloro-4-hydroxy-2-(4-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate.

(tert-Butoxy)-N-[(4-pyridylmethyl)amino]carboxamide (2).

5 To a stirred solution of t-butylcarbazate (174 g, 1.36 mole) and dry N, N-dimethylformamide (400 mL) under nitrogen was added triethylamine (108 mL, 0.78 mole) followed by 4-picolyl chloride hydrochloride (1, 40.0 g, 0.243 mole). The reaction mixture was then heated at 75 °C for 5 hours and allowed to cool to room temperature. The reaction mixture was diluted with water (2 L) and the resulting mixture extracted with ethyl acetate (4
10 x 500 mL). The combined ethyl acetate extracts were concentrated under reduced pressure and the residue was dissolved in ether (1 L). The resulting solution was washed successively with water (3 x 400 mL) and brine (400 mL) and then dried over Na₂SO₄. The Na₂SO₄ was filtered off and the filtrate was concentrated under reduced pressure to give an amber oil (130.4 g). This product was purified by flash chromatography on silica gel eluting with
15 hexane:ethyl acetate (1:1) to give the title compound (2) as a solid off-white foam (24.46 g, 45%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.36 (s, 9H); 3.90 (d, 2H, J = 4.0 Hz); 5.04 (d, 1H, J = 4.0 Hz); 7.34 (d, 1H, J = 4.5 Hz); 8.48 (d, 1H, J = 4.5 Hz).

N-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(4-pyridylmethyl)carboxamide (4).

20 To a stirred mixture of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (3, 24.29 g, 75.73 mmole) and dry tetrahydrofuran (1175 mL) under nitrogen was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (50.55 g, 119.34 mmole) in portions (35 g followed by 15.55 g after 10 min.). After stirring the reaction mixture for an additional 20 minutes, a solution of (tert-butoxy)-N-[(4-
25 pyridylmethyl)amino]carboxamide (2, 22.0 g, 98.5 mmole) and tetrahydrofuran (580 mL) was rapidly added and the mixture was stirred overnight. The reaction mixture was filtered and the filter cake was washed with methylene chloride (300 mL). The filtrate and washings were combined and additional methylene chloride (800 mL) was added. The resulting solution was washed with water (2 x 500 mL) and then dried over Na₂SO₄, filtered and concentrated under
30 reduced pressure to give 28.90 g of yellow foam. This foam was treated with ether (800 mL) and the resulting mixture was stirred and then filtered. The filter cake was dried at 45 °C under vacuum to give the desired N-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-

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(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(4-pyridylmethyl)carboxamide (4) (24.3 g, 61%) as a yellow powder.

7-Chloro-4-hydroxy-2-(4-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5).

To a stirred mixture of N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(4-pyridylmethyl)carboxamide (4, 24.0 g, 45.62 mmole) and dry tetrahydrofuran (960 mL) under nitrogen was added methanesulfonic acid (120 mL, 177.7 g, 1.85 moles) all at once. The mixture was stirred overnight and then filtered to separate the solids. The collected solids were successively washed with tetrahydrofuran (2 x 100 mL), methanol (2 x 50 mL), and ether (100 mL). The filter cake (13.4 g) was then suspended in methanol (250 mL) and the resulting mixture sonicated for 20 minutes and then filtered. The collected solids were washed with methanol (2 x 100 mL) and ether (100 mL) and then dried at 45 °C under vacuum to give 7-chloro-4-hydroxy-2-(4-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5, 12.1 g, 59%) as a yellow powder, m.p. > 250 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.32 (s, 3H), 5.36 (s, 2H); 7.49 (dd, 1H, J = 8.1 Hz, J = 2.1 Hz); 7.86 (d, 1H, J = 6.6 Hz); 8.06 (d, 1H, J = 2.1 Hz); 8.12 (d, 1H, J = 8.1 Hz); 8.82 (d, 1H, J = 6.6 Hz); 12.6 (br s, 1H); 12.84 (br s, 1H).

Calc'd. for C₁₇H₁₁ClN₄O₃•CH₃SO₃H•0.8H₂O: C, 46.47; H, 3.60; N, 12.04 Found: C, 46.39; H, 3.65; N, 11.98

Example 29: 7-Chloro-4-hydroxy-2-(3-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate.

(*tert*-Butoxy)-N-(3-pyridylmethyl)amino]carboxamide (2).

To a stirred solution of *t*-butylcarbazate (203.6 g, 1.54 mole) and dry N,N-dimethylformamide (300 mL) under nitrogen was added triethylamine (128 mL, 0.92 mole) followed by 3-picolyl chloride hydrochloride (1, 50.0 g, 0.30 mole) as a slurry in N,N-dimethylformamide (300 mL). The reaction mixture was heated at 75 °C for 3 hours, cooled to room temperature, and diluted with water (2.4 L). The resulting mixture was extracted with ether (3 x 800 mL). The aqueous layer was saturated with salt and extracted with ether (3 x 800 mL). The combined extracts were washed with water (1 x 1L), brine (1 x 1L) and then dried over Na₂SO₄. The Na₂SO₄ was filtered off and the filtrate was concentrated under reduced pressure. The product was purified by flash chromatography on silica gel eluting

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with ether to give the title compound (2) as an off-white solid (23.3 g, 34%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.36 (s, 9H); 3.88 (d, 2H, J = 4.0 Hz); 4.96 (d, 1H, J = 4.0 Hz); 7.33 (dd, 1H, J = 7.7 Hz, J = 4.8 Hz); 7.71 (d, 1H, J = 7.7 Hz); 7.44 (d, 1H, J = 4.7 Hz); 8.49 (s, 1H).

N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinoly)]-N-(3-pyridylmethyl)carboxamide (4).

To a stirred mixture of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (3, 20 g, 62.4 mmole) and tetrahydrofuran (800 mL) under nitrogen was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (40.0 g, 94.4 mmole). A solution of (*tert*-butoxy)-N-[(3-pyridylmethyl)amino]carboxamide (2, 20.9 g, 93.6 mmole) and tetrahydrofuran (450 mL) was rapidly added and the mixture stirred overnight. The reaction mixture was filtered and the filter cake was washed with tetrahydrofuran. The filter cake was slurried with methylene chloride and filtered. The filtrates were combined and evaporated under reduced pressure. The residue was dissolved in methylene chloride, dried over Na₂SO₄, filtered and evaporated under reduced pressure to give a foam. The foam was stirred with ether (200 mL) and filtered. The filter cake was sonicated with ether (200 mL), filtered, washed with ether and was dried at 40 °C under vacuum to give N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinoly)]-N-(3-pyridylmethyl)carboxamide (4) as an off-white powder (32.8 g, 100%).

7-Chloro-4-hydroxy-2-(3-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5).

To a stirred solution of N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinoly)]-N-(3-pyridylmethyl)carboxamide (4, 32.8 g, mole) and tetrahydrofuran (1L) under nitrogen was added methanesulfonic acid (150 mL, 222 g, 2.31 moles) over 10 minutes. The mixture was stirred overnight and then filtered to separate the solids. The collected solids were washed with tetrahydrofuran. The filter cake was suspended in methanol, sonicated (30 minutes) and filtered. The solids were resuspended in methanol, sonicated (30 minutes) and filtered. The collected solids were washed with methanol and then dried at 100 °C under vacuum to give 7-chloro-4-hydroxy-2-(3-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5) (19.4, 66%) as a white solid, m.p. > 300 °C. ¹H NMR (300 MHz, DMSO-d₆): δ 2.33 (s, 3H); 5.29 (s, 2H); 7.46 (dd, 1H, J = 9.0 Hz, J = 2.1 Hz); 7.94 (dd, 1H, J = 9.0 Hz, J = 5.6 Hz);

8.04 (d, 1H, J = 1.8 Hz); 8.16 (d, 1H, J = 8.7 Hz); 8.37 (d, 1H, J = 8.1 Hz); 8.82 (d, 1H, J = 4.8 Hz); 8.89 (s, 1H).

Calc'd. for $C_{17}H_{11}ClN_4O_3 \cdot CH_3SO_3H \cdot H_2O$: C, 46.11; H, 3.66; N, 11.95 Found: C, 46.34; H, 3.61; N, 11.94

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Example 30: 7-Chloro-4-hydroxy-2-(2-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate.

(tert-Butoxy)-N-[(2-pyridylmethyl)amino]carboxamide (2).

To a stirred solution of t-butylcarbazate (174 g, 1.53 mole) and dry N, N-dimethylformamide (400 mL) under nitrogen was added triethylamine (130 mL, 0.94 mole) followed by 2-picolyl chloride hydrochloride (1, 54.0 g, .33 mole). The reaction mixture was allowed to stir at ambient temperature for 1 hour, then heated at 70 °C for 3 hours and allowed to cool to room temperature. The reaction mixture was diluted with 1:1 mixture of ethyl acetate/ether and washed with brine and extracted. The aqueous layer was monitored by TLC (eluent: 100% ether) and was extracted several times with ethyl acetate (200 mL) until no product was observed. The combined organic extracts were washed with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give an amber oil (~100 g) which crystallized. The material was triturated with 1:1 ether/hexanes, filtered and dried under reduced pressure to afford the title compound (2) as an off-white solid (33.4 g, 45% yield). 1H NMR (300 MHz, DMSO- d_6): δ 1.38 (s, 9H); 3.96 (d, 2H, J = 4.0 Hz); 4.98 (d, 1H, J = 4.0 Hz); 7.24 (dd, 1H, J = 7.8 Hz, J = 7.8 Hz); 7.48 (d, 1H); 7.74 (dd, 1H, J = 7.5 Hz, J = 7.8 Hz); 8.32 (s, br, 1H); 8.47 (d, 1H, J = 4.8 Hz).

N-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(2-pyridylmethyl)carboxamide (4).

To a stirred mixture of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (3, 17.5 g, 54.7 mmole) and dry tetrahydrofuran (900 mL) under nitrogen was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (35.7 g, 81.2 mmole) in portions (25.0 g followed by 10.7 g after 10 minutes). After stirring the reaction mixture for an additional hour, a solution of (tert-butoxy)-N-[(2-pyridylmethyl)amino]carboxamide (2, 16.5 g, 73.9 mmole) and tetrahydrofuran (400 mL) was added and the mixture was stirred vigorously overnight. The reaction was monitored by TLC (10% methanol/methylene chloride) and determined to be complete. To separate the

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precipitated solids, the reaction mixture was filtered and the collected solids were washed with tetrahydrofuran. The filtrate and washings were combined and concentrated *in vacuo*. The filter cake was suspended in aqueous bicarbonate and brine solutions and extracted with methylene chloride (3 x 300 mL). These extracts were combined with the previously concentrated organic extracts and were washed with bicarbonate, brine (3x) and dried over Na₂SO₄. The Na₂SO₄ was filtered off and the filtrate was concentrated under reduced pressure to provide a residue which was purified by flash chromatography on silica gel eluting with 5% isopropanol/chloroform. After concentration of the desired fractions *in vacuo* the desired N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(2-pyridylmethyl)carboxamide (4) was isolated as a light tan powder (24.3 g, 61% yield).

7-Chloro-4-hydroxy-2-(2-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5).

To a stirred mixture of N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(2-pyridylmethyl)carboxamide (4, 24.0 g, .045 mole) and dry tetrahydrofuran (800 mL) under nitrogen was added methanesulfonic acid (100 mL, 148 g, 1.54 moles) all at once. The mixture was stirred overnight and then filtered to separate the solids. The collected solids were successively washed with tetrahydrofuran (2 x 100 mL) and ether (2 x 100 mL). The filter cake (15.8 g) was then suspended in methanol (250 mL) and the resulting mixture sonicated for 30 minutes and then filtered. The collected solids were washed with methanol (2 x 100 mL) and ether (100 mL) and then dried at 35 °C under vacuum to give the methane sulfonate salt of 7-chloro-4-hydroxy-2-(2-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (5, 12.1 g, 59%) as an orange powder, m.p. >300 °C. ¹H NMR (300 MHz, DMSO-d₆): δ 2.33 (s, 3H), 5.35 (s, 2H); 7.46 (d, 1H, J = 8.7 Hz); 7.64 (d, 1H, J = 7.8 Hz); 7.68 (dd, 1H, J = 4.8 Hz, J = 6.6 Hz); 8.02 (s, 1H); 8.14 (d, 1H, J = 8.7 Hz); 8.19 (dd, 1H, J = 6.6 Hz, J = 7.8 Hz); 8.73 (d, 1H, J = 4.8 Hz); 10.06 (s, br, 1H); 12.84 (s, br, 1H).

Calc'd. for C₁₇H₁₁ClN₄O₃•CH₃SO₃H: C, 47.95; H, 3.35; N, 12.43 Found: C, 47.93; H, 3.42; N, 12.01

Example 31: 7-Chloro-4-hydroxy-2-[3-hydroxy-5(hydroxymethyl)-2-methyl(4-pyridyl)]methyl-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate

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N-{1-Aza-2-[3-hydroxy-5(hydroxymethyl)-2-methyl(4-pyridyl)]vinyl}(tert-butoxy)-carboxamide hydrochloride (2).

To a stirred solution of *tert*-butylcarbazate (1.22 g, 9.82 mmol) in tetrahydrofuran (40 mL) was added pyridoxal hydrochloride (2.00 g, 9.82 mmol). After 2 h at room temperature, the reaction was refluxed for 4 h, and the solvent was removed *in vacuo*. The resultant solid was triturated with hexanes and filtered to give the title compound (2) as a white solid (3.00 g, 96%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.50 (s, 9H); 2.59 (s, 3H); 4.70 (s, 2H); 8.19 (s, 1H); 8.51 (s, 1H); 12.06 (s, 1H); 12.72 (s, 1H).

(+/-)-(tert-Butoxy)-N-[3-hydroxy-5(hydroxymethyl)-2-methyl(4-pyridylmethyl)]carboxamide (3).

N-{1-Aza-2-[3-hydroxy-5(hydroxymethyl)-2-methyl(4-pyridyl)]vinyl}(tert-butoxy)-carboxamide hydrochloride (3.0 g, 9.46 mmol) was dissolved in methyl alcohol (90 mL) and placed in a Parr shaker bottle. To this was added 10% palladium-on-carbon (300 mg) and the reaction was hydrogenated at 40 psi for 24 h. The mixture was filtered through celite, which was then washed with methyl alcohol (3 x 100 mL). The combine filtrate and washes were concentrated *in vacuo*. The resulting solid residue was dissolved in methylene chloride (400 mL) and the resulting solution washed with saturated sodium bicarbonate. The organic layer was washed with brine, dried over MgSO₄ and concentrated to give the title compound (3) as a white solid (1.4 g, 52%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.39 (s, 9H); 2.53 (s, 3H); 3.16 (s, 1H); 4.16 (s, 2H); 4.61 (s, 2H); 8.11 (s, 1H); 8.76 (br s, 1H).

(+/-)-N-[(tert-Butoxy)carboxylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-[3-hydroxy-5(hydroxymethyl)-2-methyl(4-pyridylmethyl)]carboxamide (4).

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (1.5 g, 4.94 mmol) in tetrahydrofuran (100 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (2.4 g, 5.68 mmol) and the reaction was stirred for five minutes. To this mixture was added via dropwise addition a solution of (+/-)-(tert-butoxy)-N-[3-hydroxy-5(hydroxymethyl)-2-methyl(4-pyridylmethyl)]carboxamide (1.4 g, 4.94 mmol) and 4-dimethylaminopyridine (0.250 g, 2.04 mmol) in tetrahydrofuran (20 mL), and the mixture was stirred at room temperature for 1 hour. The reaction mixture was filtered and the collected solids washed with methylene chloride (2 x 150 mL). The combined filtrate and washes were washed with water (100 mL).

and brine and then dried over MgSO_4 and filtered. The filtrate was concentrated to dryness to give the title compound (4) as a yellow foam (1.7 g, 58%).

7-Chloro-4-hydroxy-2-[3-hydroxy-5(hydroxymethyl)-2-methyl(4-pyridyl)methyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5).

To a stirred solution of (+/-)-N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)-N-[3-hydroxy-5(hydroxymethyl)-2-methyl(4-pyridylmethyl)] carboxamide (1.72 g, 1.5 mmol) in tetrahydrofuran (40 mL) was added methanesulfonic acid (5 mL) and the reaction was stirred overnight. The volatiles were removed *in vacuo* and the resultant oil was poured on to crushed ice. To this mixture was carefully added 10N sodium hydroxide until a solid precipitate formed. The solution was filtered to give an orange solid. The resultant aqueous layer formed a fine precipitate which was collected by filtration, washed with diethyl ether and dried to give the title compound (5) as an off-white powder (m.p. dec at 275 °C). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 2.30 (s, $\text{CH}_2\text{SO}_3\text{H}$); 2.39 (s, 3H); 4.84 (s, 2H); 5.21 (s, 2H); 7.45 (dd, 1H, $J = 2.1, 9.0$ Hz); 8.03 (m, 2H); 8.13 (d, 1H, $J = 7.2$ Hz).

Calc'd. for $\text{C}_{18}\text{H}_{13}\text{ClN}_4\text{O}_5 \cdot 0.5\text{CH}_3\text{SO}_3\text{H} \cdot \text{H}_2\text{O}$: C, 49.63; H, 3.87; N, 11.84. Found: C, 49.74; H, 3.84; N, 11.74.

Example 32: Dimethyl-carbamic acid 7-chloro-1,10-dioxo-2-pyridin-4-ylmethyl-1,2,5,10-tetrahydro-pyridazino[4,5-b]quinolin-4-yl ester (2)

To a stirred slurry of 7-chloro-4-hydroxy-2-pyridin-4-ylmethyl-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (1, 0.50 g, 1.07 mmol) in pyridine (5 mL) was added *N,N*-dimethylcarbonyl chloride (0.15 g, 0.13 mL, 1.45 mmol). The mixture was heated to reflux for 8 hours, cooled to room temperature and stirred for 16 hours. The resultant solid was collected by filtration and washed with water (20 mL) and diethyl ether (20 mL). This material was subjected to chromatography (SiO_2 , 90/10 chloroform/methyl alcohol) to give the title compound as a yellow powder (0.10 g, 22%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 2.97 (s, 3H); 3.15 (s, 3H); 5.22 (s, 2H); 7.24 (d, 2H, 5.7 Hz, $J = 5.7$ Hz); 7.46 (d, 1H, $J = 8.7$ Hz); 7.91 (s, 1H); 8.16 (d, 1H, $J = 8.7$ Hz); 8.50 (d, 1H, $J = 5.7$ Hz); 11.87 (br s, 1H). MS (-CI) m/z 424/425.

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Example 33: 7-Chloro-4-hydroxy-2-(4-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione

4-(2-Chloroethyl)pyridine (2)

To a stirred solution of 4-(2-hydroxyethyl)pyridine (4.95 g, 40.19 mmole) and chloroform (30 mL) under nitrogen was added thionyl chloride (8.04 g, 67.58 mmole). The reaction mixture was then heated at 60 °C for 0.5 hour. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in water. The resulting solution was neutralized with concentrated ammonium hydroxide and extracted with ethyl acetate (2 x 30 mL). The combined ethyl acetate extracts were dried over Na₂SO₄. The Na₂SO₄ was filtered off and the filtrate was concentrated under reduced pressure to give the title compound (2) as an amber oil (5.47 g, 96%). ¹H NMR (300 MHz, DMSO-d₆): δ 3.16 (t, 2H, J = 6.9 Hz); δ 3.93, (± 2, J = 6.9 Hz); δ 7.34 (d, 2H, J = 1.5 Hz); δ 8.50 (d, 2H, J = 1.5 Hz)

(tert-Butoxy)-N-[4 pyridylethyl]aminocarboxamide (3)

To a stirred solution of t-butylcarbazate (12.64 g, 95.34 mmole) and N,N-dimethylformamide (50 mL) under nitrogen was added 2-chloroethylpyridine 2 (5.4 g, 38.14 mmole). The reaction mixture was then heated at 75 °C for 18 hours and allowed to cool to room temperature. The reaction mixture was diluted with water (400 mL) and the resulting mixture extracted with ethyl acetate (2 x 125 mL). The combined ethyl acetate extracts were concentrated under reduced pressure and the residue was dissolved in ether (200 mL). The resulting solution was washed with 1N sodium bicarbonate (2 x 50 mL) and concentrated under reduced pressure to give an amber oil (10.24 gms). This product was purified by flash chromatography on silica gel eluting with chloroform:methanol (93:7 to 95:5) to give the title compound (3) as a solid light amber foam (4.88 g, 54%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.4 (m, 9H); δ 2.66 (t, 2H, J = 6.9 Hz); δ 2.90 (t, 2H, J = 6.91 Hz); δ 4.84 (s, 1H); δ 7.26 (d, 2H, J = 4.80 Hz); δ 8.28 (s, 1H); δ 8.43 (d, 2H, J = 4.80 Hz)

N-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(4-pyridylethyl)carboxamide (5)

To a stirred mixture of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (4, 2.7 g, 8.42 mmole) and dry tetrahydrofuran (132 mL) under nitrogen was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (7.13 g, 16.24 mmole) in portions (10.0 g followed by 6.84 g after 10 min). After stirring the reaction mixture for an additional 20 minutes, a solution of (tert-butoxy)-N-[4-

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pyridylethyl)amino]carboxamide (3, 2.0 g, 8.43 mmole) and tetrahydrofuran (64 mL) was rapidly added and the mixture was stirred overnight. The reaction mixture was filtered and the filter cake was washed with methylene chloride (40 mL). The filtrate and washings were combined and additional methylene chloride (80 mL) was added. The resulting solution was washed with water (2 x 50 mL) and then dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give 3.8 g of yellow foam. This foam was treated with ether (80 mL) and the resulting mixture was stirred and filtered. This product was purified by flash chromatography on silica gel eluting with chloroform:methanol (95:5) to give the title compound (5) as a solid yellow foam (1.20 g, 34%). MS m/z 538 (M-1); TLC, R_f = 0.62 chloroform:methanol (90:10) silica

7-Chloro-4-hydroxy-2-(4-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (6)

To a stirred mixture of *N*-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-*N*-(4-pyridylethyl)carboxamide (5, 1.15 g, 2.18 mmole) and dry tetrahydrofuran (40 mL) under nitrogen was added methanesulfonic acid (5 mL, 7.4 g, 77 mmoles) all at once. The mixture was stirred overnight and then concentrated under reduced pressure. The residue (oil) was washed with ether (2 x 50 mL) and the ether was decanted off. The residual oil was treated with water (60 mL) and stirred for 1 hr. then filtered. The filter cake was washed with water (2 x 20 mL) and dried under vacuum at 45 °C to give 7-chloro-4-hydroxy-2-(4-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (6, 620 mg, 48% yield) as a yellow powder, m.p. 223-225 °C. ^1H NMR (300 MHz, DMSO- d_6 , TFAD) δ 3.37 (t, 2H, J = 6.0 Hz); δ 4.38 (t, 2H, J = 6.0 Hz); δ 7.43 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz); δ 7.94 (d, 2H, J = 6.6 Hz); δ 8.03 (d, 1H, J = 1.8 Hz); δ 8.12 (d, 1H, J = 8.4 Hz) δ 8.78 (d, 2H, J = 6.6 Hz)

Calc'd for $\text{C}_{19}\text{H}_{13}\text{ClN}_4\text{O}_3 \cdot \text{CH}_3\text{SO}_3\text{H} \cdot 1.25 \text{H}_2\text{O}$: C, 46.82; H, 4.03; N, 11.50. Found: C, 46.71; H, 4.02; N, 11.48.

