

Clarke, Modet & Co.

COLOMBIA
E-MAIL: clarkemodet@clarkemodet.com.co
SANTAFÉ DE BOGOTÁ, A.A. 02397

152
153

SUPERINTENDENCIA Y COMERCIO
Radicación: 00097184 00000001 Folios: 131
Fecha (REC): 2001-03-28 16:36:35
Trámite: C02 PATENTES D I REGISTRO/ 329 COMPLETAR
Dependencia: 2200 DIVISION DE NUEVAS CREACIONES

SEÑORES
DIVISION DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
E. S. D.

COD:01

RE.: EXPEDIENTE N°. 00097184'
FECHA DE LA SOLICITUD: DIC. 21, 2000
SOLICITANTE: SUGEN, INC.
TITULO: 7-AZA-INDOLIN.2.ONAS SUSTITUIDAS EN 4 Y SU USO
COMO INHIBIDORES DE PROTEINA QUINASA

ASUNTO: PRESENTACIÓN PRIORIDAD

DILIA RODRIGUEZ D'ALEMAN, en mi condición de apoderada de la sociedad
solicitante, en esta oportunidad por el presente escrito me permito presentar:

- Copia certificada de la prioridad Estadounidense reivindicada N°. 60/171.288 /
- Traducción al Español de la mencionada prioridad

Esta prioridad está siendo presentada dentro del término de 3 meses que exige el Convenio de Paris, toda vez que la solicitud fué radicada el 21 de Diciembre de 2.000.

Ruego a usted continuar con el presente trámite.

Señor Jefe,



DILIA RODRIGUEZ D'ALEMAN
LCD/srr
P- 396

TRADUCCIÓN**ESTADOS UNIDOS DE AMÉRICA****A quien corresponda:****DEPARTAMENTO DE COMERCIO - OFICINA DE PATENTES Y MARCAS****01 de febrero del 2001**

CERTIFICO QUE ADJUNTA A LA PRESENTE SE ENCUENTRA UNA COPIA FIEL DE LOS REGISTROS EN LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE ABAJO IDENTIFICADA QUE REUNIAN LOS REQUISITOS PARA LA ASIGNACIÓN DE UNA FECHA DE PRESENTACIÓN EN VIRTUD DE 35 USC 111.

SOLICITUD NUMERO: 60/171.288**FECHA DE DEPÓSITO: 21 de diciembre de 1999****.En nombre del COMISARIO DE PATENTES Y MARCAS.****(Firmado) W. Montgomery Certificaciones.**

(La presente certificación se encuentra unida en forma inviolable a sus adjuntos y lleva una oblea dorada que dice): OFICINA DE PATENTES Y MARCAS - ESTADOS UNIDOS DE NORTEAMÉRICA.

Carátula para Presentación Provisional de Patente

Esta solicitud es para presentación de una SOLICITUD DE PATENTE PROVISIONAL bajo el CRF 37, art 1.53 (c)

Nro. de expediente: 7683-156-888**INVENTOR/ES SOLICITANTE/S**

Apellido, Nombre e Inicial	Residencia (Ciudad y Estado o País Extranjero)
Liang, Congxin	Sunnyvale, CA

25
157
154

Sun, Il	Foster City, CA
Wei, Chung C	Foster City, CA
Tang, Peng C.	Moraga, CA
McMahon, Gerald	Kenwood, CA
Hurth, Klas P.	San Francisco, CA
Cui, Jingrong	Foster City, CA

**Título de la Invención : 7-AZA-INDOLIN-2-ONAS SUSTITUÍDAS EN 4 Y SU
USO COMO INHIBIDORES DE PROTEÍNA QUINASA**

Dirección Postal: Pennie & Edmonds LLP

1667 K Street, N.W.

Washington, D.C. 20006

(202) 496-4400

Rogamos dirigir toda la correspondencia relacionada con esta Solicitud a:

Adjuntos:

Memoria descriptiva: 88 páginas

Dibujos: páginas

Forma de pago:

**Se autoriza al Comisario a cobrar los aranceles de presentación a través de la
cuenta número : 16-1150. Arancel : \$150,00**