Example 34: 7-Chloro-4-hydroxy-2-(pyrazin-2-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5)

2-Chloromethylpyrazine (1).

2-Methylpyrazine (1.0 mL; 22 mmol) in carbon tetrachloride (80 mL) was treated with *N*-chlorosuccinimide (4.27 g, 31.5 mmol) and benzoyl peroxide (0.26 g, 1.1 mmol). The

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mixture was heated to reflux for 7 hours, and then cooled to room temperature. The solids were filtered through celite and washed with methylene chloride. The filtrate was washed with aqueous sodium thiosulfate (sat., 1X), aqueous sodium bicarbonate (sat., 1X), water (1X), and aqueous sodium chloride (sat., 1X). The organic layer was dried over sodium sulfate, filtered, concentrated under reduced pressure, and used directly in the following reaction. (Contains 5-10% of the a,a,-dichlorinated material). ¹H NMR (300 MHz, DMSO-d₆): δ 4.88 (s, 2H), 8.65-8.68 (m, 2H); 8.85 (s, 1H).

(tert-Butoxy)-N-[(pyrazin-2-ylmethyl)amino]carboxamide (2).

To a stirred solution of 2-chloromethylpyrazine (1, 1.2 g, 93 mmol) and dry N,N-dimethylformamide (16 mL) was added t-butylcarbazate (6.3 g, 48 mmol) and triethylamine (2.6 mL, 19 mmol) under nitrogen. The reaction mixture was then heated at 75 °C for 17 hours and cooled to room temperature. The reaction mixture was diluted with water and extracted with ether (5X). The combined ether extracts were washed with brine and dried over Na₂SO₄. The Na₂SO₄ was filtered off and the filtrate was concentrated under reduced pressure to give a brown oil (1 g). The oil was subjected to flash chromatography (silica gel, 2-5% gradient of methanol in methylene chloride) to give the title compound as a waxy brown solid (2, 1.64 g, 79%). ¹H NMR (300 MHz, DMSO-d₆, TFA shake): δ 1.44 (s, 9H); 4.53 (s, 2H); 8.71-8.78 (m, 2H); 8.81 (s, 1H).

N-[(tert-butoxycarbonylamino)[7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]]-N-(pyrazin-2-ylmethyl)carboxamide (4).

To a stirred mixture of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (3, 2.3 g, 7.2 mmol) and dry tetrahydrofuran (100 mL) under nitrogen was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (4.56 g, 10.8 mmol). After stirring 20 minutes, the reaction mixture was treated with a solution of (tert-butoxy)-N-[(pyrazin-2-ylmethyl)amino]carboxamide (2, 1.6 g, 7.1 mmol) and 4-dimethylaminopyridine (46 mg, 0.4 mmol) in tetrahydrofuran (10 mL). The reaction mixture was stirred at reflux for 2 days and cooled to room temperature. The reaction mixture was filtered and the filter cake was washed with tetrahydrofuran. The combined filtrate and washes were concentrated to give a brown foam (4.9 g). The foam was subjected to flash chromatography (silica gel, 2% methanol/methylene chloride) to give the title compound (4, 3.1 g, 82%).

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7-Chloro-4-hydroxy-2-(pyrazin-2-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5).

To a stirred mixture of N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(pyrazin-2-ylmethyl)carboxamide (4, 3.1 g, 5.9 mmol) and dry tetrahydrofuran (100 mL) under nitrogen was added methanesulfonic acid (13.5 mL, 0.21 mol). The mixture was stirred overnight, filtered, and the collected solid washed with tetrahydrofuran. The solid was treated with methanol and the mixture sonicated for 1 h. The solid was again collected by filtration, washed with methanol, and dried at 50 °C under vacuum to give the title compound (5, 2.0 g, 80%) as a white powder, m.p. 235-245 °C.

¹H NMR (300 MHz, DMSO-*d*₆): δ 2.38 (CH₃CO₂H, s, 3H), 5.27 (s, 2H); 7.44 (dd, 1H, J = 1.8, 8.7 Hz); 8.04 (d, 1H, J = 1.8 Hz); 8.14 (d, 1H, J = 8.7 Hz); 8.58 (dd, 2H, J = 2.4, 7.5 Hz); 8.63 (s, 1 H). Calc'd. for C₁₈H₁₆ClN₄O₅•CH₃SO₃H•H₂O: C, 43.46; H, 3.43; N, 14.90. Found: C, 43.28; H, 3.34; N, 15.17

Example 35: 7-Chloro-4-hydroxy-2-(furan-2-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione.

N-1-aza-2-(2-furanyl)vinyl)(*tert*-butoxy)carboxamide (1).

To a stirred slurry of *tert*-butylcarbazate (131.5 g, 0.99 mol) in hexane (1000 mL) was added 2-furaldehyde (91.9 g, 0.95 mol). The slurry was refluxed for 2.5 h, and then cooled to room temperature. The resultant tan solid was filtered, dried and used without further purification in the following reaction (1, 200 g, 99%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.52 (s, 9H); 6.45 (dd, 1H, J = 3.3, 1.2 Hz); 6.71 (d, 1H, J = 3.3 Hz); 7.47 (d, 1H, J = 1.2 Hz); 7.91 (s, 1H).

(*tert*-Butoxy)-N-[(2-furanylmethyl)amino]carboxamide (2).

A one liter, three-neck round bottom flask was equipped with an addition funnel, nitrogen inlet and an overhead mechanical stirrer. The apparatus was dried under vacuum and flushed with a steady stream of nitrogen gas. The flask was charged with lithium aluminum hydride (7.75 g, 0.20 mol) and tetrahydrofuran (30 mL). N-1-Aza-2-(2-furanyl)vinyl)(*tert*-butoxy)carboxamide (20 g, 0.095 mol) was dissolved in tetrahydrofuran (250 mL) and then slowly added to the stirred lithium aluminum hydride suspension over a 30 minute period. Any residual material remaining in the addition funnel was washed into the flask by rinsing with tetrahydrofuran (2 x 30 mL). The reaction was stirred overnight, cooled with an ice bath

and then carefully quenched with a saturated aqueous solution of Na_2SO_4 . The resulting mixture was filtered and the collected solids washed with tetrahydrofuran. The combined filtrate and washes were concentrated to an oil, which was stirred for 18 hours with hexanes (ca. 600 mL). The resulting mixture was filtered and the filtrate concentrated to give the
 5 desired material 2 as a yellow oil (10.0 g, 50%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.46 (s, 9H); 3.99 (d, 2H, $J = 4.9$ Hz); 4.21 (br s, 1H); 6.17 (br s, 1H); 6.24 (d, 1H, $J = 3.0$ Hz); 6.32 (dd, 1H, $J = 3.0, 1.2$ Hz); 7.38 (d, 1H, $J = 1.2$ Hz).

(+/-)-N-[(*tert*-Butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)-N-(2-furanylmethyl)carboxamide (3).

10 To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (26.99 g, 84.3 mmol) in tetrahydrofuran (1300 mL) was added diisopropylcarbodiimide (13.94 g, 110 mmol) and the reaction was stirred for ten minutes. To this mixture was added dropwise a solution of (*tert*-butoxy)-N-(2-furanylmethyl)amino]carboxamide (22.9 g, 103 mmol) in tetrahydrofuran (200 mL). After
 15 stirring the reaction for 18 hours, it was concentrated *in vacuo* to a brown tar which was triturated with chloroform. The resulting mixture was filtered, washed with chloroform and dried. This material (3) was used without further purification in the following reaction (mass of material approximately 30 g).

7-Chloro-4-hydroxy-2-(2-furanylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (4).

20 To a stirred solution of (+/-)-N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)-N-(2-furanylmethyl)carboxamide. (29.00 g, 56.3 mmol) in tetrahydrofuran (1000 mL) was added methanesulfonic acid (118 mL) and the reaction was stirred for 18 hours at room temperature. The reaction mixture was poured on 3
 25 liters of ice-water and the resulting mixture was filtered to give an off-white solid. This material was dissolved in hot tetrahydrofuran (ca. 1 liter) and then concentrated to half-volume. The resultant slurry was poured on ice-water (2 liters) and after twenty minutes the mixture was filtered. The collected material was dried to give the title compound (4) as a
 30 white solid (14.4 g, 74%; m.p. >265 °C). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 5.08 (s, 2H); 6.37 (d, 1H, $J = 3.0$ Hz); 6.42 (dd, 1H, $J = 3.0, 1.5$ Hz); 7.43 (d, 1H, $J = 7.8$ Hz); 7.54 (s, 1H); 8.00 (d, 1H, $J = 1.5$ Hz); 8.14 (d, 1H, $J = 8.7$ Hz).

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Calc'd. for $C_{16}H_{18}ClN_2O_4$: C, 55.91; H, 2.93; N, 12.23 Found: C, 56.10; H, 2.98; N, 12.03.

Example 36: 7-Chloro-4-hydroxy-2-[2,5-dimethyl(3-furanyl)methyl-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (4)

2,5-dimethyl-3-furanmethanol (1):

To a 0 °C solution of methyl 2,5-dimethyl-3-furoate (2.0 g, 13.0 mmol) in THF (130 mL) under N_2 was added lithium aluminum hydride (39 mL of a 1.0 M solution in THF, 38.9 mmol) dropwise. The reaction was stirred at 0 °C for 3.5 h, at which time it was quenched cautiously with saturated aqueous Na_2SO_4 solution and allowed to warm to room temperature. The reaction mixture was filtered and rinsed with ethyl acetate, the filtrate was diluted with more ethyl acetate and the layers separated. The organic layer was washed with saturated aqueous NH_4Cl , water and brine and dried over Na_2SO_4 . The solution was filtered and concentrated to afford 2,5-dimethyl-3-furanmethanol as a clear liquid (1.54 g, 12.2 mmol, 94 %). 1H NMR (300 MHz, $DMSO-d_6$): δ 5.93 (s, 1H); 4.18 (s, 2H); 2.17 (s, 3H); 2.15 (s, 3H).

***N'*-(2,5-Dimethyl-furan-3-ylmethyl)-hydrazinecarboxylic acid *tert*-butyl ester (2):**

To a 0 °C solution of 2,5-dimethyl-3-furanmethanol (750 mg, 5.95 mmol) in THF (60 mL) under N_2 was added phosphorus tribromide (169 mL, 1.78 mmol). After 0.5 hour, water was added and the solution was warmed to room temperature. The reaction was diluted with ethyl acetate and washed with saturated aqueous $NaHCO_3$, water and brine and dried over Na_2SO_4 . It was then filtered and concentrated to afford a yellow-brown oil which was used in the following reaction without further purification.

To a stirred solution of 2,5-dimethyl-3-bromomethylfuran (5.95 mmol theor.) in DMF (30 mL) was added K_2CO_3 (1.64 g, 11.9 mmol) and *tert*-butylcarbazate (3.1 g, 23.8 mmol). The reaction was heated to 65 °C for 1 h. The reaction was cooled to room temperature, diluted with ethyl acetate and the layers separated. The organic layer was washed with saturated aqueous NH_4Cl , water and brine and dried over Na_2SO_4 . The mixture was filtered, concentrated and distilled on a Kuglerohr apparatus at 100 °C and 35 mTorr to remove the excess DMF and *tert*-butylcarbazate. This material was then purified using silica gel column chromatography (hexanes - 4:1 hexanes:ethyl acetate as eluant) to afford *N'*-(2,5-dimethyl-furan-3-ylmethyl)-hydrazinecarboxylic acid *tert*-butyl ester as a dark yellow oil (670 mg; 2:79

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mmol, 47 % for the two steps). ¹H NMR (300 MHz, DMSO-d₆): δ 8.11 (br s, 1H); 5.91 (s, 1H); 4.38 (br s, 1H); 3.54 (s, 2H); 2.16 (s, 3H); 2.14 (s, 3H); 1.39 (s, 9H).

N'-[7-Chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-N'-(2,5-dimethylfuran-3-ylmethyl)-hydrazinecarboxylic acid tert-butyl ester (3):

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (900 mg, 2.79 mmol) in THF (10 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (2.36 g, 5.58 mmol). To this canary yellow mixture was added a solution of N'-(2,5-dimethyl-furan-3-ylmethyl)-hydrazinecarboxylic acid *tert*-butyl ester (670 mg, 2.79 mmol) and *N,N*-dimethyl-aminopyridine (51 mg, 420 μmol) in THF (20 mL). The resultant mixture was refluxed under N₂ for 3.5 h, then cooled and filtered. The filtrate was concentrated and filtered through a small column of silica gel (CH₂Cl₂ - 5% MeOH/CH₂Cl₂) to afford N'-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-N'-(2,5-dimethylfuran-3-ylmethyl)-hydrazinecarboxylic acid *tert*-butyl ester as a yellow solid; this material was used in the following reaction without further purification. MS *m/z* 543.2 (M+1)

7-Chloro-4-hydroxy-2-[2,5-dimethyl(3-furanyl)methyl]-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione (4):

To a stirred solution of N'-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-N'-(2,5-dimethylfuran-3-ylmethyl)-hydrazinecarboxylic acid *tert*-butyl ester (2.79 mmol theor.) in THF (60 mL) was added methanesulfonic acid (7.25 mL, 112 mmol) dropwise. This solution was stirred at room temperature overnight, at which time water (ca. 150 mL) was added to induce precipitation of the product. The material was filtered to give a tan powder; this powder was dried at 30 °C under 500 mTorr overnight to afford the title compound as a tan powder (680 mg, 1.82 mmol, 66 % for two steps). ¹H NMR (300 MHz, DMSO-d₆): δ 12.51 (br s, 1H); 11.88 (br s, 1H); 8.14 (d, *J* = 8.7 Hz, 1H); 8.01 (d, *J* = 2.1 Hz, 1H); 7.42 (dd, *J* = 1.8, 8.7 Hz, 1H); 5.94 (s, 1H); 4.79 (s, 2H); 2.27 (s, 3H); 2.14 (s, 3H). MS *m/z* 370.0 (M-1)

Example 37: 7-Chloro-4-hydroxy-2-(tetrahydro-furan-2-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione:

4-(2-Hydroxy-ethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (2)

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1-(2-Hydroxy-ethyl)-piperazine (1, 2.00 g, 1.88 mL, 15.3 mmol) was dissolved in methylene chloride (80 mL) and to this was added di-*tert*-butyl dicarbonate (3.35 g, 15.3 mmol). The reaction was stirred at room temperature for 72 hours, diluted with methylene chloride (100 mL) and extracted with water (1 x 50 mL). The organic layer was then washed
5 with aqueous sodium chloride (saturated, 1 x 50 mL) and dried over Na₂SO₄. The organic layer was collected and removed under reduced pressure to give the title compound as an oil (3.5 g, 99%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.38 (s, 9H); 2.39 (m, 6H); 3.28 (t, 4H, J = 5.1 Hz); 3.46 (dd, 2H J = 6.0 Hz); 4.39 (s, 1H, J = 6.0 Hz).

4-(2-Hydroxy-ethyl)-piperazine, polystyrene bound (3)

4-(2-Hydroxy-ethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (2, 3.3 g, 14.3 mmol) was dissolved in tetrahydrofuran (80 mL) and to this was added solid potassium *tert*-butoxide (1.57 g, 13.9 mmol). The mixture was stirred for 30 minutes at 0 °C, and then at room temperature for 2 hours. The reaction mixture was then transferred to an Erlenmeyer flask which was charged with chloromethylated styrene/divinylbenzene copolymer (1% cross
10 linked, 1 meq/gram, 2.8 g). The reaction flask was shaken for 48 hours, and the resin collected by filtration. The resin was washed with tetrahydrofuran (2 x 50 mL), water (2 x 50 mL), water/*N,N*-dimethylformamide (1/1, 2 x 50 mL), methylene chloride (2 x 50 mL) and ethyl acetate (2 x 50 mL). The resin was dried under reduced pressure to give polystyrene resin bound 4-(2-hydroxy-ethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (3.1 g). IR (on
15 resin) 1696 (s); 1492 (w); 1452 (w); 1242 (s); 1172 (s) cm⁻¹.

Polystyrene bound 4-(2-hydroxy-ethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (3.1 g) was slurried in methylene chloride (50 mL) and trifluoroacetic acid (10 mL) at room temperature for 2 hours. The resin was collected by filtration and washed with ammoniated methanol (2N, 2 x 30 mL), *N,N*-dimethylformamide (1 x 40 mL), and methylene chloride (3 x
20 30 mL). The resin was dried under reduced pressure to give the title compound (2.45 g). IR (on resin) 1601 (w); 1493 (w); 1452 (w).

7-Chloro-2-chlorocarbonyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid methyl ester (3)

7-Chloro-4-oxo-1,4-dihydro-quinoline-2,3-dicarboxylic acid 3-methyl ester (1.00 g, 3.55 mmol) was slurried in chloroform (25 mL, ethanol free) and to this was added thionyl chloride (~2 mL). The mixture was heated to reflux for 6 hours, and then cooled to room
30 temperature. The volatiles were removed under reduced pressure to give the title compound

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as a solid. This material was used in the following reaction without further purification. ¹H NMR (300 MHz, CDCl₃): δ 4.06 (s, 3H); 7.58 (dd, 1H, J = 8.1 Hz); 8.06 (dd, 1H, J = 1.8 Hz); 8.29 (d, 1H, J = 8.1 Hz); 12.59 (s, 1H).

7-Chloro-2-[4-(2-hydroxy-ethyl)-piperazine-1-carbonyl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid, polystyrene bound (5)

Polystyrene bound 1-(2-hydroxy-ethyl)-piperazine (3, 2.45 g) was slurried in tetrahydrofuran (50 mL) and to this was added 7-chloro-2-chlorocarbonyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid methyl ester (1.0 g, 3.55 mmol). The mixture was shaken for 16 hours, and the resin was collected by filtration. The resin was washed with tetrahydrofuran (50 mL), *N,N*-dimethylformamide/water (1/1, 50 mL), *N,N*-dimethylformamide (50 mL), methyl alcohol (50 mL), and methanolic ammonia (0.5 N, 2 x 50 mL). The resin was dried under reduced pressure to give polystyrene resin bound 7-chloro-2-[4-(2-hydroxy-ethyl)-piperazine-1-carbonyl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid methyl ester (2.10 g). IR (on resin) 1656 (w); 1601 (w); 1492 (w); 1467 (w); 1452 (w).

Polystyrene bound 7-chloro-2-[4-(2-hydroxy-ethyl)-piperazine-1-carbonyl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid methyl ester (2.10 g) was added to a mixture of tetrahydrofuran (10 mL) and water (4 mL). To this was added potassium hydroxide (0.35 g, 6.3 mmol) and the reaction stirred at reflux for 6 hours. The reaction was quenched by the addition of aqueous hydrochloric acid (3.10 mL, 2N) and the resin collected by filtration. The resin was washed with water (30 mL), *N,N*-dimethylformamide/water (1/1, 50 mL), *N,N*-dimethylformamide (50 mL), methyl alcohol (50 mL), and methylene chloride (50 mL). The resin was then dried under reduced pressure to give the title compound (0.75 g). IR (on resin) 1666 (w); 1602 (w); 1582 (w); 1492 (w); 1452 (w).

N'-(Tetrahydro-furan-2-ylmethyl)-hydrazinecarboxylic acid *tert*-butyl ester (6)

A solution of *N'*-furan-2-ylmethylene-hydrazinecarboxylic acid *tert*-butyl ester (2.05 g, 9.80 mmol) was dissolved in 50 methyl alcohol (50 mL) and charged into a Parr shaker bottle. To this was added palladium on carbon (10%, 0.310 g) and the mixture shaken at 40 psi for 4 hours. The mixture was filtered on CELITE and solvent removed under reduced pressure to give the title compound as an oil. The material was used in the following reaction without further purification.

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N-(7-Chloro-2-[4-(2-hydroxy-ethyl)-piperazine-1-carbonyl]-4-oxo-1,4-dihydro-quinoline-3-carbonyl)-*N*-(tetrahydro-furan-2-ylmethyl)-hydrazinecarboxylic acid *tert*-butyl ester, polystyrene bound (7)

Polystyrene resin bound 7-chloro-2-[4-(2-hydroxy-ethyl)-piperazine-1-carbonyl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (5, 0.50 g) was added to tetrahydrofuran (30 mL) and to this was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (1.0 g, 2.3 mmol). The reaction was allowed to shake for 15 minutes at which point the resin turned bright yellow. To this was added *N*-(tetrahydro-furan-2-ylmethyl)-hydrazinecarboxylic acid *tert*-butyl ester (6, 0.44 g, 2.05 mmol) and *N,N*-dimethylaminopyridine (0.10 g, 0.08 mmol) and the mixture was shaken for 16 hours. The resin was collected by filtration and washed with water (30 mL), *N,N*-dimethylformamide/water (1/1, 50 mL), *N,N*-dimethylformamide (30 mL), methyl alcohol (2 x 30 mL) and methylene chloride (2 x 30 mL). The resin was dried under reduced pressure to give the title compound (0.40 g). IR (on resin) 1666 (w); 1602 (w); 1582 (w); 1492 (w); 1451 (w).

7-Chloro-4-hydroxy-2-(tetrahydro-furan-2-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione (8)

Polystyrene resin bound *N*-(7-chloro-2-[4-(2-hydroxy-ethyl)-piperazine-1-carbonyl]-4-oxo-1,4-dihydro-quinoline-3-carbonyl)-*N*-(tetrahydro-furan-2-ylmethyl)-hydrazinecarboxylic acid *tert*-butyl ester (7, 0.40 g) was added to tetrahydrofuran (15 mL) and to this was added methanesulfonic acid (1 mL). The mixture was shaken at room temperature for 24 hours. The resin was filtered and washed with tetrahydrofuran. The solvent was evaporated and the residual oil diluted with of tetrahydrofuran (3 mL). This mixture was poured into mixture of ice/water (80 mL) and stirred for 10 minutes. The resultant solid that formed was filtered and washed with diethylether (5 mL). The material was dried under reduced pressure to give the title compound as an off-white solid (0.01 g). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.65 (m, 1H); 1.85 (m, 3H); 3.61 (m, 1H); 3.75 (m, 1H); 3.87 (m, 1H); 3.95 (m, 1H); 4.20 (m, 1H); 7.42 (d, 1H, *J* = 8.1 Hz); 8.02 (s, 1H); 8.14 (d, 1H, *J* = 8.1 Hz); 12.35 (br, s). MS (+CI) *m/z* 348/349.

Example 38: 7-Chloro-4-hydroxy-2-(5-isoxazolino)methyl-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione (3)

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N'-isoxazol-5-ylmethyl-hydrazinecarboxylic acid *tert*-butyl ester (1)

A mixture of 5-bromomethyl-isoxazole (1.62 g, 10 mmol), *tert*-butylcarbazate (5.29 g, 40 mmol) and sodium carbonate (2.76 g, 20 mmol) in DMF (25 mL) was heated to 80°C under a nitrogen atmosphere for 6 hours. The mixture was cooled and partitioned between ethyl acetate (100 mL) and water (200 mL). The organic layer was washed with brine (3 X 50 mL) and dried over MgSO₄. The solvent was removed by rotary evaporation. The residual DMF and excess *tert*-butylcarbazate was removed by vacuum distillation (50 mTorr, 80°C). The residue was subjected to chromatography (silica gel, 1/1 ethyl acetate/hexane) to give the title compound (1) as a white solid (1.01 g, 49%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.38 (s, 9H); 4.01 (s, 2H); 5.13 (bs, 1H); 6.38 (s, 1H); 8.38 (s, 1H); 8.48 (s, 1H).

N'-[7-chloro-4-hydroxy-2-(pyrrolidine-1-carbonyl)-quinoline-3-carboxyl]-*N'*-isoxazol-5-ylmethyl-hydrazinecarboxylic acid *tert*-butyl ester (2)

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (1.51 g, 4.7 mmol) in tetrahydrofuran (50 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (4.24 g, 10 mmol) and the reaction was stirred for five minutes. To this mixture was added a solution of *N'*-isoxazol-5-ylmethyl-hydrazinecarboxylic acid *tert*-butyl ester (1.0 g, 4.7 mmol) and 4-dimethylaminopyridine (0.06 g, 0.5 mmol) in tetrahydrofuran (10 mL). The mixture was heated to reflux for 1.5 hours then allow to stand at room temperature for 16 hours. The solids were filtered off and washed with methylene chloride (2 X 50 mL). The combined filtrate was evaporated to dryness by rotary evaporation. The residual solid was subjected to chromatography (silica gel, 1/9 methanol/methylene chloride) to give the title compound (2) as an off-white foam (2.09 g, 86%). MS (CI) *m/z* 514/516.

7-Chloro-4-hydroxy-2-(5-isoxazolino)methyl-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione (3)

To a solution of *N'*-[7-chloro-4-hydroxy-2-(pyrrolidine-1-carbonyl)-quinoline-3-carboxyl]-*N'*-isoxazol-5-ylmethyl-hydrazinecarboxylic acid *tert*-butyl ester (1.0 g, 1.94 mmol) in THF (50 mL) was added methanesulfonic acid (5.2 mL). After 18 hours the solvent was removed by rotary evaporation and the product was precipitated by the addition of water (100 mL). The solid was collected by vacuum filtration and washed with water (2 X 50 mL) then dried *in vacuo* (500 mTorr; 30°C) for 16 hours. The solid was suspended in ether (45 mL) and methanol (5 mL) and sonicated for 10 minutes. The solid was collected by vacuum

filtration, washed with ether (2 X 30 mL), and dried *in vacuo* (500 mTorr, 30°C) for 18 hours. This gave the title compound as a yellow solid (0.54 g, 81%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 5.27 (s, 2H); 6.44 (s, 1H); 7.45 (dd, 1H, *J*_o=8.7 Hz, *J*_m=1.8 Hz); 8.03 (d, 1H, *J*_m=1.8 Hz); 8.15 (d, 1H, *J*_o=8.7 Hz); 8.53 (s, 1H, *J*_m=1.8 Hz); 11.99 (s, 1H); 12.82 (s, 1H).

5 Calc'd. for C₁₇H₉ClN₄O₄·0.40H₂O: C, 51.20; H, 2.81; N, 15.92; Found: C, 51.48-51.33; H, 2.79-2.77; N, 15.60-15.57.

Example 39: 7-Chloro-4-hydroxy-2-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-ylmethyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (6)

10 2-Chloromethyl-5-(4-methoxy-phenyl)-[1,3,4]oxadiazole (2)

4-Methoxybenzoic hydrazide (1, 0.70 g, 4.21 mmol) was dissolved in ethyl alcohol (10 mL) and to this was added 2-chloromethyl acetimidate hydrochloride (1.0 g, 6.36 mmol). The solution was stirred and heated to reflux for 2 hours. The mixture was dissolved in ethyl acetate (100 mL) and extracted with aqueous sodium bicarbonate (saturated, 1 x 20 mL),
15 water (1 x 20 mL) and aqueous sodium chloride (saturated, 1 x 20 mL). The organic layer was dried over Na₂SO₄ and solvent removed under reduced pressure to give the title compound as a white solid. (0.83 g, 94%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.86 (s, 3H); 5.12 (s, 2H); 7.16 (d, 2H, *J* = 8.4 Hz); 7.92 (d, 2H, *J* = 8.4 Hz).

20 *N*-[5-(4-Methoxy-phenyl)-[1,3,4]oxadiazol-2-ylmethyl]-hydrazinecarboxylic acid *tert*-butyl ester (3)

2-Chloromethyl-5-(4-methoxy-phenyl)-[1,3,4]oxadiazole (2, 0.83 g, 3.70 mmol) was dissolved in *N,N*-dimethylformamide (5 mL) and to this was added *tert*-butyl carbazate (1.22 g, 9.26 mmol) and *N,N*-diisopropylethylamine (0.71 g, 0.96 mL, 5.55 mmol). The solution was stirred and heated to 90 °C for 5 hours. The mixture was dissolved in ethyl acetate (150
25 mL) and extracted with water (3 x 15 mL), and brine (1 x 20 mL). The material was dried over Na₂SO₄ and the organic solvent removed under reduced pressure. The residual oil was triturated with hexanes/ethyl acetate (7:3, 10 mL) giving rise to a white precipitate. The precipitate was filtered and washed with small portions of hexanes/ethyl acetate (3 x 5 mL) giving the title compound as a white solid (0.75 g, 64%). ¹H NMR (300 MHz, DMSO-*d*₆): δ
30 1.34 (s, 9H); 3.85 (s, 3H); 4.08 (d, 2H, *J* = 4.2 Hz); 5.27 (m, 1H); 7.15 (d, 2H, *J* = 8.4 Hz); 7.93 (d, 2H, *J* = 8.4 Hz); 8.39 (br s, 1H);

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N-[7-Chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N*-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-ylmethyl]-hydrazinecarboxylic acid *tert*-butyl ester (5)

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (4, 0.54 g, 1.66 mmol) in tetrahydrofuran (10 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (0.87 g, 2.07 mmol) and the reaction was stirred for five minutes. To this mixture was added, via dropwise addition, a solution of *N*-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-ylmethyl]-hydrazinecarboxylic acid *tert*-butyl ester (3, 0.56 g, 1.75 mmol) and 4-dimethylaminopyridine (0.01 g, 0.08 mmol) in tetrahydrofuran (2 mL), and the mixture was then refluxed for 6 hours. The solution was filtered and the insolubles washed with tetrahydrofuran. The tetrahydrofuran was removed under reduced pressure to give a sticky yellow oil. The material was used without further purification in the following reaction (~ 1 gram material isolated).

7-Chloro-4-hydroxy-2-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-ylmethyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (6)

To a stirred solution of *N*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-*N*-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-ylmethyl]-hydrazinecarboxylic acid *tert*-butyl ester (5, 0.30 g, 0.48 mmol) in tetrahydrofuran (15 mL) was added methanesulfonic acid (1.0 mL) and the reaction was stirred at room temperature for 2 hours. The tetrahydrofuran was removed under reduced pressure to yield an orange oil. To this was added diethyl ether (50 mL) and the reaction stirred for 10 minutes. The diethyl ether was carefully decanted to leave a thick orange oil. To this was added water (20 mL) and a fluffy yellow precipitate formed. The mixture was stirred for 10 minutes and then filtered. The solids were washed with water (10 mL) followed by diethyl ether (20 mL). The solids were collected and sonicated in methyl alcohol/diethyl ether (1:9, 5 mL) for 10 minutes. The solids were collected and dried under reduced pressure to give the title compound as a cream-colored solid (0.53 g, 25%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.83 (s, 3H); 5.42 (s, 2H); 7.13 (d, 2H, *J* = 8.7 Hz); 7.45 (d, 1H, *J* = 8.7 Hz); 7.89 (d, 2H, *J* = 8.7 Hz); 8.04 (s, 1H); 8.15 (d, 1H, *J* = 8.7 Hz); 12.03 (br s, 1H); 12.89 (br s, 1H). MS (+CI) *m/z* 452/454.

Example 40: 2-(Benzo[b]thiophen-2-ylmethyl)-7-chloro-4-hydroxy-2,5-dihydropyridazino[4,5-b]quinoline-1,10-dione:

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105Benzo[b]thiophene-2-carbaldehyde (2).

To a solution of benzo[b]thiophene (10 g, 74.5 mmole) in dry tetrahydrofuran (12 mL) at -78 °C was added 65 mL of 1.6M n-butyl lithium in hexanes. Ten minutes later N,N-dimethylformamide (23 mL, 298 mmole) was added. The reaction was warmed to room temperature and then refluxed for 3 hours. The tetrahydrofuran was evaporated and the residue was poured into 1N HCl and ice. The acidic solution was extracted with ether (2x). The combined ether solution was washed with 1 N HCl (3x), saturated NaHCO₃ (1x), brine (1x), and then dried with MgSO₄. The MgSO₄ was filtered off and the filtrate concentrated to an oil which was treated with NaHSO₃. The solid that formed was collected, treated with aqueous NaHCO₃, then extracted with methylene chloride. The methylene chloride solution was dried over MgSO₄ and evaporated to give the title compound (2) as a yellow oil (3.2 g, 26% yield). ¹H NMR (300 MHz, CDCl₃): δ 7.44 (dd, 1H, J = 6.9, 7.2 Hz); 7.51 (dd, 1H, J = 6.9, 8.1 Hz); 7.91 (d, 1H, J = 8.1 Hz); 7.95 (d, 1H, J = 7.8 Hz); 8.04 (s, 1H); 10.12 (s, 1H).

(tert-Butoxy)-N-((1E)-1-aza-2-benzo[b]thiophen-2-ylvinyl)carboxamide (3).

To a stirred solution of benzo[b]thiophene-2-carbaldehyde (2, 3.2 g, 19.7 mmole) and t-butylcarbazate (2.6 g, 19.7 mmole) in ethanol (20 mL) was added 3 drops of concentrated HCl. After 30 minutes the mixture was filtered, the solid washed with ether, and dried under vacuum to give the title compound (3, 3.8 g, 70% yield). ¹H NMR (300 MHz, CDCl₃): δ 1.54 (s, 9H); 7.30 - 7.36 (m, 2H); 7.40 (s, 1H); 7.71 - 7.74 (m, 1H); 7.78-7.81 (m, 1H); 7.89 (s, 1H); 8.31 (br s, 1H).

(tert-Butoxy)-N-[(benzo[b]thiophen-2-ylmethyl)amino]carboxamide (4).

To a slurry of (tert-butoxy)-N-((1E)-1-aza-2-benzo[b]thiophen-2-ylvinyl)carboxamide (3, 1.8 g, 6.5 mmole) in tetrahydrofuran (6 mL) was added sodium cyanoborohydride (0.75 g, 11.9 mmole). A solution of p-toluene sulfonic acid (1.86g, 9.8 mmole) in tetrahydrofuran (6 mL) was added dropwise. After stirring overnight, the reaction was diluted with ethyl acetate and washed with saturated NaHCO₃ (1x) and saturated NaCl (1x). The ethyl acetate solution was dried over K₂CO₃. The K₂CO₃ was filtered off and the filtrate was concentrated under reduced pressure. The resulting solid was treated with saturated NaHCO₃ overnight and extracted with methylene chloride. The methylene chloride solution was dried over Na₂SO₄. The Na₂SO₄ was filtered off and the filtrate concentrated to give the title compound (4) as a white solid (1.7 g, 76% yield). ¹H NMR (300 MHz, CDCl₃): δ 1.44 (s, 9H); 4.68 (s, 2H); 7.36 - 7.45 (m, 2H); 7.45 (s, 1H); 7.78 - 7.86 (m, 2H).

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N'-Benzo[b]thiophen-2-ylmethyl-N'-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydroquinoline-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (6).

To a stirred mixture of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (5, 1.59 g, 5.0 mmole) and dry tetrahydrofuran (60 mL) under nitrogen was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (3.19 g, 7.0 mmole). This was followed by a solution of (tert-butoxy)-N-[(benzo[b]thiophen-2-ylmethyl)amino]carboxamide (4, 1.37 g, 5.0 mmole) and dimethylaminopyridine (27.8 mg, 0.21 mmole) in tetrahydrofuran (15 mL). The reaction was heated at reflux overnight and the mixture was filtered. The concentrated filtrate was purified by chromatography (MeOH/CH₂Cl₂, 5/95, v/v) to give the title compound (6) as a yellow solid (771 mg, 24% yield).

2-(Benzo[b]thiophen-2-ylmethyl)-7-chloro-4-hydroxy-2,5-dihydropyridazino[4,5-b]quinoline-1,10-dione (7).

To a stirred mixture of N'-benzo[b]thiophen-2-ylmethyl-N'-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydroquinoline-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (6, 760 mg, 1.3 mmole) and dry tetrahydrofuran (16 mL) at 0 °C under nitrogen was added methanesulfonic acid (3.0 mL, 4.44 g, 46.2 mmole). After stirring overnight, the tetrahydrofuran was evaporated and the residue cooled in an ice bath. Water was added and the resulting precipitate was collected, sonicated with methanol, and dried under vacuum to give the title compound (7) as an off-white solid (470 mg, 88% yield), m.p. > 300 °C. ¹H NMR (300 MHz, DMSO-d₆): δ 5.38 (s, 2H); 7.30-7.38 (m, 2H); 7.40 (s, 1H); 7.44 (d, 1H, J = 8.7 Hz); 7.90 (d, 1H, J = 7.2 Hz); 7.82 (d, 1H, 7.2 Hz); 8.06 (s, 1H); 8.15 (d, 1H, J = 8.7 Hz).

Calc'd. for C₂₈H₁₇ClN₄O₂S: C, 58.61; H, 2.95; N, 10.25. Found: C, 58.42; H, 3.19; N, 10.20.

Example 41: 7-Chloro-10-hydroxy-2-[(3-methyl(2-thienyl)methyl)-2,3-dihydropyridazino[4,5-b]quinoline-1,4-dione.

(tert-Butoxy)-N-[(1E)-1-aza-2-(3-methyl(2-thienyl)vinyl]carboxamide (2).

To a stirred solution of 3-methylthiophene-2-carbaldehyde (1, 5.03 g, 39.86 mmole) and absolute ethanol (75 mL) under nitrogen was added 3 drops of concentrated HCl, followed by the addition of t-butylcarbazate (4.75 g, 35.94 mmole). After 30 minutes the

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reaction mixture was filtered and washed with ethanol (10 mL). The product was dried at 30 °C under vacuum to give the title compound (2) as an off-white powder (6.92 g, 80% yield). ¹H NMR (300 MHz, DMSO-d₆): δ 1.45 (s, 9H); 2.24 (s, 3H); 6.91 (d, 1H, J = 5.1 Hz); 7.46 (d, 1H, J = 5.1 Hz); 8.25 (s, 1H); 10.70 (s, 1H).

5 (tert-Butoxy)-N-([(3-methyl(2-thienyl))methyl]amino)carboxamide (3).

To a stirred solution of (tert-butoxy)-N-[(1E)-1-aza-2-(3-methyl(2-thienyl)vinyl)]carboxamide (2, 2.60 g, 10.82 mmole) and dry tetrahydrofuran (40 mL) under nitrogen was added p-toluene sulfonic acid monohydrate (2.09 g, 10.99 mmole) followed by sodium cyanoborohydride (1.78 g, 28.33 mmole). The reaction mixture was stirred at room temperature for 4 hours and then diluted with 2N NaOH (40 mL). The resulting solution was extracted with ethyl acetate (3 x 40 mL) and the combined organic extracts were washed with brine (50 mL), then dried over Na₂SO₄. The Na₂SO₄ was filtered off and the filtrate was concentrated under reduced pressure to give an off-white solid (2.79 g). This product was purified by flash chromatography on silica gel eluting with hexane:ethyl acetate (3:1) to give the title compound (3) as a colorless oil (2.44 g, 92% yield). ¹H NMR (300 MHz, DMSO-d₆): δ 1.39 (s, 9H); 2.14 (s, 3H); 3.95 (d, 2H, J = 4.5 Hz); 4.69 (d, 1H, J = 3.9 Hz); 6.82 (d, 1H, J = 5.1 Hz); 7.29 (d, 1H, J = 5.1 Hz); 8.26 (s, 1H).

15 (tert-Butoxy)-N-([7-chloro-4-hydroxy-2-(pyrrolidinylcarbonyl)(3-quinolyl)]-N-[(3-methyl(2-thienyl))methyl]carbonylamino)carboxamide (5).

To a stirred mixture of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (4, 3.56 g, 11.10 mmole) and dry tetrahydrofuran (80 mL) under nitrogen was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (7.11 g, 16.78 mmole). After stirring the reaction mixture for an additional 20 minutes, a solution of (tert-butoxy)-N-([(3-methyl(2-thienyl))methyl]amino)carboxamide (3, 2.44 g, 10.07 mmole) and tetrahydrofuran (15 mL) was rapidly added. The mixture was heated to 70 °C and stirred overnight. The reaction was cooled to room temperature, filtered, and the filter cake was washed with tetrahydrofuran (30 mL). The filtrate and washings were combined and concentrated under reduced pressure to give a yellow solid (6.72 g). The product was purified by flash chromatography on silica gel eluting with methylene chloride:methanol (95:5) to give the desired compound (tert-butoxy)-N-([7-chloro-4-hydroxy-2-(pyrrolidinylcarbonyl)(3-quinolyl)]-N-[(3-methyl(2-thienyl))methyl]carbonylamino)carboxamide (5, 3.22 g, 59% yield) as a yellow powder.

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7-Chloro-10-hydroxy-2-[(3-methyl(2-thienyl)methyl)-2,3-dihydropyridazino[4,5-b]quinoline-1,4-dione (6).

To a stirred mixture of (*tert*-butoxy)-N-{[7-chloro-4-hydroxy-2-(pyrrolidinylcarbonyl)(3-quinolyl)]-N-[(3-methyl(2-thienyl)methyl)carbonylamino]carboxamide (5, 3.68 g, 6.77 mmole) and dry tetrahydrofuran (150 mL) under nitrogen was added methanesulfonic acid (10 mL, 14.8 g, 77.8 mmole) all at once. The mixture was stirred overnight and then concentrated under pressure. The residue was diluted with water (80 mL) and then filtered to separate the solids. The collected solids were successively washed with water (30 mL), methanol (20 mL), and ether (150 mL) and then dried at 50 °C under vacuum to give 7-chloro-10-hydroxy-2-[(3-methyl(2-thienyl)methyl)-2,3-dihydropyridazino[4,5-b]quinoline-1,4-dione (6, 1.98 g, 78% yield) as an off-white powder, m.p. > 250 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.32 (s, 3H), 5.17 (s, 2H); 6.84 (d, 1H, *J* = 5.1 Hz); 7.32 (d, 1H, *J* = 5.1 Hz); 7.43 (d, 1H, *J* = 8.7 Hz); 8.02 (s, 1H); 8.14 (d, 1H, *J* = 8.7 Hz); 11.92 (br s, 1H); 12.62 (br s, 1H).

Calc'd. for C₁₇H₁₂ClN₄O₃S•0.05CH₃SO₃H•0.05H₂O: C, 54.62; H, 3.24; N, 11.24. Found: C, 53.96; H, 3.27; N, 11.07.

Example 42: 7-Chloro-4-hydroxy-2-[4-(carbomethoxy)phenyl-2-ethyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione;

4-[1-(*tert*-Butoxycarbonyl-hydrazino)-ethyl]-benzoic acid methyl ester (1)

To a solution of methyl 4-acetylbenzoate (5.20 g, 29.2 mmol) and *tert*-butylcarbazate (3.85 g, 29.2 mmol) in THF (70 mL) was added concentrated HCl (3 drops). Upon stirring 2 hours a solid formed. At that time the mixture was evaporated to dryness by rotary evaporation. The resulting solid was triturated with hexanes (3 X 50 mL) and dried *in vacuo* (500 mTorr, 30°C) for 30 minutes to give the title compound 1 as an off-white solid (8.36 g, 98%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.49 (s, 9H); 2.22 (s, 3H); 3.34 (s, 1H); 3.86 (s, 3H); 7.87 (d, 2H, *J*_o = 8.4 Hz); 7.97 (d, 2H, *J*_o = 8.4 Hz); 9.97 (s, 1H).

4-[1-(*tert*-Butoxycarbonyl-hydrazino)-ethyl]-benzoic acid methyl ester (2)

To a suspension of 4-[1-(*tert*-butoxycarbonyl-hydrazino)-ethyl]benzoic acid methyl ester (8.35 g, 28.6 mmol) in ethanol (200 mL) was added 10 % palladium on carbon (ca. 0.5 g) and the mixture was shaken under hydrogen at 50 psig on a Parr shaker for 3.5 hours. All the organic material dissolved during this time. The catalyst was removed by vacuum

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filtration through celite. The filtrate was evaporated by rotary evaporation. The residual solid was subjected to chromatography (silica gel, gradient of ethyl acetate/methylene chloride beginning from 10/90 and ending at 20/80, respectively) to give the title compound 2 as a white solid (6.41 g, 76%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.18 (d, 3H, *J*=6.6 Hz); 1.35 (s, 9H); 3.84 (s, 3H); 4.16 (m, 1H); 4.71 (m, 1H); 7.48 (d, 2H, *J*_o=8.3 Hz); 7.89 (d, 2H, *J*_o=8.3 Hz); 8.15 (s, 1H).