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

Atentamente, firmado Laura A. Coruzzi

Nro. de registro: 30.742

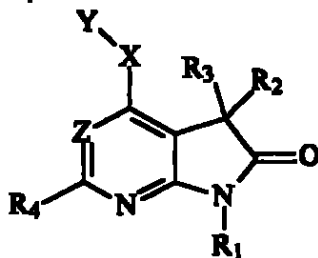
Fecha: 21 de diciembre de 1999

Por: Max Bachrach, nro. de registro: P-45.479, Pennie & Edmonds LLP

4
158
155

REIVINDICACIONES

1. Un compuesto que tiene la Fórmula 1



o una sal aceptable para uso farmacéutico, solvato, clatrato, o prodroga del mismo, donde:

R₁ es H o metilo;

cada uno de R₂ y R₃ es independientemente H, halógeno, (C₁-C₃)alquilo, o (C₁-C₃)alcoxi; o R₂ y R₃ tomados juntos de un metilindeno opcionalmente sustituido o un anillo de 3 a 7 miembros que comprende opcionalmente 0-3 heteroátomos;

R₄ es H, metilo, trifluorometilo, (C₁-C₄)alquilo, alcoxi, amido, amino, o ariilo opcionalmente sustituido;

X es un enlace químico, etínilo, -O-, -S-, -S(O)-, -S(O₂)-, -NR₅C(O)-, o -NR₅-, donde R₅ es H, metilo, o metileno sustituido;

Y es un anillo mono o bicíclico de 5 a 10 miembros, saturado, insaturado o aromático que comprende 0-3 heteroátomos y opcionalmente sustituido; y

Z es N o CR₆, donde R₆ es H, halógeno, nitro, ciano, alcoxilo, sulfonamida, amino, o amida.

2. El compuesto de la reivindicación 1 en el cual X es un enlace químico, -O-, -S-, o -NR₅-.

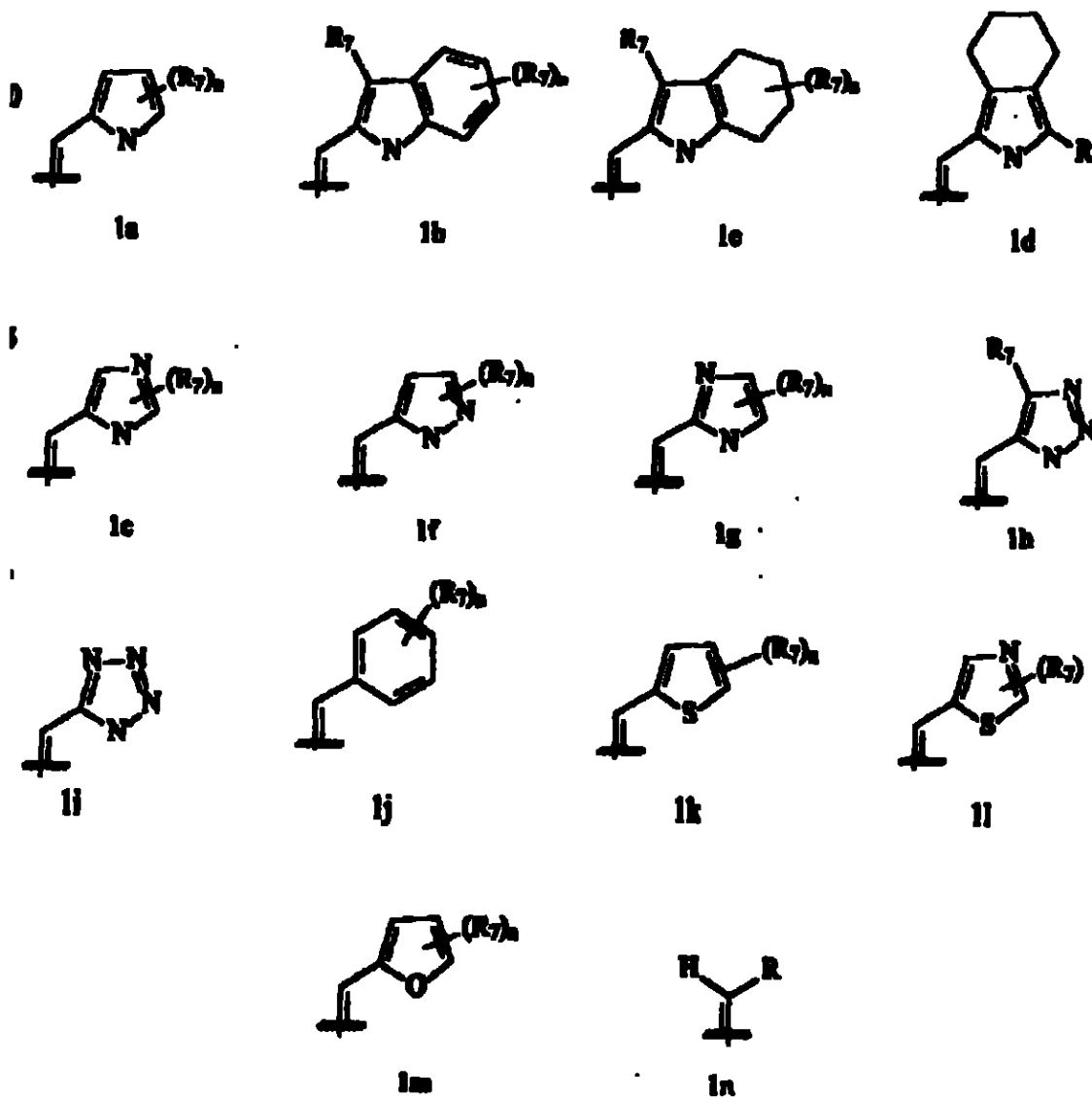
3. El compuesto de la reivindicación 1 en el cual Y se selecciona entre el grupo que consiste en fenilo, indolilo, indolinilo, 1H-indazolilo, 2,3-

5
159
156

dihidro-1H-indazolilo, 1H-bencimidazolilo, 2,3-dihidro-1H-bencimidazolilo, benzotriazolilo, piridilo, pirimidilo, 4-substituted piperazin-1-ilo, morfolino, piperidinilo, pirrolidin-1-ilo, furanilo, tiofenilo, pirrolilo, pirazolilo, imidazolilo, piridopirrolilo, pirdazopirrolilo, pirimidopirrolilo, pirazopirrolilo, piridofuranilo, y derivados del mismo.

4. El compuesto de la reivindicación 1 en el cual Z es N o CH.
5. El compuesto de la reivindicación 1 en el cual R₂ y R₃ son ambos H, halógeno, o metilo.
6. El compuesto de la reivindicación 1 en el cual R₂ y R₃ se toman juntos para formar un anillo seleccionado entre el grupo que consiste en 1,3-dioxolano, 1,3-dioxano, ciclopropilo, ciclobutilo, ciclopentilo, y ciclohexilo.
7. El compuesto de la reivindicación 1 en el cual R₂ y R₃ se toman juntos para formar un metilindeno opcionalmente sustituido seleccionado entre aquellos de las fórmulas 1a-1n:

6
160
159



donde:

n es un entero de 0-3;

cada R_7 es independientemente H, alquilo, ácido carboxílico, amina, halógeno, nitro, ciano, X_1 , X_2 -(C-C₄)alquil- R_8 , X_2 -(C₁-C₄)alquenil- R_8 , o X_2 -(C₁-C₄)alquinil- R_8 ;

7
161
133

X_1 es $-C(O)NR_g$, $-NR_gC(O)-$, $-C(O)O-$, $C(O)R_{11}$, $-OC(O)-$, $-O-$, $-NR_g$, $-S-$, $-S(O_2)$, o $-S(O_2)NR_g$;

X_2 es un enlace químico, $-C(O)NR_g$, $-NR_gC(O)-$, $-C(O)O-$, $C(O)R_{11}$, $-OC(O)-$, $-O-$, $-NR_g$, $-S-$, $-S(O_2)$, o $-S(O_2)NR_g$;

R_g se selecciona entre el grupo que consiste en hidrógeno, dialquilamino, carboxilo, hidroxilo, alcoxi, sulfonamida, urea, carbamato, diol, alquilsulfonilo, y R_{10} ;

R_g es H o (C_1-C_3) alquilo;

R_{10} es un heterociclo saturado, insaturado, o aromático de 5 o 6 miembros opcionalmente sustituido que comprende de 1 a 4 heteroátomos; y

R_{11} es un anillo heterocíclico saturado de 5 a 6 miembros opcionalmente sustituido.