4-(1-(*N'*-*tert*-Butoxycarbonyl-*N*-[7-chloro-4-hydroxy-2-(pyrrolidine-1-carbonyl)-quinoline-3-carboxyl]-hydrazino)-ethyl)-benzoic acid methyl ester (3).

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (2.15 g, 6.7 mmol) in THF (50 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (5.72 g, 13.5 mmol) and the reaction was stirred for five minutes. To this mixture was added a solution of 4-[1-(*tert*-butoxycarbonyl-hydrazino)-ethyl]-benzoic acid methyl ester (1.97 g, 6.7 mmol) and 4-dimethylaminopyridine (0.10 g, 0.84 mmol) in THF (10 mL), and the mixture was heated to reflux for 3 hours. The reaction mixture was allowed to cool, then the solids were filtered off and washed with methylene chloride (2 X 50 mL). The combined filtrates were evaporated to dryness by rotary evaporation. The residual solid was subjected to chromatography (silica gel, 5/95 methanol/methylene chloride) to give the title compound (3) as an off-white solid (3.53 g, 71%). MS (CI) *m/z* 597/599.

7-Chloro-4-hydroxy-2-[4-(carbomethoxy)phenyl-2-ethyl]-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione (4)

To a solution of 4-(1-(*N'*-*tert*-butoxycarbonyl-*N*-[7-chloro-4-hydroxy-2-(pyrrolidine-1-carbonyl)-quinoline-3-carboxyl]-hydrazino)-ethyl)-benzoic acid methyl ester (3.45 g, 5.78 mmol) in THF (50 mL) was added methanesulfonic acid (5 mL). After 18 hours the solvent was removed by rotary evaporation and the product was precipitated by addition of water (100 mL). The solid was collected by vacuum filtration and washed with water (2 X 50 mL) then ether (50 mL), and dried *in vacuo* (500 mTorr, 30 °C) for 1.5 hours. The solid was suspended in ether (175 mL) and methanol (25 mL) and sonicated for 20 minutes. The solid was collected by vacuum filtration and dried *in vacuo* (500 mTorr, 30°C) for 18 hours. The resulting yellow solid (2.05 g, 83%) was found to be of insufficient purity by HNMR. A portion of this material (1.73 g) was suspended in acetonitrile (10 mL) and water (10 mL) to which was added 20% choline hydroxide in water dropwise until all the solids dissolved, then

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purified by preparative HPLC (41.4 mm X 25 cm C-18 Dynamax 60A column using a gradient 10-35% of acetonitrile in water with 0.1% TFA over 50 minutes with a flow rate of 20 ml/min, monitoring 210 nm). Product was precipitated from the fractions containing pure material by addition of 6M HCl to pH 3. The solid was collected by vacuum filtration, washed with water (2 X 50 ml) then ether (2 X 50 ml) and dried *in vacuo* (500 mTorr, 30 °C) for 18 hours to give the title compound as a yellow solid (1.23 g, 59%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.69 (d, 3H, J=6.9 Hz); 3.84 (s, 3H); 6.26 (q, 1H, J=7.2 Hz); 7.38-7.50 (m, 3H); 7.92 (d, 2H, J=8.4 Hz); 8.03 (d, 1H, J=1.8 Hz); 8.15 (d, 1H, J=8.7 Hz); 11.94 (s, 1H); 12.57 (s, 1H). MS (CI) *m/z* 426/428.

Calc'd. for C₂₁H₁₈ClN₃O₅·0.20H₂O: C 58.74 H 3.85 N 9.79. Found C 58.71-58.85 H 3.89-3.90 N 9.93-9.95.

Example 43: (+/-)-7-Chloro-4-hydroxy-2-(3-pyridyleth-1-yl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate:

N-((1Z)-1-Aza-2-(3-pyridyl)prop-1-enyl)(tert-butoxy)carboxamide (2).

To a stirred solution of *tert*-butylcarbazate (2.18 g, 16.5 mmol) in tetrahydrofuran (40 mL) was added 3-acetylpyridine (2.00 g, 16.5 mmol), followed by 3 drops of hydrochloric acid (conc.). After 1 h, the reaction turned cloudy, and the solvent was removed *in vacuo*. The resultant solid was triturated with hexanes and filtered to give the title compound as a white solid (3.58 g, 93%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.48 (s, 9H); 7.43 (dd, 1H, J = 4.2, 8.0 Hz); 8.10 (dt, 1H, J = 1.5, 8.0 Hz); 8.55 (d, 1H, J = 4.2 Hz); 8.90 (s, 1H); 10.00 (s, 1H).

(+/-)-(tert-Butoxy)-N-[(3-pyridylethyl)amino]carboxamide (3).

N-((1Z)-1-Aza-2-(3-pyridyl)prop-1-enyl)(tert-butoxy)carboxamide (1.0 g, 4.2 mmol) was dissolved in methyl alcohol (80 mL) and placed in a Parr shaker bottle. To this was added 10% palladium-on-carbon (250 mg) and the reaction was hydrogenated at 40 psi for 24 h. The mixture was filtered through celite, which was washed with methyl alcohol (3 x 100 mL). The combined filtrate and washes were concentrated *in vacuo*. The resultant oil (ca. 1.0 g) was used in the following reaction without further purification. ¹H NMR (300 MHz, DMSO-d₆): δ 1.19 (d, 3H, J = 9 Hz); 1.33 (s, 9H); 4.14 (m, 1H); 4.81 (m, 1H); 7.31 (m, 1H); 7.31 (m, 1H); 8.43 (dd, 1H, J = 1.5, 2.4 Hz); 8.49 (d, 1H, J = 1.5 Hz).

(+/-)-N-[(tert-Butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)-N-(3-pyridylethyl)carboxamide (4).

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To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (1.2 g, 3.82 mmol) in tetrahydrofuran (40 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (2.42 g, 5.73 mmol) and the reaction was stirred for five minutes. To this mixture was added, via dropwise addition, a solution of (+/-)-(tert-butoxy)-N-[(3-pyridylethyl)amino]carboxamide (1.0 g, 4.21 mmol) and 4-dimethylaminopyridine (0.080 g, 0.65 mmol) in tetrahydrofuran (20 mL). The mixture was stirred overnight at room temperature and then refluxed for three hours. The reaction mixture was filtered and the collected solids washed with methylene chloride (2 x 150 mL). The combined filtrate and washes were concentrated *in vacuo* to dryness. The resultant yellow foam was subjected to chromatography (silica gel, 95/5 chloroform/methyl alcohol) to give the title compound (4) as a yellow foam (0.860 g, 41%).

(+/-)-7-Chloro-4-hydroxy-2-(3-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5).

To a stirred solution of (+/-)-N-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(3-pyridylethyl)carboxamide (0.860 g, 1.50 mmol) in tetrahydrofuran (30 mL) was added methanesulfonic acid (4 mL) and the reaction was stirred overnight. The volatiles were removed *in vacuo* and the resultant oil was poured on to crushed ice. To this mixture was carefully added 10N sodium hydroxide until a solid precipitate formed. The solution was filtered to give an orange solid. This material was washed with diethyl ether, and then sonicated in 20 mL of 1/1 diethyl ether/methyl alcohol for fifteen minutes. The insoluble materials were collected and washed with the same solvent system to give the title compound (5) (0.402 g, 54%) as an off-white powder (m.p. >280 °C). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.75 (d, 3H, J = 6.9 Hz); 2.34 (s, 3H, CH₃SO₃H); 6.33 (q, 1H, J = 6.9 Hz); 7.45 (dd, 1H, J = 1.5, 8.7 Hz); 7.96 (m, 1H); 8.03 (s, 1H); 8.15 (d, 1H, J = 8.7 Hz); 8.42 (d, 1H, J = 7.8 Hz); 8.82 (d, 1H, J = 4.8 Hz); 8.91 (s, 1H); 12.04 (s, 1H); 12.72 (s, 1H).

Calc'd for C₁₉H₁₃ClN₄O₃•CH₃SO₃H•1.5H₂O: C, 46.39; H, 4.09; N, 11.38. Found: C, 46.35; H, 3.94; N, 11.21.

Example 44: (+/-)-7-Chloro-4-hydroxy-2-(2-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate.

N-((1Z)-1-Aza-2-(2-pyridyl)prop-1-enyl)(tert-butoxy)carboxamide (1).

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To a stirred solution of *tert*-butylcarbazate (2.18 g, 16.5 mmol) in tetrahydrofuran (40 mL) was added 2-acetylpyridine (2.00 g, 16.5 mmol), followed by 3 drops of hydrochloric acid (conc.). After 1 h, the reaction turned cloudy, and the solvent was removed *in vacuo*. The resultant solid was triturated with hexanes and filtered to give the title compound as a white solid (3.12 g, 80%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.49 (s, 9H); 7.38 (dd, 1H, *J* = 4.8, 6.7 Hz); 7.94 (m, 1H); 7.99 (d, 1H, *J* = 7.5 Hz); 8.58 (d, 1H, *J* = 4.2 Hz); 10.04 (s, 1H).

(+/-)-(tert-Butoxy)-N-[(2-pyridylethyl)amino]carboxamide (2).

N-((1*Z*)-1-Aza-2-(2-pyridyl)prop-1-enyl)(*tert*-butoxy)carboxamide (2.0 g, 8.5 mmol) was dissolved in methyl alcohol (80 mL) and placed in a Parr shaker bottle. To this was added 10% palladium-on-carbon (850 mg) and the reaction was hydrogenated at 40 psi for 24 h. The mixture was filtered through celite, which was washed with methyl alcohol (3 x 100 mL). The combined filtrate and washes were concentrated *in vacuo*. The resultant oil (2, ca. 1.8 g) was used in the following reaction without further purification. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.19 (d, 3H, *J* = 6.6 Hz); 1.33 (s, 9H); 4.11 (m, 1H); 4.79 (m, 1H); 7.22 (m, 1H); 7.49 (d, 1H, *J* = 7.8 Hz); 8.22 (m, 1H, *J* = 1.5, 2.4 Hz); 8.49 (d, 1H, *J* = 4.2 Hz).

(+/-)-N-[(tert-Butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(2-pyridylethyl)carboxamide (3).

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (2.43 g, 7.57 mmol) in tetrahydrofuran (100 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (3.69 g, 8.72 mmol) and the reaction was stirred for five minutes. To this mixture was added dropwise a solution of (+/-)-(tert-butoxy)-N-[(2-pyridylethyl)amino]carboxamide (1.8 g, 7.59 mmol) and 4-dimethylaminopyridine (0.160 g, 1.30 mmol) in tetrahydrofuran (20 mL). The mixture was stirred at room temperature for 45 minutes and then refluxed overnight. The cooled solution was filtered and the collected insolubles washed with methylene chloride (2 x 150 mL). The combined filtrate and washes were concentrated *in vacuo* to dryness. The resultant yellow foam was subjected to chromatography (silica gel, 95/5 chloroform/methyl alcohol) to give the title compound (3) as a yellow foam (3.3 g, 81%).

(+/-)-7-Chloro-4-hydroxy-2-(2-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (4).

To a stirred solution of (+/-)-N-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(2-pyridylethyl)carboxamide (3.0 g, 5.56 mmol) in

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tetrahydrofuran (100 mL) was added methanesulfonic acid (15 mL) and the reaction was stirred overnight. The volatiles were removed *in vacuo* and the resultant oil was poured on to crushed ice. A fine precipitate formed which was filtered to give an orange solid. This material was washed with diethyl ether, and then sonicated in 20 mL of 1/1 diethyl ether/methyl alcohol for fifteen minutes and filtered. The collected solids were sonicated again in 85 mL of the same solvent system for an additional fifteen minutes. The insoluble materials were collected, washed with the same solvent system and dried at 55 °C for 12 h to give the title compound (4, 1.29 g, 48%) as an off-white powder (m.p. >290 °C). ¹H NMR (300 MHz, DMSO-d₆): δ 1.77 (d, 3H, J = 6.9 Hz); 2.34 (s, 3H, CH₃SO₃H); 6.38 (q, 1H, J = 6.9 Hz); 7.44 (dd, 1H, J = 1.5, 8.7 Hz); 7.82 (m, 2H); 7.90 (d, 1H, J = 8.1 Hz); 8.04 (d, 1H, J = 8.7 Hz); 8.38 (app t, 1H, J = 7.5 Hz); 8.82 (d, 1H, J = 5.1 Hz); 11.98 (s, 1H); 12.80 (s, 1H).

Calc'd. for C₁₈H₁₅ClN₄O₅·CH₃SO₃H·H₂O: C, 47.25; H, 3.96; N, 11.60. Found: C, 47.26; H, 3.67; N, 11.50.

Example 45: (+/-)-7-Chloro-4-hydroxy-2-(4-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate.

N-((1Z)-1-aza-2-(4-pyridyl)prop-1-enyl)(tert-butoxy)carboxamide (1).

To a stirred solution of *tert*-butylcarbazate (2.18 g, 16.5 mmol) in tetrahydrofuran (40 mL) was added 4-acetylpyridine (2.00 g, 16.5 mmol), followed by 3 drops of hydrochloric acid (conc.). After 1 h, the reaction turned cloudy, and the solvent was removed *in vacuo*. The resultant solid was triturated with hexanes and filtered to give the title compound 1 as a white solid (3.88 g, 100%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.47 (s, 9H); 7.55 (dd, 2H, J = 1.5, 4.5 Hz); 7.97 (s, 1H); 7.99 (d, 2H, J = 4.5 Hz); 11.22 (s, 1H).

(+/-)-(tert-Butoxy)-N-[(4-pyridylethyl)amino]carboxamide (2).

N-((1Z)-1-Aza-2-(4-pyridyl)prop-1-enyl)(*tert*-butoxy)carboxamide (2.0 g, 8.51 mmol) was dissolved in ethyl alcohol (90 mL) and placed in a Parr shaker bottle. To this was added 10% palladium-on-carbon (500 mg) and the reaction was hydrogenated at 40 psi for 24 h. The mixture was filtered through celite, which was washed with methyl alcohol (3 x 100 mL). The combined filtrate and washes were concentrated *in vacuo*. The resultant material was triturated with 90/10 hexanes/methylene chloride to give the title compound (2) as a white solid (1.34 g, 66%). ¹H NMR (300 MHz, DMSO-d₆): δ 1.19 (d, 3H, J = 6.6 Hz); 1.34 (s, 9H); 4.09 (m, 1H); 4.86 (m, 1H); 7.35 (d, 2H, J = 5.7 Hz); 8.21 (s, 1H); 8.47 (d, 2H, J = 5.7 Hz).

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(+/-)-N-[(*tert*-Butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(4-pyridylethyl)carboxamide (3).

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (1.66 g, 5.17 mmol) in tetrahydrofuran (40 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (2.82 g, 6.72 mmol) and the reaction was stirred for five minutes. To this mixture was added dropwise a solution of (+/-)-(*tert*-butoxy)-N-[(4-pyridylethyl)amino]carboxamide (1.3 g, 5.69 mmol) and 4-dimethylaminopyridine (0.080 g, 0.65 mmol) in tetrahydrofuran (20 mL). After stirring the reaction mixture at room temperature for 2 hours, an additional portion of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (0.500 g) was added and the mixture was refluxed overnight. The solution was cooled to 50 °C, another portion of diimide added (0.500 g) and the mixture was refluxed for 3h. The cooled reaction mixture was filtered and the collected insolubles washed with methylene chloride (2 x 150 mL). The combined filtrate and washes were concentrated *in vacuo* to dryness. The resultant yellow foam was subjected to chromatography (silica gel, 92/8 chloroform/methyl alcohol) to give the title compound (3) as a yellow foam (2.04 g, 73%).

(+/-)-7-Chloro-4-hydroxy-2-(4-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione methanesulfonate (4).

To a stirred solution of (+/-)-N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)]-N-(4-pyridylethyl)carboxamide (2.04 g, 3.8 mmol) in tetrahydrofuran (100 mL) was added methanesulfonic acid (13.5 mL) and the reaction was stirred overnight. The volatiles were removed *in vacuo* and the resultant oil was poured on to crushed ice. A fine precipitate formed which was filtered to give an orange solid. This material was washed with diethyl ether, and then sonicated in 40 mL of 1/1 diethyl ether/methyl alcohol for fifteen minutes. The material was washed with diethyl ether to give the title compound (4, 0.715 g, 40%) as an off-white powder (m.p. 245-248 °C). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.74 (d, 3H, *J* = 6.9 Hz); 2.31 (s, 3H, CH₃SO₃H); 6.24 (q, 1H, *J* = 6.9 Hz); 7.45 (dd, 1H, *J* = 1.8, 8.7 Hz); 7.86 (m, 2H); 8.05 (d, 1H, *J* = 1.8 Hz); 8.14 (d, 1H, *J* = 8.7 Hz); 8.82 (m, 2H); 12.03 (s, 1H); 12.71 (s, 1H).

Calc'd. for C₁₉H₁₁ClN₄O₅•CH₃SO₃H•0.8H₂O: C, 47.61; H, 3.91; N, 11.68. Found: C, 47.84; H, 3.79; N, 11.54.

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Example 46: (+/-)-7-Chloro-4-hydroxy-2-(4-pyridylpropyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione sodium methanesulfonate

(+/-)-(tert-Butoxy)-N-[(4-pyridylpropyl)amino]carboxamide

To a stirred solution of N-1-aza-2-(4-pyridyl)vinyl(*tert*-butoxy)carboxamide (2.0 g, 9.40 mmol) in tetrahydrofuran at 0 °C was slowly added ethylmagnesium bromide (18.0 mL, 18.8 mmol, 2M solution in tetrahydrofuran). This solution was stirred 4h during which time it was warmed to room temperature. The volatiles were removed, and the reaction was carefully quenched with saturated ammonium chloride solution (30 mL). The aqueous layer was extracted with ethyl acetate (300 mL) and then the organic layers washed with brine. The organic layer was then dried over MgSO₄ and the solution concentrated *in vacuo*. The material was chromatographed (SiO₂, ethyl acetate as eluent) to give the title compound as a yellow oil (0.400 g, 18%). ¹H NMR (300 MHz, DMSO-d₆): δ 0.73 (t, 3H, J = 7.5 Hz); 1.44 (m, 2H); 3.92 (br s, 1H); 4.78 (m, 1H); 7.31 (m, 2H); 8.48 (m, 2H).

(+/-)-N-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinoly)]-N-(4-pyridylpropyl)carboxamide

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (1.22 g, 3.82 mmol) in tetrahydrofuran (80 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (1.77 g, 4.20 mmol) and the reaction was stirred for five minutes. To this mixture was added, via dropwise addition, a solution of (+/-)-(tert-butoxy)-N-[(4-pyridylpropyl)amino]carboxamide (0.96 g, 3.82 mmol) and 4-dimethylaminopyridine (0.080 g, 0.65 mmol) in tetrahydrofuran (20 mL), and the mixture was stirred at room temperature for 1 hour. An additional portion of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (0.300 g) was added and the mixture was refluxed overnight. The solution was filtered and the insolubles washed with methylene chloride (2 x 150 mL). The mother liquor was collected and concentrated to dryness. The resultant yellow foam was subjected to chromatography (SiO₂, 95/5 chloroform/methyl alcohol) to give the title compound as a yellow foam (1.72 g, 81%).

(+/-)-7-Chloro-4-hydroxy-2-(4-pyridylpropyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione sodium methanesulfonate

To a stirred solution of (+/-)-N-[(tert-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinoly)]-N-(4-pyridylpropyl)carboxamide (1.72 g, 1.5 mmol) in tetrahydrofuran (60 mL) was added methanesulfonic acid (9 mL) and the reaction was

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stirred overnight. The volatiles were removed *in vacuo* and the resultant oil was poured on to crushed ice. To this mixture was carefully added 10N sodium hydroxide until a solid precipitate formed. The solution was filtered to give an orange solid. This material was washed with diethyl ether, and then sonicated in 40 mL of 3/1 diethyl ether/methyl alcohol for fifteen minutes. The material was washed with diethyl ether to give the title compound (0.557 g, 28%) as an off-white powder (m.p. >265 °C). ¹H NMR (300 MHz, DMSO-d₆): δ 0.88 (t, 3H, J = 7.2 Hz); 2.16 (m, 2H); 2.31 (s, 3H, CH₃SO₃H); 5.98 (dd, 1H, J = 5.7, 9.46 Hz); 7.44 (m, 3H); 8.05 (d, 1H, J = 1.5 Hz); 8.15 (d, 1H, J = 8.7 Hz); 8.58 (m, 2H); 12.04 (s, 1H); 12.69 (s, 1H).

Calc'd. for C₁₉H₁₅ClN₄O₃•2CH₃SO₃Na•H₂O: C, 39.59; H, 3.63; N, 8.79. Found: C, 39.73; H, 3.33; N, 8.94.

Example 47: 7-Chloro-4-hydroxy-2-(2-benzofuranyl)methyl-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione:

N'-(1-Benzofuran-2-yl-ethylidene)-hydrazinecarboxylic acid tert-butyl ester (1):

To a solution of benzofuran-2-yl methyl ketone (5.0 g, 31 mmol) in THF (200 mL) was added *tert*-butyl carbazate (4.1 g, 31 mmol), followed by conc. HCl (10 drops). This reaction was stirred overnight at room temperature, at which time the THF was removed *in vacuo* and the resultant solid was triturated with hexanes and filtered to afford *N'*-(1-benzofuran-2-yl-ethylidene)-hydrazinecarboxylic acid *tert*-butyl ester as a white solid (8.2 g, 30 mmol, 96 %). ¹H NMR (300 MHz, DMSO-d₆): δ 10.03 (s, 1H); 7.65 (d, J = 7.8 Hz, 1H); 7.60 (d, J = 8.1 Hz, 1H); 7.35 (dd, J = 7.2, 7.2 Hz, 1H); 7.26 (s, 1H); 7.26 (m, 1H); 2.17 (s, 3H); 1.50 (s, 9H).

N'-(1-Benzofuran-2-yl-ethyl)-hydrazinecarboxylic acid tert-butyl ester (2):

To a solution of *N'*-(1-benzofuran-2-yl-ethylidene)-hydrazinecarboxylic acid *tert*-butyl ester (3.0 g, 11 mmol) in MeOH (55 mL) was added sodium cyanoborohydride (3.4 g, 55 mmol) followed by enough acetic acid to adjust to pH 3 (ca. 7 mL). This solution was heated to 65 °C; at 1 h and 3 h, more sodium cyanoborohydride was added (ca. 2 g each time). After a reaction time of 4 h, the reaction was then cooled to room temperature, diluted with water and the methanol was removed under reduced pressure. The remaining material was diluted with ethyl acetate and washed with saturated aqueous NaHCO₃, water, brine and dried over Na₂SO₄. The mixture was filtered and concentrated to give *N'*-(1-benzofuran-2-yl-ethyl)-

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hydrazinecarboxylic acid *tert*-butyl ester as a white solid (3.1 g, 11 mmol, 100 %). This material was used without further purification.

N'-(1-Benzofuran-3-yl-ethyl)-*N'*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-hydrazinecarboxylic acid *tert*-butyl ester (3):

To a stirred slurry of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (1.77 g, 5.51 mmol) in THF (30 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (4.67 g, 11.0 mmol). To this canary yellow mixture was added a solution of 2 (1.5 g, 5.51 mmol) and *N,N*-dimethylaminopyridine (100 mg, 827 mmol) in THF (25 mL). The resultant mixture was refluxed under N₂ for 3 h, then cooled and filtered. The filtrate was concentrated and filtered through a small column of silica gel (10% MeOH/CH₂Cl₂) to afford *N'*-(1-benzofuran-3-yl-ethyl)-*N'*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-hydrazinecarboxylic acid *tert*-butyl ester as a pale yellow solid; this material was in the following reaction without further purification. MS *m/z* 579.2 (M+1)

7-Chloro-4-hydroxy-2-(2-benzofuranyl)methyl-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione (4):

To a 0 °C solution of *N'*-(1-benzofuran-3-yl-ethyl)-*N'*-[7-chloro-4-oxo-2-(pyrrolidine-1-carbonyl)-1,4-dihydro-quinoline-3-carbonyl]-hydrazinecarboxylic acid *tert*-butyl ester (1.0 g, 1.74 mmol) in MeOH (20 mL) was added dropwise a room temperature solution of methanesulfonic acid (3.4 mL, 52.3 mmol) in MeOH (10 mL). This mixture was allowed to warm to room temperature and stirred for 24 h, at which time water (ca. 100 mL) was added and the precipitate collected. This solid was sonicated (50 mL of 10% MeOH/Et₂O) for 20 min and the solid collected. This sonication step was repeated, and ultimately a tan solid was collected. This material was dried at 30 °C at 500 mTorr for 1.5 h to afford pure 7-chloro-10-hydroxy-2-(2-benzofuranyl)methyl-2,3-dihydropyridazino[4,5-*b*]quinoline-1,10-dione (260 mg, 630 mmol, 36 %). ¹H NMR (300 MHz, DMSO-*d*₆): δ 12.56 (s, 1H); 11.93 (s, 1H); 8.16 (d, *J* = 8.7 Hz, 1H); 8.02 (d, *J* = 1.8 Hz, 1H); 7.60 (dd, *J* = 1.5, 5.7 Hz, 1H); 7.50 (d, *J* = 7.5 Hz, 1H); 7.44 (d, *J* = 7.2 Hz, 1H); 7.25 (m, 2H); 6.88 (s, 1H); 6.41 (q, *J* = 7.2 Hz, 1H); 1.71 (d, *J* = 6.9 Hz, 3H).

Calc'd. for C₂₇H₁₄ClN₃O₄·0.2H₂O: C, 61.31; H, 3.53; N, 10.21. Found: C, 61.14, 61.19; H, 3.54, 3.53; N, 10.25, 10.25.

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118**TESTS FOR BIOLOGICAL FUNCTION:****Test A: [³H]-glycine binding at the N-methyl-D-aspartate receptor.**

This test measures the ability of ligands to inhibit the binding of [³H]-glycine to the N-methyl-D-aspartate receptor. In the [³H]-glycine binding assay, neuronal synaptic membranes are prepared from adult (about 250 g) male Sprague-Dawley rats. Freshly dissected cortices and hippocampi are homogenised in 0.32 M sucrose (110 mg/ml). Synaptosomes are isolated by centrifugation (1000 xg, 10 min), the supernatant is pelleted (20,000 xg, 20 min) and resuspended in double-distilled water. The suspension was centrifuged for 20 minutes at 8,000 xg. The resulting supernatant and buffy coat are washed twice (48,000 xg, 10 mins, resuspension in double-deionized water). The final pellet is quickly frozen (dry ice/ethanol bath) under double-deionized water and stored at -70 °C.

On the day of the experiment, thawed synaptic membranes are homogenized with a Brinkmann Polytron (tm, Brinkmann Instruments, Westbury, N.Y.) tissue homogenizer in 50 millimolar tris(hydroxymethyl)aminomethane citrate, pH 7.1. The membranes are incubated with 0.04% Sufact-AMPS X100 (tm, Pierce, Rockford, IL) in buffer for 20 minutes at 37 °C and washed six times by centrifugation (48,000 xg, 10 min) and resuspended in buffer. The final pellet is homogenized at 200 mg wet weight/ml of the buffer for the binding assay.

For [³H]-glycine binding at the N-methyl-D-aspartate receptor, 20 nanomolar [³H]-glycine (40-60 Ci/mmol, New England Nuclear, Boston, MA) is incubated with the membranes suspended in 50 millimolar tris (hydroxymethyl)aminomethane citrate, pH 7.1 for 30 minutes at 4 °C. Glycine, 1 millimolar, is used to define the non-specific binding. Bound [³H]-glycine is isolated from the free form using a Brandel (Biomedical Research and Development Laboratories, Gaithersburg, MD) cell harvester for vacuum filtration over glass fibre filters (Whatman GF/B from Brandel, Gaithersburg, MD) pre-soaked in 0.025% polyethylenimine. The samples retained on the glass fibre filters are rinsed 3 times with a total of 2.5 ml ice cold buffer. Radioactivity is estimated by liquid scintillation counting. IC₅₀ values are obtained from a least-squares regression of a logit-log transformation of the data. Typical IC₅₀ values for compounds of the invention are usually less than 50 µM (micromolar). The examples disclosed herein are glycine antagonists.

Test B: N-Methyl-D-aspartate (NMDA) Evoked Behavior Test:

The NMDA-evoked behavior test is a functional assay performed with rats to determine antagonism of the NMDA receptor *in vivo*. This test measures the ability of

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various unknown compounds to inhibit aversive behaviors evoked by the injection of NMDA into the spinal cord.

Male rats weighting 90-150 gm are used in this study. Animals are pretreated with glycine antagonists (by either intrathecal, systemic or oral routes) at various times before the intrathecal injection (0.25 - 10 nmol/rat) of NMDA which elicits aversive behaviors. NMDA is injected into the spinal cord by free hand intrathecal injection. The aversive behaviors typically observed immediately following the intrathecal NMDA injection include: biting at the injection site, biting response lasting longer than 90 seconds, rigid tail weave, scratching behavior, vocalization, abnormal motor activity, back arching behavior, circling behavior, flipping or rolling behavior, stretching behavior, kicking and jumping behaviors. The manifestation of each behavior is scored and the maximum possible score is 13 behaviors. In a standard assay used for compound screening, 2.5 nanomolar NMDA is injected intrathecally into animals and the average aversive behavioral score observed is typically 10 out of 13. All data for a specific compound of interest are calculated as percentage inhibition compared with a vehicle group. The statistical analysis of vehicle and compound-treated groups is the t-test. Compounds are considered active based on their ability to inhibit the NMDA-evoked behavior and their potency by the route of interest.

Test C: Formalin test:

The Formalin test assesses the inhibitory effects of orally administered glycine antagonists on formalin-induced nocifensive behaviors in rats. The formalin test is a well established pain test (Dubuisson and Dennis, 1977; Wheeler-Aceto *et al.*, 1990;Coderre *et al.*, 1993). This test consists of two distinctive phases of formalin-induced behaviors. The first phase response, occurring between 0 to 5 minutes, is caused by acute nociception to the noxious chemical (formalin) injected into the paw. There is a quiescent period between 5 to 15 min post injection. A second phase response, occurring after 15 minutes and lasting up to 60 minutes, is caused by sensitization of the central neurons in the dorsal horn. Central sensitization augments the noxious afferent input and a stronger pain barrage is transmitted into the brain. Inhibition of the second phase response indicates a spinal mechanism of drug action.

The procedure for the formalin test is as follows: male rats are placed in a plexiglass chamber and observed for 30-45 min. to observe their baseline activity. Multiple groups of animals are pretreated with either vehicle or different doses of a test compound. Animals are

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dosed 3 hours prior to injection of formalin into a hind paw (under the dorsal skin) with 0.05ml of sterile 1% formalin. The number of paw flinches and licks during first phase (0-5 min.) and second phase (20-35 min.) are scored and recorded. Flinch and lick responses are calculated as percentage of inhibition compared with the mean score of a saline control group.

- 5 Student t-tests are used for statistical analysis to determine the significance of drug effects.
- Compounds are considered active based on their ability to inhibit both flinch and lick responses.

Test D: Neuropathic pain model (Chronic Constriction Injury):

- 10 The Chronic Constriction Injury ("CCI") test assesses neuropathic pain associated with nerve injuries that can arise directly from trauma and compression, or indirectly from a wide range of diseases such as infection, cancer, metabolic conditions, toxins, nutritional deficiencies, immunological dysfunction, and musculoskeletal changes. A unilateral peripheral neuropathy is produced in rats by the nerve ligation in the chronic constriction injury (CCI) model (Bennett and Xie, 1988).

- 15 Sprague-Dawley rats (250-350 g) are anesthetized with sodium pentobarbital and the common sciatic nerve is exposed at the level of the mid thigh by blunt dissection through the biceps femoris. A section of nerve (about 7 mm), proximal to the sciatic trifurcation, is freed of tissue and ligated 4 times with chromic gut suture. The suture is tied with about 1 mm spacing between ligatures. The incision is closed in layers and the animals are allowed to recover.
- 20 Thermal hyperalgesia is measured using the paw-withdrawal test (Hargreaves et al, 1988). Animals are habituated on an elevated glass floor. A radiant heat source is aimed at the mid-plantar hindpaw (sciatic nerve territory) through the glass floor. The latencies for the withdrawal reflex in both paws are recorded. A 20 second cut-off is used to prevent permanent injury to the skin. Injured paws with ligated nerves show shorter paw withdrawal latencies compared to the uninjured or sham operated paws. Response to test compounds are evaluated by time following oral administration to determine onset and duration of drug effect. Dose response studies are conducted with multiple groups of CCI rats dosed orally with either vehicle or the test compound for 5 days. Paw withdrawal latencies are measured each day prior to the first daily dose. Data analysis is performed by multiple means
- 30 comparison (Dunnett's test) and compound potencies are expressed by their minimum effective dose.

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Table 2 shows the results from Tests A, B, C and D for certain compounds of the invention. In table 1, results from test A are given as the nanomolar ("nM") concentration of compound that provided a 50% inhibition of [³H]-glycine binding; results from test B are given as concentration mg/Kg administered intrathecally that gave an inhibition (%) of NMDA-evoked behaviors in the test; results from test C are given as concentration mg/Kg administered orally that gave an inhibition (%) of formalin-induced nocifensive behaviors in the test, and results from test D are given as concentration mg/Kg administered orally that gave an inhibition (%) of heat-induced responses in the test. Where no data is provided the test was not performed.

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Table 2:

Ex. No.	Test A IC ₅₀ (nM)	Test B mg/Kg (%)	Test C mg/Kg (%)	Test D mg/Kg (%)
1	39.9	0.004 (81)		
2	108.4	0.004 (56)	200 (55)	
3		0.005 (50)	190 (59)	2 (83 ± 6)
4		0.004 (100)	200 (33)	
5	95.8			
6	87.7	0.014 (22)		
7	42.7	0.013 (63)		
10	67.9			
11	62.5			
12				
13	59	0.013 (63)		
14	165			
15	146			
16	86.6			
17	75.9			
18	106			
19	166			
20	83.4			
21	248			

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22	57.4	0.014 (39)		
23	158			
24	205			
25	37.3			
26	27.9			
27	31.9			
28		0.005 (52)	200(48)	Active*
29	99.7	0.005 (58)	200 (74)	30 (59 ± 15)
30	146.3	0.005 (44)	200 (56)	
31	1150	0.014 (41)		
32	5960			
33	793			
34				30 (64 ± 21)
35	70.8	0.1 (78)	200 (59)	30 (124 ± 5)
36	24.8			
37	449			
38	30.5	0.011 (33)		
39	146			
40	65.2			
41	12.7	0.011 (74)		
42	268			
43	272	0.015 (54)		
44	282	0.005 (48)	200 (83)	3 (56 ± 10)
45	249	0.014 (35)		
46	1940	0.06 (41)		
47	63.5			

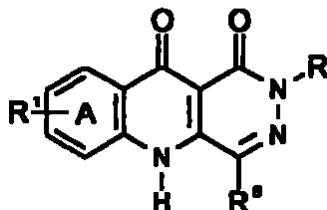
* compound showed measurable activity.

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CLAIMS

1. A method for treatment of pain comprising, administering to an individual in need thereof an effective amount of any compound in accord with structural diagram I,



I

or pharmaceutically-acceptable salts of such compounds, tautomers, or optical isomers of such compounds, wherein:

R¹ at each occurrence is independently selected from hydrogen, halo and NO₂; and
 ring-A has four R¹ groups;
 R is selected from (CH₂)ₙL, and C(R²)ₓ(CH₂)ₙL wherein n is an integer selected from the range 0 to 6 and R² at each occurrence is independently selected from hydrogen, halogen, C₁-₄alkyl and C₃-₆cycloalkyl;

L is phenyl substituted with one, two, three or four groups selected from: OR³ where R³ is selected from hydrogen and pyridyl-methyl, N(R⁴)₂ where R⁴ at each occurrence is independently selected from hydrogen and C₁-₄alkyl, halogen, carboxyl, C₁-₄alkyl, O-C₁-₄alkyl, C₂-₄alkenyl, C₂-₄alkynyl, a heteroaryl group selected from furanyl, oxazolyl, oxadiazolyl, thiadiazole, tetrazolyl, morpholinyl and benzimidazolyl, morpholino-N-carbonyl, the group OC(O)N(R⁵)₂ where R⁵ at each occurrence is independently selected from hydrogen, C₁-₄alkyl and phenyl; or

L is heteroaryl selected from pyridyl, isooxazolyl, oxadiazolyl, thiophenyl, benzo[b]thiophenyl, benzofuranyl and pyrazinyl; where any said heteroaryl is substituted with one, two or three groups selected from: hydroxy, C₁-₄alkyl, hydroxyC₁-₄alkyl and methoxyphenyl; or

L is tetrahydrofuranyl; and

R⁶ is selected from OH or OC(O)N(R⁷)₂ wherein R⁷ at each occurrence is independently selected from hydrogen or C₁-₄alkyl.

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2. A method according to Claim 1, wherein in said compound in accord with structural diagram I:

R^1 and ring-A in combination are 7-halophenyl and L is selected from substituted or unsubstituted phenyl, pyridyl, isooxazolyl, oxadiazolyl, thiophenyl, benzo[b]thiophenyl, benzofuranyl and pyrazinyl groups, where, when substituted, said groups are substituted with one, two or three moieties independently selected at each occurrence from halo, hydroxy, oxo, carbonyl, carbonylamino, C_{1-4} alkyl and hydroxy C_{1-4} alkyl.

3. A method according to Claim 2, wherein in said compound in accord with structural diagram I:

R^1 and ring-A in combination are 7-chlorophenyl.

4. A method according to Claim 1, wherein in said compound in accord with structural diagram I:

R is $(CH_2)_nL$, wherein n is an integer selected from the range 0 to 6, and L is phenyl substituted with one, two, three or four groups selected from: OR^3 where R^3 is selected from hydrogen and pyridyl-methyl, $N(R^4)_2$ where R^4 at each occurrence is independently selected from hydrogen and C_{1-4} alkyl, halogen, carboxyl, C_{1-4} alkyl, $O-C_{1-4}$ alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, a heteroaryl group selected from furanyl, oxazolyl, oxadiazolyl, thiadiazole, tetrazolyl, morpholinyl and benzimidazolyl, morpholino-N-carbonyl, the group $OC(O)N(R^5)_2$ where R^5 at each occurrence is independently selected from hydrogen, C_{1-4} alkyl and phenyl, and R^6 is hydroxy.

5. A method according to Claim 1, wherein in said compound in accord with structural diagram I:

R is $(CH_2)_nL$, wherein n is an integer selected from the range 0 to 6, and L is heteroaryl selected from pyridyl, isooxazolyl, oxadiazolyl, thiophenyl, benzo[b]thiophenyl, benzofuranyl and pyrazinyl; where any said heteroaryl is substituted with one, two or three groups selected from: hydroxy, C_{1-4} alkyl, hydroxy C_{1-4} alkyl and methoxyphenyl and R^6 is hydroxy.

6. A method according to Claim 1, wherein in said compound in accord with structural diagram I:

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R is $(CH_2)_nL$, wherein n is an integer selected from the range 0 to 6, L is tetrahydrofuranyl and R⁶ is hydroxy.

7. A method according to Claim 1, wherein in said compound in accord with structural diagram I:

R is $C(R^2)_x(CH_2)_nL$ wherein n is an integer selected from the range 0 to 6 and R² at each occurrence is independently selected from hydrogen, halogen, C₁₋₄alkyl and C₃₋₆cycloalkyl.

8. A method according to Claim 1, wherein in said compound is selected from

7-chloro-4-hydroxy-2-(2,4,6-trimethylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione;

7,9-dichloro-4-hydroxy-2-(4-methoxy-2-methylphenyl)-1,2,5,10-

tetrahydropyridazino[4,5-b]quinoline-1,10-dione;

7-chloro-4-hydroxy-2-(2-chloro-4-methyl phenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione;

methyl 4-[(7-chloro-4-hydroxy-1,10-dioxo-2,5-dihydropyridazino[4,5-b]quinolin-2-yl)methyl]benzoate;

7-chloro-4-hydroxy-2-(4-[1,3,4]oxadiazol-2-yl-benzyl)-1,2,5,10-

tetrahydropyridazino[4,5-b]quinoline-1,10-dione (7)

7-chloro-4-hydroxy-2-[4-(morpholine-4-carbonyl)-benzyl]-1,2,5,10-

tetrahydropyridazino[4,5-b]quinoline-1,10-dione (7)

7-chloro-4-hydroxy-2-[3-(1h-tetrazol-5-yl)-benzyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (7)

4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-n-furan-2-ylmethyl-benzamide;

preparation of 7-chloro-4-hydroxy-2-(4-dimethylaminobenzyl)-1,2,5,10-

tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (4);

7-chloro-4-hydroxy-2-(benzimidazol-5-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate;

7-chloro-4-hydroxy-2-(4-hydroxy-3-methyl-benzyl)-1,2,5,10-

tetrahydropyridazino[4,5-b]quinoline-1,10-dione (2)

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7-chloro-4-hydroxy-2-[3-methyl-4-(pyridin-2-ylmethoxy)-benzyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (2)

dimethyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methyl-phenyl ester (7) (method a)

5 dimethyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methyl-phenyl ester (7) (method b)

dimethyl-carbamic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester;

10 methyl-phenyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester;

diethyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester;

diethyl-carbamic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester;

15 morpholine-4-carboxylic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester;

methyl-phenyl-carbamic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester;

20 diphenyl-carbamic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester;

pyrrolidine-1-carboxylic acid 3-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-phenyl ester;

morpholine-4-carboxylic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methyl-phenyl ester;

25 methyl-phenyl-carbamic acid 4-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methyl-phenyl ester;

dimethyl-carbamic acid 5-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methoxy-phenyl ester;

30 diethyl-carbamic acid 5-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methoxy-phenyl ester;

morpholine-4-carboxylic acid 5-(7-chloro-4-hydroxy-1,10-dioxo-5,10-dihydro-1h-pyridazino[4,5-b]quinolin-2-ylmethyl)-2-methoxy-phenyl ester;

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7-chloro-4-hydroxy-2-(4-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate;

7-chloro-4-hydroxy-2-(3-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate;

5 7-chloro-4-hydroxy-2-(2-pyridylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate;

7-chloro-4-hydroxy-2-[3-hydroxy-5(hydroxymethyl)-2-methyl(4-pyridyl)]methyl-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate;

10 dimethyl-carbamic acid 7-chloro-1,10-dioxo-2-pyridin-4-ylmethyl-1,2,5,10-tetrahydro-pyridazino[4,5-b]quinolin-4-yl ester (2)

7-chloro-4-hydroxy-2-(4-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione;

7-chloro-4-hydroxy-2-(pyrazin-2-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate (5)

15 7-chloro-4-hydroxy-2-(furan-2-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione;

7-chloro-4-hydroxy-2-[2,5-dimethyl(3-furanyl)]methyl-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (4)

20 7-chloro-4-hydroxy-2-(tetrahydro-furan-2-ylmethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione;

7-chloro-4-hydroxy-2-(5-isoxazolino)methyl-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (3)

7-chloro-4-hydroxy-2-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-ylmethyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione (6)

25 2-(benzo[b]thiophen-2-ylmethyl)-7-chloro-4-hydroxy-2,5-dihydropyridazino[4,5-b]quinoline-1,10-dione;

7-chloro-10-hydroxy-2-[(3-methyl(2-thienyl))methyl]-2,3-dihydropyridazino[4,5-b]quinoline-1,4-dione;

30 7-chloro-4-hydroxy-2-[4-(carbomethoxy)phenyl-2-ethyl]-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione;

(+/-)-7-chloro-4-hydroxy-2-(3-pyridyleth-1-yl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate;

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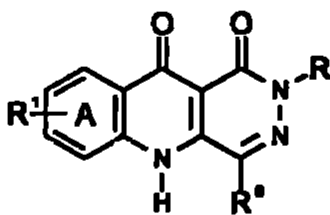
(+/-)-7-chloro-4-hydroxy-2-(2-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate;

(+/-)-7-chloro-4-hydroxy-2-(4-pyridylethyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione methanesulfonate;

5 (+/-)-7-chloro-4-hydroxy-2-(4-pyridylpropyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione sodium methanesulfonate; and

7-chloro-4-hydroxy-2-(2-benzofuranyl)methyl-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione.