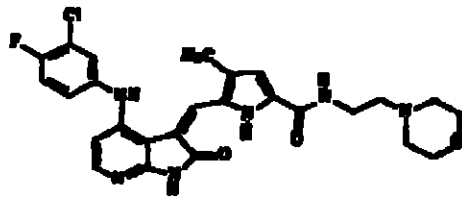
8. El compuesto de la reivindicación 7 en el cual R_7 es $X_2-(C_1-C_4)$ alquil- R_g , $X_2-(C_1-C_4)$ alquenil- R_g , o $X_2-(C_1-C_4)$ alquini- R_g , y R_g se selecciona entre el grupo que consiste en alquilsulfonilo, alcoxi, carboxilo, morfolino, 1-alquil-piperazin-4-ilo, pirrolidinilo, piperidinilo, piridilo, imidazol, triazol, tetrazol, y tiazol.

9. El compuesto de la reivindicación 1 donde R_4 es H, metilo, o trifluorometilo.

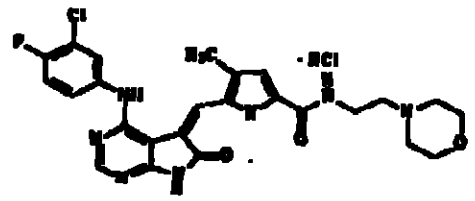
10. El compuesto de la reivindicación 1 donde si Z es CH, R_1 es CH_3 o R_3 y R_2 no forma un metilindeno opcionalmente sustituido.

11. Un compuesto que se selecciona del grupo que consiste en:

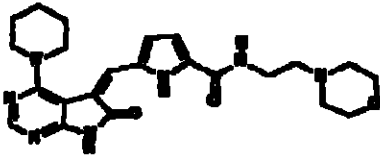
162
159



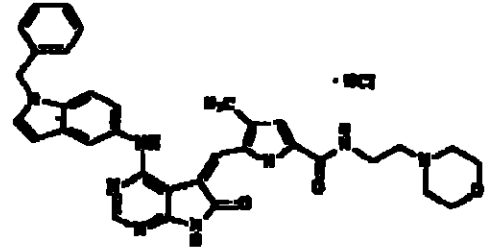
3



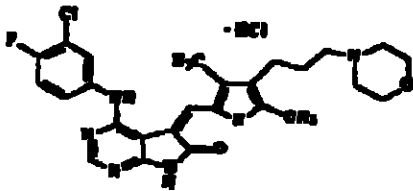
4



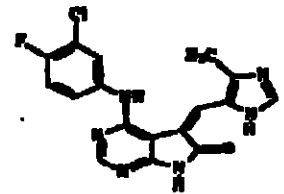
5



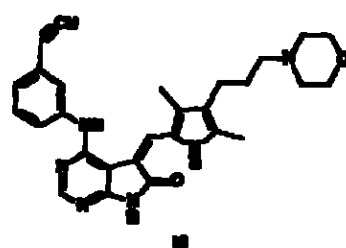
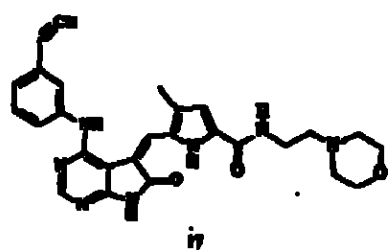
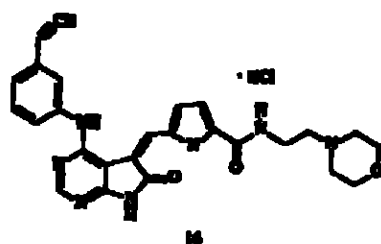
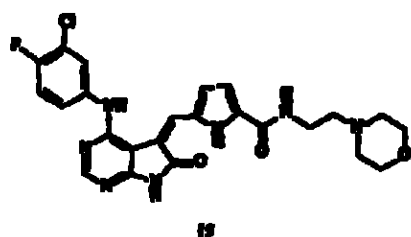
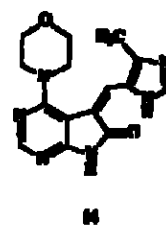
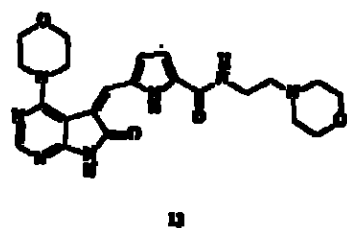
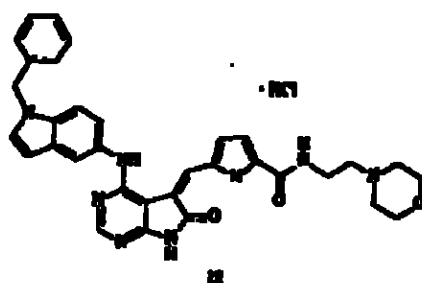
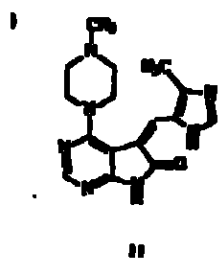
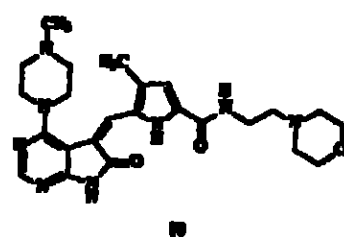
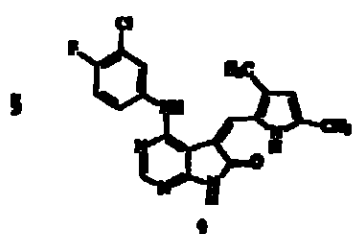
6