10 9. A pharmaceutical composition having as an active ingredient a compound in accord with structural diagram I



I

or pharmaceutically-acceptable salts of such compounds, tautomers of such compounds or optical isomers of such compounds, wherein:

15 R^1 at each occurrence is independently selected from hydrogen, halo and NO_2 , and ring-A has four R^1 groups;

R is selected from $(\text{CH}_2)_n\text{L}$, and $\text{C}(\text{R}^3)_2(\text{CH}_2)_n\text{L}$ wherein n is an integer selected from the range 0 to 6 and R^2 at each occurrence is independently selected from hydrogen, halogen, C_{1-4} alkyl and C_{3-6} cycloalkyl;

20 L is phenyl substituted with one, two, three or four groups selected from: OR^3 where R^3 is selected from hydrogen and pyridyl-methyl, $\text{N}(\text{R}^4)_2$ where R^4 at each occurrence is independently selected from hydrogen and C_{1-4} alkyl, halogen, carboxyl, C_{1-4} alkyl, $\text{O}-\text{C}_{1-4}$ alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, a heteroaryl group selected from furanyl, oxazolyl, oxadiazolyl, thiadiazole, tetrazolyl, morpholinyl and benzimidazolyl, morpholino-N-carbonyl, the group $\text{OC}(\text{O})\text{N}(\text{R}^5)_2$ where R^5 at each occurrence is independently selected from hydrogen, C_{1-4} alkyl and phenyl; or

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L is heteroaryl selected from pyridyl, isooxazolyl, oxadiazolyl, thiophenyl, benzo[b]thiophenyl, benzofuranyl and pyrazinyl; where any said heteroaryl is substituted with one, two or three groups selected from: hydroxy, C₁₋₄alkyl, hydroxyC₁₋₄alkyl and methoxyphenyl; or

5 L is tetrahydrofuranyl; and

R⁶ is selected from OH or OC(O)N(R')₂, wherein R' at each occurrence independently selected from hydrogen or C₁₋₄alkyl, and
a pharmaceutically-acceptable excipient or diluent.

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Intendente General
Ministerio del Poder Judicial

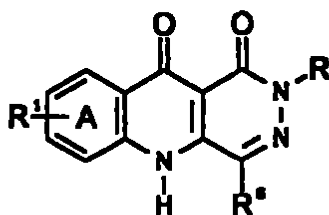
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A B S T R A C T

A method for treatment of pain comprising administering an effective amount of a compound in accord with structural diagram I,



I

or pharmaceutically-acceptable salts, tautomers or optical isomers of such compounds,
 wherein: R¹ is selected from hydrogen, halo and other groups as disclosed in the specification,
 R is selected from (CH₂)_nL, and C(R²)_n(CH₂)_nL wherein n is an integer selected from the range
 0 to 6 and R² is selected from hydrogen, halogen, C₁₋₄alkyl and C₃₋₆cycloalkyl and L is
 substituted or unsubstituted phenyl, heteroaryl or other groups as disclosed in the specification
 whichm, when substituted, are substituted with one, two, three or four groups selected from a
 variety of substituents as disclosed in the specification.

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A CUANTOS LLEGAREN LAS PRESENTES:

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de Fábrica de los Estados Unidos

5.799
Marta Leticia Bragado de Rostrop
Intérprete Oficial
Exp. No. 0762 del Mto. de Justicia

4 de diciembre del 2000

POR LA PRESENTE SE CERTIFICA QUE ANEXA AQUI SE ENCUENTRA UNA COPIA FIEL, TOMADA DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE FABRICA DE LOS ESTADOS UNIDOS, DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA ABAJO QUE SATISFACEN LOS REQUISITOS PARA QUE SEA OTORGADA UNA FECHA DE REGISTRO SEGUN 35 USC 111.

NUMERO DE SOLICITUD: 60/236,783

FECHA DE REGISTRO: 29 de septiembre del 2000.

Por Autorización del

COMISIONADO DE PATENTES Y MARCAS DE FABRICA

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(Firma)

L. EDELEN

Funcionario Certificador

Sello: OFICINA DE PATENTES Y MARCAS DE FABRICA - ESTADOS UNIDOS
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SOLICITUD PROVISIONAL PARA HOJA DE CUBIERTA DE PATENTE

Esta es una solicitud de registro de una SOLICITUD PROVISIONAL DE PATENTE
según 37 CFR 1.53 (c)

RECEIVED. OFFICIAL NO. 5299
Marta Leden
Registradora Oficial
de Patentes

INVENTOR(ES)		
Nombre	Apellido	Residencia (Ciudad y Estado o País Extranjero)
WENHWA	XIAO	WILMINGTON, DELAWARE
☒ Los inventores adicionales están mencionados en la hoja numerada separadamente anexa a la presente.		
TITULO DE LA INVENCION		
COMPUESTO Y METODO PARA EL TRATAMIENTO DEL DOLOR		
DIRECCION DE CORRESPONDENCIA		
Dirigir toda la correspondencia a:		
☒ Número del Cliente 22466		

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X Descripción: Número de Páginas <u>17</u>	
METODO DE PAGO DE LOS DERECHOS DE REGISTRO DE ESTA SOLICITUD PROVISIONAL DE PATENTE	
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¿La invención fué hecha por una agencia del Gobierno de los Estados Unidos o bajo un contrato con una agencia del Gobierno de los Estados Unidos?	
X No.	

MANEJO OFICIAL 5.769
Marta Luisa Brizuela Rodríguez
Interventora Oficial
Cm. No. 8708 del 10 de Agosto

Presentada respetuosamente,

FIRMA (Firma) _____

Fecha 29/9/00

NO. DE REGISTRO: 42,007

NOMBRE EN LETRA DE IMPRENTA: KENNETH F. MITCH

TELEFONO 302 - 886 - 7466

Número de Expediente: 100 178

ANEXO:

SOLICITUD PROVISIONAL PARA HOJA DE CUBIERTA

Página Adicional

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134

Número del Expediente		100178	Escriba un signo más (+) dentro de esta casilla -	+
INVENTOR(ES)/SOLICITANTE(S)				
Nombre	Apellido	Residencia (Ciudad y Estado o País Extranjero)		
THOMAS MICHAEL	BARE	WILMINGTON, DELAWARE		
MEGAN	MURPHY	WILMINGTON, DELAWARE		
REBECCA	URBANEK	WILMINGTON, DELAWARE		
DEAN GORDON	BROWN	WILMINGTON, DELAWARE		

Número 1 de 1

5799
Marta Luisa Briceño Rodríguez
Interventora General
Dep. Ma. GTR del Min. de Sanidad

ANEXO:

100178

REIVINDICACIONES:

1. 7-Cloro-4-hidroxi-2-(2-cloro-4-metilfenil)-1,2,5,10-tetrahidropiridazino[4,5-b)quinolino-1,10-diona o sales farmacéuticamente aceptables de esta.
2. El compuesto de la Reivindicación 1 en el que dicha sal farmacéuticamente aceptable es una sal de colina.

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3. Un método para tratar el dolor, que comprende. la administración de una cantidad efectiva para aliviar el dolor del compuesto acorde con la Reivindicación 1.
4. Una composición farmacéutica que comprende un compuesto acorde con la Reivindicación 1 como ingrediente activo junto con uno o varios aditivos farmacéuticamente aceptables.
5. Un método para tratar el dolor, que comprende. la administración de una cantidad efectiva para aliviar el dolor del compuesto acorde con la Reivindicación 2.
6. Una composición farmacéutica que comprende un compuesto acorde con la Reivindicación 2 como ingrediente activo junto con uno o varios aditivos farmacéuticamente aceptables.

ES TRADUCCION FIEL Y COMPLETA, de la cual queda copia en los archivos de esta oficina de traducciones, para su confrontación y a la que me remito.

Derechos de Traducción: \$45.000,00

BOGOTA, 29 DE DICIEMBRE DEL 2000.

RECIBIDO. OFICINA 5.799
Maria Luisa Brigard de Restrepo
Maria Luisa Brigard de Restrepo
Interpretadora
Cm. No. 6782 del 10to. de Justicia

AstraZeneca
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WA 337742

THE UNITED STATES OF AMERICA

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United States Patent and Trademark Office

December 04, 2000

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OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/236,783

FILING DATE: September 29, 2000

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS



L. Edelen

L. EDELEN
Certifying Officer

10-200
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09/29/00
35390 U.S. PTO

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET
This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.63(c).

35390 U.S. PTO
09/23/03
09/23/00

INVENTOR(S)				
Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)		
WENHUA	XIAO	WILMINGTON, DELAWARE		
☒ Additional inventors are being named on the <u>1</u> separately numbered sheets attached hereto				
TITLE OF THE INVENTION (200 characters max) COMPOUND AND METHOD FOR THE TREATMENT OF PAIN				
Direct all correspondence to: CORRESPONDENCE ADDRESS				
☒ Customer Number		22466 → Place Customer Number Bar Code Label here		
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ENCLOSED APPLICATION PARTS (check all that apply)				
☒ Specification Number of Pages		17	☐ CD(s), Number	
☐ Drawing(s) Number of Sheets			☐ Other (specify)	
☐ Application Data Sheet. See 37 CFR 1.78				
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT				
☐ Applicant claims small entity status. See 37 CFR 1.27.		FILING FEE AMOUNT (\$)		
☐ A check or money order is enclosed to cover the filing fees				
☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number:		26-0166	150.00	
☐ Payment by credit card. Form PTO-2038 is attached.				
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.				
☒ No.				
☐ Yes, the name of the U.S. Government agency and the Government contract number are _____				

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Respectfully submitted,
SIGNATURE K F Mitchell
TYPED or PRINTED NAME KENNETH F. MITCHELL
TELEPHONE 302-886-7466

Date 9/27/00
REGISTRATION NO. 42,007
(if appropriate)
Docket Number: 100178

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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PROVISIONAL APPLICATION COVER SHEET
Additional Page

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Docket Number

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INVENTOR(S)/APPLICANT(S)

Given Name (First and middle (if any))	Family or Surname	Residence (City and either State or Foreign Country)
THOMAS MICHAEL	BARE	Wilmington, DELAWARE
MEGAN	MURPHY	Wilmington, DELAWARE
REBECCA	URBANEK	Wilmington, DELAWARE
DEAN GORDON	BROWN	WILMINGTON DELAWARE

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Mark L. Linder, Esq.
Intellectual Property
Attorney at Law
P.O. Box 5708, San Diego, CA 92161

Number 1 of 1

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60236783-092900

COMPOUND AND METHOD FOR THE TREATMENT OF PAIN**FIELD OF THE INVENTION**

This invention relates to the treatment or prevention of pain or nociception.

RELATED ART

5 Pain is a sensory experience distinct from sensations of touch, pressure, heat and cold. It is often described by sufferers by such terms as bright, dull, aching, pricking, cutting or burning and is generally considered to include both the original sensation and the reaction to that sensation. This range of sensations, as well as the variation in perception of pain by different individuals, renders a precise definition of pain difficult, however, many individuals
10 suffer with severe and continuous pain.

Pain that is caused by damage to neural structures is often manifest as a neural hypersensitivity or hyperalgesia and is termed "neuropathic" pain. Pain can also be "caused" by the stimulation of nociceptive receptors and transmitted over intact neural pathways, such pain is termed "nociceptive" pain.

15 The level of stimulation at which pain becomes noted is referred to as the "pain threshold." Analgesics are pharmaceutical agents which relieve pain by raising the pain threshold without a loss of consciousness. After administration of an analgesic drug a stimulus of greater intensity or longer duration is required before pain is experienced. In an individual suffering from hyperalgesia an analgesic drug may have an anti-hyperalgesic effect.
20 In contrast to analgesics, agents such as local anaesthetics block transmission in peripheral nerve fibers thereby blocking awareness of pain. General anaesthetics, on the other hand, reduce the awareness of pain by producing a loss of consciousness.

Tachykinin antagonists have been reported to induce antinociception in animals, which is believed to be analogous to analgesia in man (Maggi et al, J. Auton. Pharmacol. (1993) 13,
25 23-93). In particular, non-peptide NK-1 receptor antagonists have been shown to produce such analgesia. For example, the NK-1 receptor antagonist RP 67,580 produced analgesia with potency comparable to that of morphine (Garret et al, Proc. Natl. Acad. Sci. USA (1993) 88, 10208-10212).

The opioid analgesics are a well-established class of analgesic agents with morphine-
30 like actions. Synthetic and semi-synthetic opioid analgesics are derivatives of five chemical classes of compound: phenanthrenes; phenylheptylamines; phenylpiperidines; morphinans; and benzomorphans. Pharmacologically these compounds have diverse activities, thus some are strong agonists at the opioid receptors (e.g. morphine); others are moderate to mild

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agonists (e.g. codeine); still others exhibit mixed agonist-antagonist activity (e.g. nalbuphine); and yet others are partial agonists (e.g. nalorphine). Whilst an opioid partial agonist such as nalorphine, (the N-alkyl analogue of morphine) will antagonize the analgesic effects of morphine, when given alone it can be a potent analgesic in its own right.

5 Of all of the opioid analgesics, morphine remains the most widely used, but, in addition to its therapeutic properties, it has a number of drawbacks including respiratory depression, decreased gastrointestinal motility (resulting in constipation), nausea and vomiting. Tolerance and physical dependence also limit the clinical uses of opioid compounds.

10 Aspirin and other salicylate compounds are frequently used in treatment to interrupt amplification of the inflammatory process in rheumatoid diseases and arthritis and temporarily relieve the pain. Other drug compounds used for these purposes include phenylpropionic acid derivatives such as Ibuprofen and Naproxen, Sulindac, phenyl butazone, corticosteroids, antimalarials such as chloroquine and hydroxychloroquine sulfate, and fenemates (J. Hosp. Pharm., 36:622 (May 1979)). These compounds, however, are ineffective for neuropathic pain.

Available therapies for pain also have drawbacks. Some therapeutic agents require prolonged use before an effect is experienced by the patient. Other existing drugs have serious side effects in certain patients, and subjects must be carefully monitored to ensure that
20 any side effects are not unduly threatening. Most existing drugs provide only temporary relief from pain and must be taken consistently on a daily or weekly basis. With disease progression the amount of medication needed to alleviate the pain often increases, thus increasing the potential for adverse side effects.

NMDA receptors are defined by the binding of N-methyl-D-aspartate (NMDA)
25 comprises a receptor/ion channel complex with several different identified binding domains. NMDA itself is a molecule structurally similar to glutamate (Glu) which binds at the glutamate binding site and is highly selective and potent in activating the NMDA receptor (Watkins (1987); Olney (1989)).

Many compounds are known that bind at the NMDA/Glu binding site (for example
30 CPP, DCP-ene, CGP 40116, CGP 37849, CGS 19755, NPC 12626, NPC 17742, D-AP5, D-AP7, CGP 39551, CGP-43487, MDL-100,452, LY-274614, LY-233536, and LY233053).
Other compounds, referred to as non-competitive NMDA antagonists, bind at other sites in the NMDA receptor complex (examples are phencyclidine, dizocilpine, ketamine, tiletamine,

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CNS 1102, dextromethorphan, memantine, kynurenic acid, CNQX, DNQX, 6,7-DCQX, 6,7-DCHQC, R(+)-HA-966, 7-chloro-kynurenic acid, 5,7-DCKA, 5-iodo-7-chloro-kynurenic acid, MDL-28,469, MDL-100,748, MDL-29,951, L-689,560, L-687,414, ACPC, ACPCM, ACPCE, arcaine, diethylenetriamine, 1,10-diaminododecane, 1,12-diaminododecane, ifenprodil, and SL-82.0715). These compounds have been extensively reviewed by Rogawski (1992) and Massieu et. al., (1993), and articles cited therein.

In addition to its physiological function, glutamate (Glu) can be neurotoxic. Glu neurotoxicity is referred to as "excitotoxicity" because the neurotoxic action of Glu, like its beneficial actions, is mediated by an excitatory process (Olney (1990); Choi (1992)).

Normally, when Glu is released at a synaptic receptor, it binds only transiently and is then rapidly removed from the receptor by a process that transports it back into the cell. Under certain abnormal conditions, including stroke, epilepsy and CNS trauma, Glu uptake fails and Glu accumulates at the receptor resulting in a persistent excitation of electrochemical activity that leads to the death of neurons that have Glu receptors. Many neurons in the CNS have Glu receptors, so excitotoxicity can cause an enormous amount of CNS damage.

Acute excitotoxicity injury can occur as a result of ischemic events, hypoxic events, trauma to the brain or spinal cord, certain types of food poisoning which involve an excitotoxic poison such as domoic acid, and seizure-mediated neuronal degeneration, which can result from persistent epileptic seizure activity (status epilepticus). A large body of evidence has implicated the NMDA receptor as one receptor subtype through which Glu mediates a substantial amount of CNS injury, and it is well established that NMDA antagonists are effective in protecting CNS neurons against excitotoxic degeneration in these acute CNS injury syndromes (Choi (1988); Olney (1990)).

In addition to neuronal damage caused by acute insults, excessive activation of Glu receptors may also contribute to more gradual neurodegenerative processes leading to cell death in various chronic neurodegenerative diseases, including Alzheimer's disease, amyotrophic lateral sclerosis, AIDS dementia, Parkinson's disease and Huntington's disease (Olney (1990)). It is generally considered that NMDA antagonists may prove useful in the therapeutic management of such chronic diseases.

In the 1980's it was discovered that PCP (also known as "angel dust") acts at a "PCP recognition site" within the ion channel of the NMDA Glu receptor. PCP acts as a non-competitive antagonist that blocks the flow of ions through the NMDA ion channel. More recently it has become evident that drugs which act at the PCP site as non-competitive NMDA

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antagonists are likely to have psychotomimetic side effects. Further, it is now recognized that certain competitive and non-competitive NMDA antagonists can cause similar pathomorphological effects in rat brain (Olney et. al., (1991); Hargreaves et. al., (1993)).

Such compounds also have psychotomimetic effects in humans (Kristensen et. al., (1992);

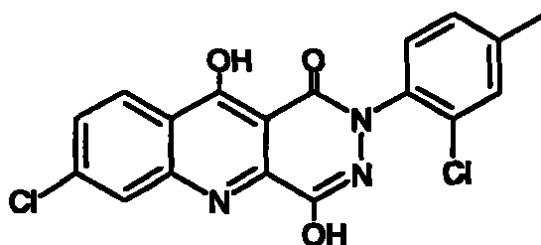
5 Herling (1994); Grotta (1994)).

The glycine binding site of the NMDA receptor complex is distinguishable from the Glu and PCP binding sites. Also, it has recently been discovered that NMDA receptors occur as several subtypes which are characterized by differential properties of the glycine binding site of the receptor. Many compounds that bind at the NMDA receptor glycine site, useful for
10 the treatment of stroke and neurodegenerative conditions, have been described in U.S. Patents 5,604,227; 5,733,910; 5,599,814; 5,593,133; 5,744,471; 5,837,705 and 6,103,721.

SUMMARY OF THE INVENTION

It has now been discovered that a certain compound which exhibits the property of binding to the NMDA receptor glycine site has a utility for the amelioration of pain and
15 particularly for the amelioration of neuropathic pain.

Therefore, in one aspect, the invention provides a compound, 7-chloro-4-hydroxy-2-(2-chloro-4-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione, according to structural diagram I;



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In another aspect, the invention provides a method for the treatment of pain using a compound in accord with structural diagram I, the method comprising administering a pain-ameliorating effective amount of the compound.

In another embodiment, the method comprises administration of a pain-ameliorating
25 effective amount of the compound according to structural diagram I in the form of a pharmaceutical composition comprising a compound according to structural diagram I as an active ingredient together with one or more pharmaceutically-acceptable additives.

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In a further embodiment, the method comprises binding the compound of the invention to the NMDA receptor glycine site of a warm-blooded animal, such as a human being, so as to beneficially inhibit the activity of the NMDA receptor.

Another aspect of the invention is a method for making the compound in accord with structural diagram I.

Yet other aspects of the invention are pharmaceutical compositions which contain the compound in accord with structural diagram I and the use of the compound in accord with structural diagram I for the preparation of medicaments and pharmaceutical compositions.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a compound, 7-chloro-4-hydroxy-2-(2-chloro-4-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione, pharmaceutically-acceptable salts thereof, methods of making the compound and its salts, pharmaceutical compositions containing the compound or salts thereof and methods for using the compound, the salts and the pharmaceutical compositions.

Suitable pharmaceutically-acceptable salts of compounds of the invention include acid addition salts such as methanesulphonate, fumarate, hydrochloride, hydrobromide, citrate, tris(hydroxymethyl)aminomethane, maleate and salts formed with phosphoric and sulphuric acid. In other embodiments, suitable salts are base salts such as an alkali metal salts for example sodium, alkaline earth metal salts for example calcium or magnesium, organic amine salts for example triethylamine, morpholine, *N*-methylpiperidine, *N*-ethylpiperidine, procaine, dibenzylamino, choline, *N,N*-dibenzylethylamine or amino acids such as lysine.

To use the compound of the invention or a pharmaceutically-acceptable salt thereof for the therapeutic treatment, which may include prophylactic treatment, of pain in mammals, which may be humans, the compound can be formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

Suitable pharmaceutical compositions that contain a compound of the invention may be administered in conventional ways, for example by oral, topical, parenteral, buccal, nasal, vaginal or rectal administration or by inhalation. For these purposes a compound of the invention may be formulated by means known in the art into the form of, for example, tablets, capsules, aqueous or oily solutions, suspensions, emulsions, creams, ointments, gels, nasal sprays, suppositories, finely divided powders or aerosols for inhalation, and for parenteral use (including intravenous, intramuscular or infusion) sterile aqueous or oily solutions or

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suspensions or sterile emulsions. A preferred route of administration is orally by tablet or capsule.

In addition to a compound of the present invention a pharmaceutical composition of this invention may also contain one or more other pharmacologically-active agents, or such
5 pharmaceutical composition may be simultaneously or sequentially co-administered with one or more other pharmacologically-active agents.

Pharmaceutical compositions of this invention will normally be administered so that a pain-ameliorating effective daily dose is received by the subject. The daily dose may be given in divided doses as necessary, the precise amount of the compound received and the route of
10 administration depending on the weight, age and sex of the patient being treated and on the particular disease condition being treated according to principles known in the art. A preferred dosage regime is once daily.

A further embodiment of the invention provides a pharmaceutical composition which contains a compound of the invention as defined herein or a pharmaceutically-acceptable salt thereof, in association with a pharmaceutically-acceptable additive such as an excipient or
15 carrier.

A yet further embodiment of the invention provide the use of the compound of the invention, or a pharmaceutically-acceptable salt thereof, in the manufacture of a medicament useful for binding to the NMDA receptor glycine site in a warm-blooded animal such as a
20 human being.

Still another embodiment of the invention provides a method of binding the compound of the invention to the NMDA receptor glycine site of a warm-blooded animal, such as a human being, in need of treatment for pain, which method comprises administering to said animal an effective amount of a compound of structural diagram I or a pharmaceutically-
25 acceptable salt thereof.

Definitions:

Generally in the methods, processes and examples described herein:
concentrations were carried out by rotary evaporation *in vacuo*;
operations were carried out at ambient temperature, that is in the range 18-26 °C and
30 under a nitrogen atmosphere;

column chromatography (by the flash procedure) was performed on Merck Kieselgel _____ silica (Art. 9385) unless otherwise stated;

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yields are given for illustration only and are not necessarily the maximum attainable; the structure of the end-products of the formula I were generally confirmed by NMR and mass spectral techniques, proton magnetic resonance spectra were determined in DMSO-d₆ unless otherwise stated using a Varian Gemini 2000 spectrometer operating at a field strength of 300 MHz; chemical shifts are reported in parts per million downfield from tetramethylsilane as an internal standard (δ scale) and peak multiplicities are shown thus: s, singlet; bs, broad singlet; d, doublet; AB or dd, doublet of doublets; t, triplet, dt, double of triplets, m, multiplet; bm, broad multiplet; fast-atom bombardment (FAB) mass spectral data were obtained using a Platform spectrometer (supplied by Micromass) run in electrospray and, where appropriate, either positive ion data or negative ion data were collected, in this application, (M+H)⁺ is quoted;

intermediates were not generally fully characterized and purity was in general assessed mass spectral (MS) or NMR analysis.

The following abbreviations and definitions when used, have the meanings, as follows:

15	CDCl ₃	is deuterated chloroform;
	CMC	is 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho- <i>p</i> -toluenesulfonate;
	DCM	is dichloromethane;
	DCU	is dicyclohexyl urea;
20	DHC	is 1,3-dicyclohexylcarbodiimide;
	DMAP	is 4-(dimethylamino)pyridine;
	DMF	is N,N-dimethylformamide;
	DMSO	is dimethylsulphoxide;
	m/s	is mass spectroscopy;
25	NMP	is N-methylpyrrolidinone;
	NMR	is nuclear magnetic resonance;
	p.o.	is per os;
	THF	is tetrahydrofuran, and
	t.i.d.	is three times daily.

30 The examples and tests described herein are intended to illustrate but not limit the invention.

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EXAMPLES:**Example 1:**

The compound of the invention, 7-chloro-4-hydroxy-2-(2-chloro-4-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione, may be prepared by the following procedure:

2-Chloro-4-methyl phenylhydrazine hydrochloride.

A suspension of 2-chloro-4-methyl aniline (10.1 mL, 11.63 g, 82.1 mmol) in 64 mL water and 60 mL 12 N HCl was cooled to -5 °C (internal temperature) and stirred with a mechanical stirrer. A solution of sodium nitrite (8.26 g, 119.7 mmol) in 56 mL water was added over 30 minutes. The solution became more clear but some solid remained. The mixture was stirred at -5 °C for 20 minutes and then cooled to -10 °C. A solution of tin(II) chloride dihydrate (53.60 g, 237.6 mmol) in 36 mL 12 N HCl was added dropwise over 30 minutes while maintaining an internal temperature of -5 to -10 °C. The resulting pinkish-brown mixture was stirred at -5 to -10 °C for 2 hours and then filtered cold through a pre-chilled fritted glass funnel. The collected solids were washed with cold 1% ethanol in ether (100 mL) followed by cold ether (500 mL) and air dried for 30 minutes. After drying *in vacuo*, the desired product was obtained as a very pale yellow crystalline solid (7.76 g, 49%). ¹H NMR δ (300 MHz, CDCl₃) δ 10.09 (bs, 2 H), 7.89 (s, 1 H), 7.25 (d, 1 H, *J*_H=1.2 Hz), 7.13 (dd, 1 H, *J*_o=8.4 Hz, *J*_H=1.2 Hz), 7.02 (d, 1 H, *J*_o=8.4 Hz), 2.24 (s, 3 H); MS (CI) *m/z* 157/159.

(*tert*-Butoxy)-N-[(2-chloro-4-methyl phenyl)aminolcarboxamide.

A suspension of 2-chloro-4-methylphenylhydrazine hydrochloride (7.74 g, 40.09 mmol) in 95 mL saturated aqueous NaHCO₃ was stirred for 10 minutes and then treated with solid K₂CO₃ (9.45 g, 68.37 mmol). The resulting fine light yellow suspension was stirred for 10 minutes. A solution of di-*t*-butyldicarbonate (12.97 g, 46.12 mmol) in 195 mL THF was added over 5 minutes and the resulting biphasic mixture was vigorously stirred for 3 hours. The reaction mixture was partitioned and the aqueous layer was extracted with ether (5 x 25 mL). The combined organic layers were washed with distilled water (2 x 75 mL), dried over MgSO₄, and concentrated under reduced pressure. Drying *in vacuo* afforded a light orange oil (14.07 g). The material was purified by flash chromatography on silica gel using 10:90 ether:hexanes as the eluant. The product was obtained as a light yellow oil which solidified upon standing (9.92 g, 96%): ¹H NMR (300 MHz, CDCl₃) δ 8.88 (s, 1 H), 7.15 (s, 1 H), 7.09

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(d, 1 H, $J_{\text{H}}=1.2$ Hz), 6.97 (d, 1 H, $J_{\text{H}}=8.1$ Hz), 6.64 (d, 1 H, $J_{\text{H}}=8.1$ Hz), 2.18 (s, 3 H), 1.41 (s, 9 H); MS (CI) m/z 279/281.

Dimethyl 7-chloro-4-hydroxyquinoline-2,3-dicarboxylate:

A stirred mixture of methyl 2-amino-4-chlorobenzoate (2.50 g, 13.5 mmol) and dimethyl acetylenedicarboxylate (2.05 g, 14.4 mmol) in *tert*-butanol (22 ml) was refluxed for 7 hours under a nitrogen atmosphere. After adding additional dimethyl acetylenedicarboxylate (1.16 g, 8.13 mmol) and refluxing another 2.5 hours, the reaction mixture was allowed to cool to room temperature and potassium *tert*-butoxide (1.56 g, 13.9 mmol) was added in one portion. A precipitate formed and the resulting mixture was refluxed for 1.5 hours. The mixture was cooled to room temperature and filtered to separate the solids, which were washed with *tert*-butanol and ether. The solids were dissolved in water and acidified with 1 N sulfuric acid to form a precipitate. The resulting mixture was extracted with methylene chloride and the combined extracts were washed with brine and water, dried over MgSO_4 , filtered and concentrated to give a green solid. Recrystallization of this material from methanol provided the title compound (1.15 g, 47%) as an off-white solid, mp 232-233 °C; MS (CI): 296 (M+H). Analysis for $\text{C}_{13}\text{H}_{10}\text{ClNO}_5$: Calc'd: C, 52.81; H, 3.41; N, 4.74; Found: C, 52.75; H, 3.47; N, 4.69.

3-Carbomethoxy-7-chloro-4-hydroxyquinoline-2-carboxylic acid:

To a stirred suspension of dimethyl 7-chloro-4-hydroxyquinoline-2,3-dicarboxylate (1.0 g, 3.38 mmol) in water (20 mL) was added an aqueous solution of sodium hydroxide (0.27 g, 6.75 mmol). Upon addition, the suspension dissolved. The reaction mixture was warmed to 60 °C for 1 hour. After this time the reaction was cooled to room temperature and acidified with concentrated hydrochloric acid. The product was then extracted into diethyl ether and ethyl acetate. The organic extracts were dried over MgSO_4 , filtered and concentrated *in vacuo* to provide the title compound as a solid (900 mg). This material was purified by recrystallization employing an ethyl acetate/hexane co-solvent system to provide the title compound (571 mg, 60%) as a white solid mp 296 °C (dec); MS (CI) = 238 (M+H). Analysis for $\text{C}_{12}\text{H}_8\text{NO}_5\text{Cl} \cdot 0.45 \text{CH}_3\text{CO}_2\text{CH}_2\text{CH}_3 \cdot 0.10 \text{H}_2\text{O}$: Calc'd: C, 51.30; H, 3.68; N 4.34, Found: C, 51.28; H, 3.62; N 3.97 ^1H NMR 8.22 (d, $J = 8.7$ Hz, 1H), 7.92 (d, $J = 1.8$ Hz, 1H), 7.28 (dd, $J = 8.7, 1.8$ Hz, 1H), 3.90 (s, 3H).

3-Carbomethoxy-2-pyrrolidinocarbamide-7-chloro-4-hydroxyquinoline:

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To a suspension of 3-carbomethoxy-7-chloro-4-hydroxyquinoline-2-carboxylic acid (2.25 g, 8.0 mmol) in THF (20 mL) at ambient temperature under a N₂ atmosphere was added dicyclohexylcarbodiimide (1.65 g, 8.0 mmol) and pyrrolidine (0.596 g, 8.4 mmol). The reaction was stirred room temperature for 15 hours after which time the by-product urea was removed via filtration. The desired product was purified via flash column chromatography employing 5% methanol in chloroform to provide the title compound (2.52 g, 94.3%) as a tan solid, mp = 215 °C; MS (CI): 335 (M+H). 300 MHz ¹H NMR (DMSO-d₆): 8.12 (d, J = 8.7 Hz, 1H), 7.60 (d, 1H, J = 1.8 Hz), 7.47 (dd, 1H, J = 8.8, 2.0 Hz), 3.69 (s, 3H), 3.40-3.49 (m, 2H), 3.27-3.33 (m, 2H), 1.80-1.96 (m, 4H).

10 7-Chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid:

To a suspension of 3-carbomethoxy-2-pyrrolidinocarbamide-7-chloro-4-hydroxy quinoline (2.52g, 7.5 mmol) in de-ionized water (40 mL) was added dropwise a solution (20 mL) of an aqueous potassium hydroxide (882 mg, 15.75 mmol). Upon complete addition, the reaction was warmed to 60 °C. After 3 hours, the reaction was filtered to remove a small amount of insoluble material. The filtrate was then acidified to pH = 1 which yield a white precipitate. The solid was isolated by vacuum filtration, washed with water, and dried at 30 °C *in vacuo* for 16 hours. This provided the title compound (1.5 g, 64%) as a white solid, mp = 225-8 °C; MS (CI): 321 (M+H). 300 MHz ¹H NMR (DMSO-d₆): 8.28 (d, J = 8.8 Hz, 1H), 7.77 (s, 1H), 7.64 (d, 1H, J = 8.7), 3.52-3.57 (m, 2H), 3.17-3.19 (m, 2H), 1.83-1.98 (m, 4H).

20 N-[(*tert*-butoxy)carbonylamino]7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinolyl)-1-N-(2-chloro-4-methyl phenyl)carboxamide.

To a stirred suspension of 7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)hydroquinoline-3-carboxylic acid (14.57 g, 45.43 mmol) in anhydrous THF (300 mL) under nitrogen was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC, 34.89 g, 82.37 mmol). The white suspension immediately became bright yellow. The (*tert*-butoxy)-N-[(2-chloro-4-methyl phenyl)amino]carboxamide (13.89 g, 54.10 mmol) was added as a solid followed by 50 mL anhydrous THF. The bright yellow reaction mixture was stirred at room temperature for 22 hours. A second portion of CMC (16.77 g, 39.59 mmol) was added to the reaction mixture. After 2.5 hours at room temperature, the reaction was heated at 60 °C for 5.5 hours. After cooling to room temperature, the reaction mixture was filtered and the collected solids were washed with THF. The filtrate and washes were concentrated and dried *in vacuo* to afford a light yellow foam. The material was dissolved in methylene chloride (400

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mL), washed with distilled water (2 x 150 mL), and extracted with 10% NaHCO₃ (2 x 500 mL). The organic layer was dried over Na₂SO₄, concentrated and dried *in vacuo* to afford a light tan foam. The material was purified by flash chromatography on silica gel using a gradient of 95:5 to 85:15 chloroform:methanol as eluant to afford 15.42 g (61%) of the desired product as a white solid. ¹H NMR (300 MHz, DMSO, d₆) δ 13.03 (bs, 1 H), 9.19 (bs, 1 H), 8.25 (d, 1 H, J_o=8.7 Hz), 7.68 (d, 1 H, J_m=1.8 Hz), 7.54 (dd, 1 H, J_o=8.7 Hz, J_m=1.8 Hz), 7.50 (d, 1 H, J_m=1.8 Hz), 7.45 (d, 1 H, J_o=7.8 Hz), 6.81 (d, 1 H, J_o=7.8 Hz), 3.47 (m, 4 H), 2.34 (s, 3 H), 1.90 (m, 4 H), 1.40 (s, 9 H); MS (-CI) m/z 559/561.

10 7-Chloro-4-hydroxy-2-(2-chloro-4-methyl phenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione.

To a stirred suspension of N-[(*tert*-butoxy)carbonylamino][7-chloro-4-oxo-2-(pyrrolidinylcarbonyl)(3-hydroquinoly)]-N-(2-chloro-4-methyl phenyl)carboxamide (21.16 g, 37.82 mmol) in 900 mL anhydrous THF under nitrogen was slowly added methanesulfonic acid (120.0 mL, 184.9 mmol). The resulting dark yellow solution was stirred at room temperature for 18 hours. The solution was poured into 7 L water, stirred 3 hours, and filtered to afford a light yellow solid. The solid was sonicated in methanol, isolated by filtration, and dried *in vacuo* (30 mm) at 40 °C to afford the product as a white solid (12.93 g, 88 %). ¹H NMR (300 MHz, DMSO, d₆) δ 12.90 (bs, 1 H), 12.10 (bs, 1 H), 8.16 (d, 1 H, J_o=8.7 Hz), 8.07 (d, 1 H, J_m=1.8 Hz), 7.47 (dd, 1 H, J_o=8.7 Hz, J_m=1.8 Hz), 7.47 (d, 1 H, J_m=1.2 Hz), 7.42 (d, 1 H, J_o=8.1 Hz), 7.29 (dd, 1 H, J_o=8.1 Hz, J_m=1.2 Hz), 2.38 (s, 3 H); MS (CI) m/z 388/390/392. Calc'd. for C₁₈H₁₁Cl₂N₃O₃: C, 55.69; H, 2.86; N, 10.82. Found C, 55.78; H, 2.89; N, 10.79.

Example 2:

7-chloro-4-hydroxy-2-(2-chloro-4-methyl phenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione choline salt.

25 A suspension of 7-chloro-4-hydroxy-2-(2-chloro-4-methyl phenyl)-1,2,5,10-tetrahydropyridazine[4,5-b]quinoline-1,10-dione (753 mg, 1.94 mmol) in methanol (50 mL) was treated with choline hydroxide (550 mL of a 45% solution in methanol, 1.94 mmol). Most of the solids dissolved immediately and the mixture was sonicated for 10 min to dissolve the rest. The solution was filtered through a 0.2 micron nylon syringe filter. The solution was reduced by rotary evaporation to 1.01 g (>100%) yellow solid. The solid was re-crystallized from refluxing ethanol (25 mL), and the solution was allowed to crystallize slowly and without agitation. After about 2 h, the crystals were collected by vacuum filtration. The

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yellow solid was air dried to give (696 mg, 73 %) of the title compound, which was re-crystallized from refluxing ethanol (20 mL). Solids were allowed to form over 16 h, and were gently scraped from the flask and collected by vacuum filtration and washed with ethanol (2 x 3 mL) to afford 500 mg of the title compound, which upon drying at 100 mTorr at 30 °C for 5 three days provided 480 mg of the title compound (50%). mp 239.5-240.5 °C (decomp.); ¹H NMR (300 MHz, DMSO-d₆) δ 8.12-8.09 (2H, m); 7.34-7.17 (4H, m); 3.86-3.80 (2H, m); 3.39 (2H, t, J = 5.25 Hz); 3.09 (9H, s); 2.35 (3H, s); Calc'd for C₁₈H₁₆N₃O₃Cl₂•1.0 C₅H₁₄NO•0.6 H₂O: C, 55.01; H, 5.06; N, 11.16; Found, C, 55.04, 54.75; H, 4.86, 4.86; N, 11.05, 11.07.

BIOLOGICAL FUNCTION TESTS

10 Test A: Inhibition of binding of [³H]-MDL105,519:

Rat Brain Membranes: The rat brain membranes used in the experiments were obtained from Analytical Biological Services Inc., and were prepared substantially in accordance with the method of B.M. Baron *et al.*, *J. Pharmacol. Exp. Ther.* 250, 162 (1989). Briefly, fresh brain tissue including cerebral cortex and hippocampus from male Sprague 15 Dawley rats was homogenized in 0.32 M sucrose and centrifuged at low speed to separate cellular membranes from other cellular components. The membranes were then washed 3 times using deionized water, followed by treatment with 0.04% Triton X-100. Finally, membranes were washed six times in 50 mM Tris citrate buffer, pH 7.4, and frozen at -80 °C until use.

20 [³H]MDL105,519 (72 Ci/mmol) was purchased from Amersham. Cold MDL105,519 was purchased from Sigma/RBL. Binding assays were performed substantially in accordance with the protocol of B.M. Baron *et al.*, *J. Pharmacol. Exp. Ther.* 279, 62 (1996), as follows. On the day of the experiment, brain membranes were thawed at room temperature and suspended in 50 mM tris acetate buffer, pH 7.4 ("TAB"). Seventy-five micro grams per 25 milliliter protein (by using the BioRad dye) were used for competition binding. The experiments were carried out using 96-well plates. Membranes were incubated with 20 µL of compounds of various concentrations and 1.2 nM [³H]MDL105,519 for 30 minutes at room temperature in a total volume of 250 µL. Non specific binding was determined by using 100 µM of unlabeled MDL105,519. The unlabeled MDL105,519 and compounds were dissolved 30 as 12.5 mM stock solutions in DMSO. Final DMSO concentration in each well was kept below 1%, which concentration was found not to alter the binding results. After incubation, unbound [³H]MDL105,519 was removed by filtration onto GF/B Unifilter plates using a

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Packard harvester. Filters were washed four times with ice cold TAB (total of 1.2 mL buffer). The plates were dried overnight at room temperature and bound radioactivity was measured on a Packard TopCount after the addition of 45 µL per well of the MICROSCINT O. Potency of a compound is expressed as the Ki and results were calculated using Microsoft Excel spreadsheet and GraphPad Prizm software.

Human Brain Membranes: Human brain membranes were obtained from Analytical Biological Services Inc., and assays were performed as described for rat membranes

Test B: Formalin test:

The Formalin test assesses the inhibitory effects of orally administered compounds on formalin-induced nociceptive behaviors in rats (D. Dubuisson, *et al.*, *Pain* 4, 161-174 (1977); H. Wheeler-Aceto *et al.*, *Psychopharmacology* 104, 35-44 (1991); T.J. Codere, *et al.*, *Pain* 54, 43-50 (1993)). In the test two distinctive phases of formalin-induced behaviors are detected. A first phase response, caused by acute nociception to the noxious chemical (formalin) injected into the paw, occurs between 0 to 5 minutes. A quiescent period of between 5 to 15 min post injection follows. After the quiescent period a second phase response, caused by sensitization of the central neurons in the dorsal horn, occurs after 15 minutes and lasts up to 60 minutes. Central sensitization augments a noxious afferent input causing a stronger pain barrage to be transmitted to the brain. Inhibition of the second phase response indicates a spinal mechanism of drug action.

The procedure for the formalin test is as follows: male rats are placed in a plexiglass chamber and observed for 30-45 min. to observe their baseline activity. Multiple groups of animals are pretreated with either vehicle or different doses of a test compound. Animals are dosed 3 hours prior to injection of formalin into a hind paw (under the dorsal skin) with 0.05 mL of sterile 1% formalin. The number of paw flinches (responses) during first phase (0-5 min.) and second phase (20-35 min.) are scored and recorded. Flinch response is calculated as percentage of inhibition compared with the mean score of a saline control group. The ED₅₀ is the dose of compound which produces 50% inhibition of nociceptive response.

% inhibition of nociceptive response

$$= 100 \times \frac{(\text{number of responses in vehicle group} - \text{number of responses in compound group})}{(\text{number of responses in vehicle group})}$$

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Student's t-test was used for statistical analysis to determine the significance of compound effects. Compounds are considered active based on their ability to inhibit flinch responses.

Test C: Neuropathic pain model (Chronic Constriction Injury):

- 5 The Chronic Constriction Injury ("CCI") test models neuropathic pain associated with nerve injuries that can arise directly from trauma and compression, or indirectly from a wide range of diseases such as infection, cancer, metabolic conditions, toxins, nutritional deficiencies, immunological dysfunction, and musculoskeletal changes. In the model a unilateral peripheral hyperalgesia is produced in rats by nerve ligation (G.J. Bennett, *et al.*,
10 *Pain* 33, 87-107 (1988)).

- Procedurally, Sprague-Dawley rats (250-350 g) are anesthetized with sodium pentobarbital and the common sciatic nerve is exposed at the level of the mid thigh by blunt dissection through the biceps femoris. A section of nerve (about 7 mm), proximal to the sciatic trifurcation, is freed of tissue and ligated at four positions with chromic gut suture. The
15 suture is tied with about 1 mm spacing between ligatures. The incision is closed in layers and the animals are allowed to recuperate. Thermal hyperalgesia is measured using a paw-withdrawal test (K. Hargreaves, *et al.*, *Pain* 32, 77-88 (1988)). To perform the test, animals are habituated on an elevated glass floor. A radiant heat source is aimed at the mid-plantar hindpaw (sciatic nerve territory) through the glass floor with a 20 second cut-off used to
20 prevent injury to the skin. The latencies for the withdrawal reflex in both hind paws are recorded.

- Injured paws with ligated nerves show shorter paw withdrawal latencies compared to the uninjured or sham operated paws. Response to test compounds are evaluated at different times after oral administration to determine onset and duration of compound effect. Dose
25 response studies are conducted with multiple groups of CCI rats dosed orally with either vehicle or the test compound three times daily with either vehicle or the test compound for 5 days. Paw withdrawal latencies are measured each day 10 min before and 2 or 3 hr after the first daily dose. Efficacy is calculated as mean percentage decrease of hyperalgesia over 5 dosing days compared to vehicle-treated group. Compound potencies are expressed as the
30 minimum effective dose (MED) in mg/Kg/day that yields a % decrease in hyperalgesia that is statistically significant, where the anti-hyperalgesic effect is determined as follows:
- $$\% \text{ of anti-hyperalgesia} = \frac{(\text{Mean of vehicle group} - \text{Mean of compound group})}{\text{Mean of vehicle group}} \times 100$$

455
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(Mean of vehicle group)

Data analysis was performed by multiple means comparison (Dunnett's test).