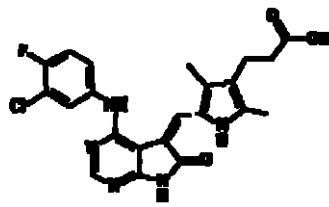
7



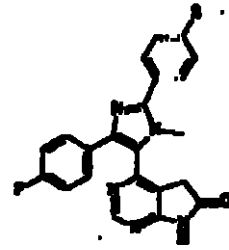
8

A
163
160

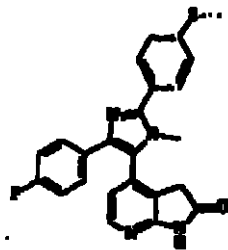
~~164~~
161



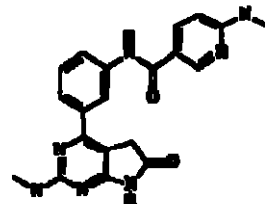
19



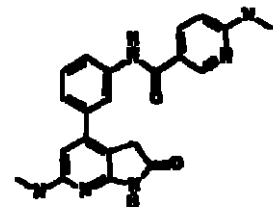
20



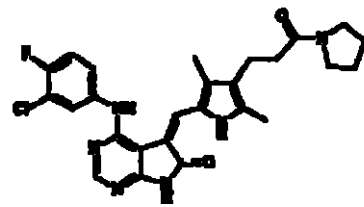
21



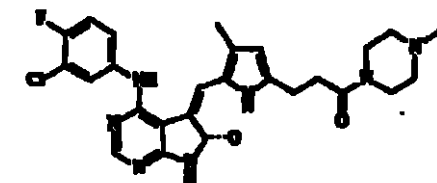
22



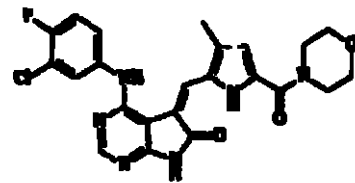
23



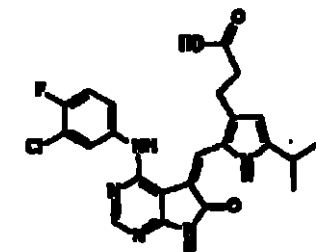
24



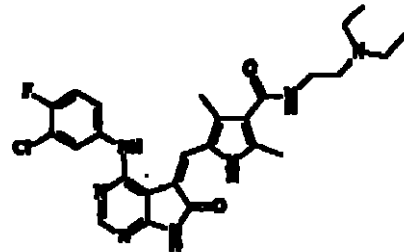
25



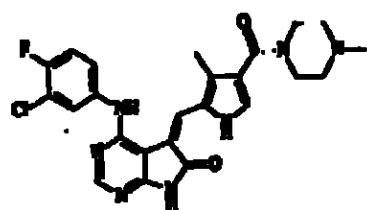
26



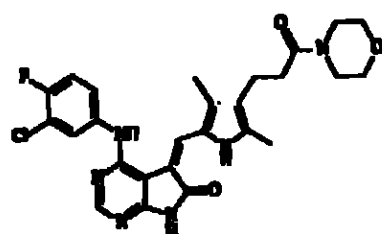
27



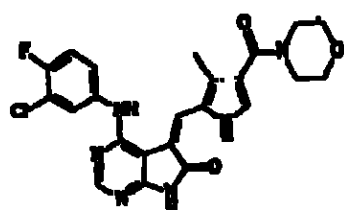
28

H
165
162