Table 1 shows the results from Tests A, B and C for the compound of the invention.

Table 1

Test:	Result:
A: Affinity for NMDA glycine site (Inhibition of ³ H-MDL-105519 binding)	56 nM (rat brain) 50 nM (human brain)
B: Efficacy in formalin pain model	ED ₅₀ ~ 100 mg/Kg
C: CCI model of neuropathic pain, heat hyperalgesia.	65% anti-hyperalgesia at MED < 2 mg/Kg/d

5

In the Formalin pain model the effective dose the compound of the invention that caused a 50% decrease in sensitivity to a painful stimulus was about 100 mg/Kg which dose was comparable to the dose of gabapentin required to achieve a similar result. In the CCI model of neuropathic pain, however, the minimal effective dose of the compound of the invention was less than 2 mg/Kg/day to achieve 65% anti-hyperalgesia. In comparison, about 90 mg/Kg/day of gabapentin is required to achieve about 46% anti-hyperalgesia.

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When administered by intrathecal injection, the compound of the invention inhibited the development of NMDA induced behavior/seizure with an ED₅₀ of 110 nmol.

The compound of the invention was also tested for binding to a panel of more than 80 non-NMDA receptors. The compound showed no significant interaction with any tested receptor other than the NMDA receptor.

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602367 3-092900

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154**CLAIMS:**

1. 7-Chloro-4-hydroxy-2-(2-chloro-4-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-b]quinoline-1,10-dione or pharmaceutically-acceptable salts thereof.
- 5 2. The compound of Claim 1, wherein said pharmaceutically-acceptable salt is a choline salt.
3. A method for treating pain, said method comprising administration of a pain-
- 10 ameliorating effective amount of the compound according to Claim 1.
4. A pharmaceutical composition comprising a compound according to Claim 1 as an active ingredient together with one or more pharmaceutically-acceptable additives.
- 15 5. A method for treating pain, said method comprising administration of a pain-ameliorating effective amount of the compound according to Claim 2.
6. A pharmaceutical composition comprising a compound according to Claim 2 as an active ingredient together with one or more pharmaceutically-acceptable additives.

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PROCESO OFICIAL 5.999
Luis J. Rodríguez
Instituto Oficial
San. De. GTR del Min. de Justicia

151
155**ABSTRACT****TITLE: COMPOUND AND METHOD FOR THE TREATMENT OF PAIN**

- 5 A compound, 7-chloro-4-hydroxy-2-(2-chloro-4-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dione, pharmaceutically-acceptable salts thereof, a method for treating pain comprising administration of a pain-ameliorating effective amount of the compound and pharmaceutical compositions containing the compound are disclosed.

602367 3-092900

AstraZeneca
00.097190
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TRADUCCION OFICIAL No. 417

**LEGALIZACION DE UN DOCUMENTO PRESENTADO SIMULTANEAMENTE EN
INGLES Y ESPAÑOL. TRASPASO DE LA INVENCION DENOMINADA
COMPUESTO Y METODO PARA EL TRATAMIENTO DEL DOLOR A FAVOR DE
AstraZeneca AB.**

**INVENCION: COMPUESTO Y METODO PARA EL TRATAMIENTO DEL
DOLOR**

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Firmado: Megan Murphy
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USA


MARIA SALAZAR DE ARSÉN
TRADUCTORA OFICIAL
DEL GOBIERNO DE PUERTO RICO

2
X6
157

Firmado: Rebecca Ann Urbanek
Rebecca Ann Urbanek
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USA

Firmado: Wenhua Xiao
Wenhua Xiao
AstraZeneca
1800 Concord Pike
P.O. Box 15437
Wilmington, DE 19850-5437 Ciudadano chino
USA

**CERTIFICADO NOTARIAL (PERSONA NATURAL) SIMULTANEAMENTE EN
INGLES Y ESPAÑOL.**

Fecha: Diciembre 06, 2000

Firmado: Frances L. Toutkaldjian

Sello: FRANCES L. TOUTKALDJIAN
NOTARIO PUBLICO
ESTADO DE DELAWARE
Mi comisión expira Octubre 26, 2002

**CERTIFICADO NOTARIAL (PERSONA NATURAL) SIMULTANEAMENTE EN
INGLES Y ESPAÑOL.**

Fecha: Diciembre 08, 2000


LAYLA SALAMASCA DE GARCIA
TRADUCTORA OFICIAL
DES. MINISTRO DE JUSTICIA 1200

161
158

Firmado: **Frances L. Toutkaldjian**

Sello: **FRANCES L. TOUTKALDJIAN**
NOTARIO PUBLICO
ESTADO DE DELAWARE
Mi comisión expira Octubre 28, 2002


KATIA BELANDIER DE ARDEN
TRADUCTORA OFICIAL
EST. MINISTECIA 3284

T62
159

ESTADO DE DELAWARE

OFICINA DEL SECRETARIO DE ESTADO

YO, **EDWARD J. FREEL**, SECRETARIO DE ESTADO DEL ESTADO DE DELAWARE, POR EL PRESENTE CERTIFICO QUE **FRANCES L. TOUTKALDJIAN**, CUYA FIRMA AUTENTICA APARECE EN EL DOCUMENTO ANTERIOR ERA EN EL MOMENTO DE HACERLO Y ES AHORA NOTARIO PUBLICO DEL ESTADO DE DELAWARE EN Y PARA EL CONDADO DE NEW CASTLE DEBIDAMENTE DESIGNADO, COMISIONADO Y CALIFICADO, Y QUE DEBE DARSE PLENA FE Y CREDITO A TODOS SUS ACTOS OFICIALES COMO TAL.

EN TESTIMONIO DE LO CUAL, HE FIRMADO EL PRESENTE DOCUMENTO Y COLOCADO EL SELLO OFICIAL EN DOVER EL 19 DE DICIEMBRE, 2000, A LAS 11:14 A.M.

Firmado : **Edward J. Freel, Secretario de Estado**

Autenticación : **0863807**

Fecha: **12-19-00**

Sello en papel dorado del Secretario de Estado del Estado de Delaware.


KATIA SALASARCA DE ABRAH
TRADUCTORA OFICIAL
REG. NOTARIAL 3288

167
160

**CONSULADO DE COLOMBIA
WASHINGTON, D.C.**

No. 3358

CERTIFICA la firma y funciones del señor **Edward J. Freel**, como **Secretario de Estado de Delaware**.

Fecha: **Diciembre 29, 2000.**

Firmado: **Lizeth Martínez Peña**
 Cónsul

Hay sello del Consulado de Colombia en Washington, D.C.

MINISTERIO DE RELACIONES EXTERIORES

CERTIFICA la firma y funciones del funcionario consular **LIZETH MARTINEZ**, bajo el número **214839**, de **Enero 30, 2001**.

Firmado: **(Firma ilegible)**
 Jefe de Legalizaciones
 Santafé de Bogotá, D.C.


LIDIA VALANDEZ DE ASATO
TRADUCTORA OFICIAL
R.E. MINISTERIO 2200

MINISTERIO DE RELACIONES
EXTERIORES

408341
Julio Robles
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2001 FEB -9 9 08

[Signature]
JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

MINISTERIO DE RELACIONES
EXTERIORES

214839,
Josef Martinez
CUNA FICHA APARECE EN EL
DOCUMENTO DESEMPENA
LAS FUNCIONES INDICADAS

NO SE ASUME
RESPONSABILIDAD DEL TIEMPO

2001 ENE 30 P 2: 39,
[Signature]
JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

State of Delaware
Office of the Secretary of State

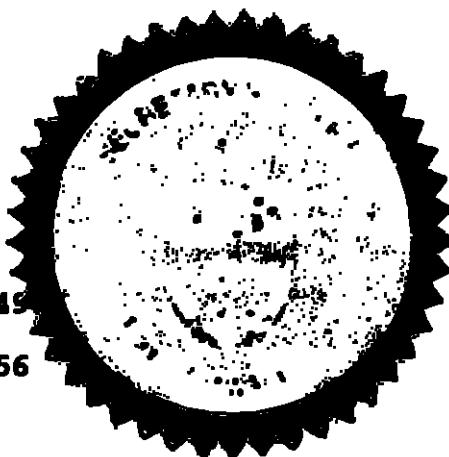
PAGE 1

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162

I, EDWARD J. FREEL, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THAT FRANCES L. TOUTKALDJIAN, WHOSE GENUINE SIGNATURE APPEARS ON THE ABOVE AND FOREGOING DOCUMENT WAS AT THE TIME OF MAKING AND NOW IS A NOTARY PUBLIC FOR THE STATE OF DELAWARE IN AND FOR NEW CASTLE COUNTY DULY APPOINTED, COMMISSIONED AND QUALIFIED, AND THAT FULL FAITH AND CREDIT AND OUGHT TO BE, GIVEN TO ALL OF HIS OFFICIAL ACTS AS SUCH.

IN TESTIMONY WHEREOF, I HAVE HEREUNTO SET MY HAND AND AFFIXED MY OFFICIAL SEAL OF OFFICE AT DOVER ON THE NINETEENTH DAY OF DECEMBER, A.D. 2000, AT 11:14 A.M.



200087949

001635556

Edward J. Freel, Secretary of State

AUTHENTICATION: 0863807

DATE: 12-19-00

100178
COL
F6
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ASSIGNMENT

The undersigned,

Please see enclosure!

domiciled in

Please see enclosure!

hereby state under oath to be the sole and true inventor of

COMPOUND AND METHOD FOR THE TREATMENT OF PAIN

We state as well that we assign, without reservations or limitation, in favor of

AstraZeneca AB

a corporation organized and existing under the laws of Sweden

domiciled in

S-151 85 Södertälje, Sweden

all rights inherent to said invention, and that the above mentioned corporation hereby is enabled to apply, in its name, for the corresponding patent in the Republic of Colombia.

TRASPASO

abajo firmado

Por favor ver adjunto!

domiciliado en

Por favor ver adjunto!

declaramos bajo juramento ser únicos y verdaderos inventores de.

COMPUESTO Y METODO PARA EL TRATAMIENTO DEL DOLOR

Declaramos asimismo que cedemos y transferimos sin reserva ni limitación, a favor de la sociedad

AstraZeneca AB

organizada y existente de conformidad con la leyes de Suecia

domiciliada en

S-151 85 Södertälje, Suecia

Todos los derechos inherentes a dicha invención y que la citada sociedad queda facultada para solicitar en su nombre la patente correspondiente en la República de Colombia.

DEC
9-1
1985

CERTIFICADO NOTARIAL (INDIVIDUO)

A los 6 días del mes de Diciembre
2000, comparecieron personalmente ante mí,
Notario Publico,
Dean Gordon Brown
Megan Murphy
Rebecca Ann Urbanek

a quienes doy fé de conocer y me consta que
las personas descritas y firmantes
del documento que precede, declarado ante mí que
otorga el mismo para los fines y propósitos que
en él se expresan.

NOTARY PUBLIC (Notario Público)

NOTARIAL CERTIFICATE (INDIVIDUAL)

On this 6th day of December
2000 personally appeared before me, a Notary Public,

Dean Gordon Brown
Megan Murphy
Rebecca Ann Urbanek

to me known and known to me to be the person
described in and who executed the foregoing document,
and they duly acknowledged to me that
they executed the same for the uses and purposes
therein expressed.

Frances L. Toutkaldjian

FRANCES L. TOUTKALDJIAN
NOTARY PUBLIC
STATE OF DELAWARE
My Commission Expires Oct. 28, 2002

CERTIFICADO NOTARIAL (INDIVIDUO)

A los 8 días del mes de Diciembre
2000, compareció personalmente ante mí,
Notario Publico,
Wenhua Xiao

a quien doy fé de conocer y me consta que
la persona descrita y firmante
del documento que precede, declarado ante mí que
otorga el mismo para los fines y propósitos que
en él se expresan.

NOTARIAL CERTIFICATE (INDIVIDUAL)

On this 8th day of December
2000 personally appeared before me, a Notary Public,

Wenhua Xiao

to me known and known to me to be the person
described in and who executed the foregoing document,
and she duly acknowledged to me that
she executed the same for the uses and purposes
therein expressed.

Frances L. Toutkaldjian

FRANCES L. TOUTKALDJIAN
NOTARY PUBLIC
STATE OF DELAWARE
My Commission Expires Oct. 28, 2002

1001106
166
164

Thomas Michael Bare
1057 Windy Knoll Road
West Chester
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An American citizen

Dean Gordon Brown
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1800 Concord Pike
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An American citizen

Megan Murphy
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USA

An American citizen

Rebecca Ann Urbanek
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Wilmington DE 19850-5437
USA

An American citizen

Wenhua Xiao
AstraZeneca
1800 Concord Pike
P.O. Box 15437
Wilmington DE 19850-5437
USA

A Chinese citizen



AstraZeneca
00.097.180
165

TRADUCCION OFICIAL No. 418

**LEGALIZACION DE UN DOCUMENTO PRESENTADO SIMULTANEAMENTE EN
INGLES Y ESPAÑOL. TRASPASO DE LA INVENCION DENOMINADA
COMPUESTO Y METODO PARA EL TRATAMIENTO DEL DOLOR A FAVOR DE
AstraZeneca AB.**

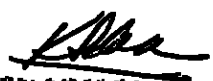
**INVENCION: COMPUESTO Y METODO PARA EL TRATAMIENTO DEL
DOLOR**

Firmado: Thomas Michael Bare
Thomas Michael Bare
West Chester
Pennsylvania 19382 Ciudadano americano
U.S.A.

**CERTIFICADO NOTARIAL (PERSONA NATURAL) SIMULTANEAMENTE EN
INGLES Y ESPAÑOL.**

Fecha: Diciembre 20, 2000
Firmado: Frances L. Toutkaldjian
Sello: FRANCES L. TOUTKALDJIAN
NOTARIO PUBLICO
ESTADO DE DELAWARE
Mi comisión expira Octubre 26, 2002

Sello en altorrelieve del Notario Público de Delaware, Frances L. Toutkaldjian


MARIA SALOMÓN DE ARREDONDO
TRADUCTORA OFICIAL
ED. MINISTERIO 3284

8
166

ESTADO DE DELAWARE

OFICINA DEL SECRETARIO DE ESTADO

YO, **EDWARD J. FREEL**, SECRETARIO DE ESTADO DEL ESTADO DE DELAWARE, POR EL PRESENTE CERTIFICO QUE **FRANCES L. TOUTKALDJIAN**, CUYA FIRMA AUTENTICA APARECE EN EL DOCUMENTO ANTERIOR ERA EN EL MOMENTO DE HACERLO Y ES AHORA NOTARIO PUBLICO DEL ESTADO DE DELAWARE EN Y PARA EL CONDADO DE NEW CASTLE DEBIDAMENTE DESIGNADO, COMISIONADO Y CALIFICADO, Y QUE DEBE DARSE PLENA FE Y CREDITO A TODOS SUS ACTOS OFICIALES COMO TAL.

EN TESTIMONIO DE LO CUAL, HE FIRMADO EL PRESENTE DOCUMENTO Y COLOCADO EL SELLO OFICIAL EN DOVER EL 29 DE DICIEMBRE, 2000, A LAS 6:09 P.M.

Firmado : **Edward J. Freel, Secretario de Estado**

Autenticación : **0886825**
Fecha: **12-29-00**

Sello en papel dorado del Secretario de Estado del Estado de Delaware.


LATIA SALASACA DE AVILA
TRADUCTORA OFICIAL
RES. MINISTROS 1200

169
167

**CONSULADO DE COLOMBIA
WASHINGTON, D.C.**

No. 0030

CERTIFICA la firma y funciones del señor **Edward J. Freel**, como **Secretario de Estado de Delaware**.

Fecha: Enero 05, 2001.

Firmado: **Jaqueline Espitia Arias**

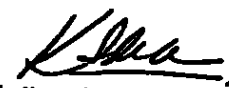
Vicecónsul

Hay sello del Consulado de Colombia en Washington, D.C.

MINISTERIO DE RELACIONES EXTERIORES

CERTIFICA la firma y funciones del funcionario consular **Jaqueline Espitia**, bajo el número **214840**, de Enero 30, 2001.

Firmado: (Firma ilegible)
Jefe de Legalizaciones
Santafé de Bogotá, D.C.


MARIA SALAZAR DE ARREB
TRADUCTORA OFICIAL
SEN. MIN. JUSTICIA 3288

MINISTERIO DE RELACIONES
EXTERIORES

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Notu. Bolívar, cu
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES DE LAS

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

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JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.



CONSULADO DE COLOMBIA
WASHINGTON, D.C.

168

No. 0030

EL CONSULADO DE COLOMBIA

CERTIFICA:

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5:30



Que es auténtica la firma de EDWARD J. FREEL
quien el día 29 DE DICIEMBRE DEL 2000
desempeñaba las funciones de SECRETARIO DE ESTADO DEL ESTADO DELAWARE
la cual aparece al pie de los documentos anexos relacionados con CERTIFICADO.

Dado en Washington, D.C. a los JAN 05 2001 días del mes de
_____ del año dos mil uno (2001)

Pagado Impuesto de Timbre

Pagado Derecho Consular

Total

Jaqueline Espitia Arias
JAQUELINE ESPITIA ARIAS
Vicecónsul

(El Consulado no asume ninguna
responsabilidad por el contenido de los
documentos anexos)

MINISTERIO DE RELACIONES
EXTERIORES

214840.
Jayme Luis Esquivel
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS.

NO SE ASUME
RESPONSABILIDAD DEL DOCUMENTO

2001 ENE 30 1973
[Signature]
JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

JOSE LLORENDA CAMACHO & CO
Ashe Zenece
00.097.100

State of Delaware

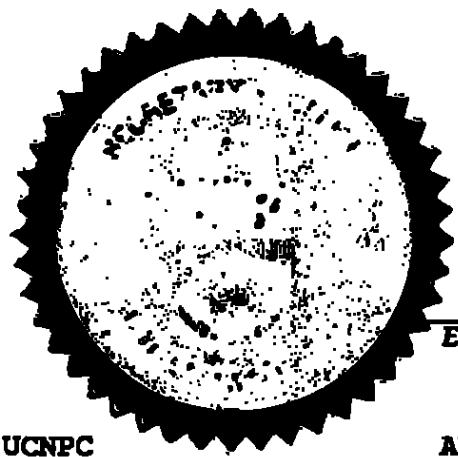
Office of the Secretary of State

PAGE 1

169

I, EDWARD J. FREEL, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THAT FRANCES L. TOUTKALDJIAN, WHOSE GENUINE SIGNATURE APPEARS ON THE ABOVE AND FOREGOING DOCUMENT WAS AT THE TIME OF MAKING AND NOW IS A NOTARY PUBLIC FOR THE STATE OF DELAWARE IN AND FOR NEW CASTLE COUNTY DULY APPOINTED, COMMISSIONED AND QUALIFIED, AND THAT FULL FAITH AND CREDIT ARE, AND OUGHT TO BE, GIVEN TO ALL OF HIS OFFICIAL ACTS AS SUCH.

IN TESTIMONY WHEREOF, I HAVE HEREUNTO SET MY HAND AND AFFIXED MY OFFICIAL SEAL OF OFFICE AT DOVER ON THE TWENTY-NINTH DAY OF DECEMBER, A.D. 2000, AT 6:09 P.M.



Edward J. Freel

Edward J. Freel, Secretary of State

200090852UCNPC

AUTHENTICATION: 0886825

001658683

DATE: 12-29-00

ASSIGNMENT

The undersigned,

Please see enclosure!

domiciled in

Please see enclosure!

hereby state under oath to be the sole and true inventor
of

COMPOUND AND METHOD FOR THE TREATMENT OF PAIN

We state as well that we assign, without reservations or
limitation, in favor of

AstraZeneca AB

a corporation organized and existing under the laws of
Sweden

domiciled in

S-151 85 Södertälje, Sweden

all rights inherent to said invention, and that the above
mentioned corporation hereby is enabled to apply, in its
name, for the corresponding patent in the Republic of
Colombia.

TRASPASO

abajo firmado

Por favor ver adjunto!

domiciliado en

Por favor ver adjunto!

declaramos bajo juramento ser único y verda-
dero inventores de.

COMPUESTO Y METODO PARA EL TRATAMIENTO DEL DOLOR

Declaramos asimismo que cedemos y
transferimos sin reserva ni limitación, a favor de la
sociedad

AstraZeneca AB

organizada y existente de conformidad con la leyes de
Suecia

domiciliada en

S-151 85 Södertälje, Suecia

Todos los derechos inherentes a dicha invención y que la
citada sociedad queda facultada para solicitar en su
nombre la patente correspondiente en la República de
Colombia.

10017 B

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CERTIFICADO NOTARIAL (INDIVIDUO)

A los 20 días del mes de Diciembre
2000, comparecí personalmente ante mi,
Notario Publico,
THOMAS MICHAEL BARE

a quien doy fé de conocer y me consta que
la persona descrita y firmante
del documento que precede, declarado ante mí que
otorga el mismo para los fines y propósitos que
en él se expresan.

NOTARY PUBLIC (Notario Público)

NOTARIAL CERTIFICATE (INDIVIDUAL)

On this 20 day of December
2000 personally appeared before me, a Notary Public,
Thomas Michael Bare

to me known and known to me to be the person
described in and who executed the foregoing document,
and he duly acknowledged to me that
he executed the same for the uses and purposes
therein expressed.

Frances L. Tolitkaldjian
FRANCES L. TOLITKALDJIAN
NOTARY PUBLIC
STATE OF DELAWARE
My Commission Expires Oct. 28, 2002

CERTIFICADO NOTARIAL (INDIVIDUO)

A los días del mes de
2000, comparecí personalmente ante mi,
Notario Publico,

a quien doy fé de conocer y me consta que
la persona descrita y firmante
del documento que precede, declarado ante mí que
otorga el mismo para los fines y propósitos que
en él se expresan.

NOTARIAL CERTIFICATE (INDIVIDUAL)

On this day of
2000 personally appeared before me, a Notary Public,

to me known and known to me to be the person
described in and who executed the foregoing document,
and duly acknowledged to me that
executed the same for the uses and purposes
therein expressed.

172
171

~~Thomas Michael Bare~~

Thomas Michael Bare
1057 Windy Knoll Road
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An American citizen

Dean Gordon Brown
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An American citizen

Megan Murphy
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USA

An American citizen

Rebecca Ann Urbanek
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USA

An American citizen

Wenhua Xiao
AstraZeneca
1800 Concord Pike
P.O. Box 15437
Wilmington DE 19850-5437
USA

A Chinese citizen

ORIGINAL

ORIGINAL