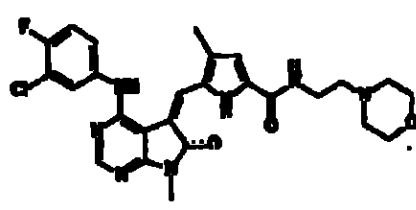
27



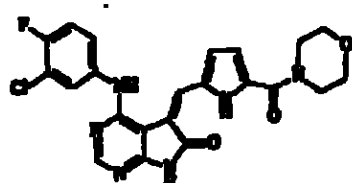
28



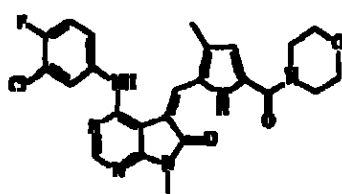
29



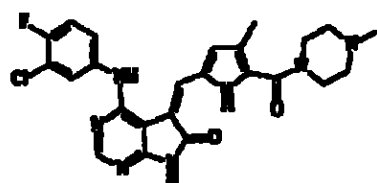
30



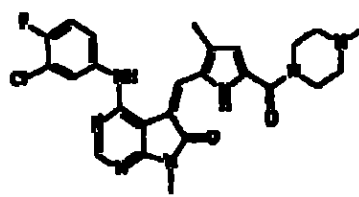
31



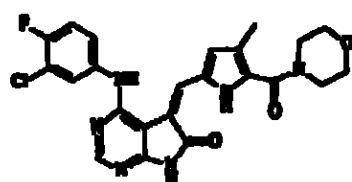
32



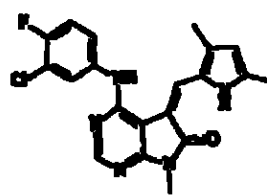
33



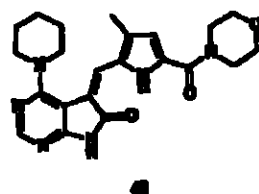
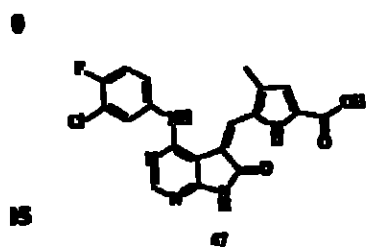
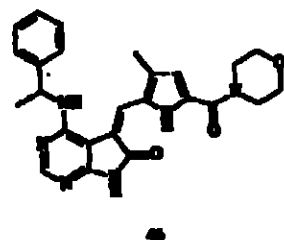
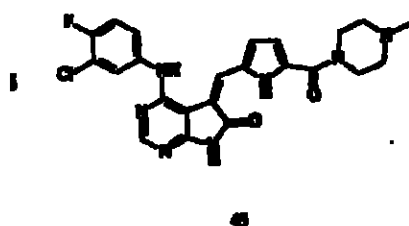
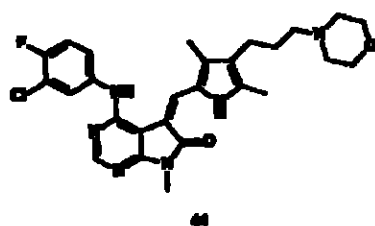
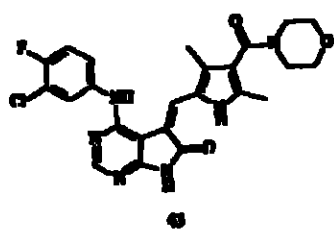
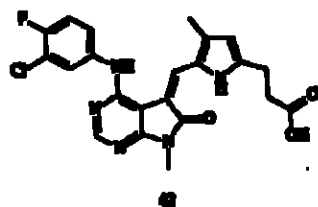
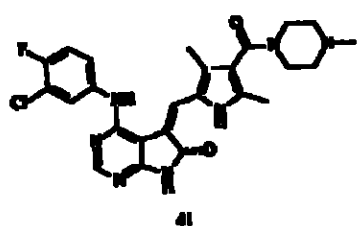
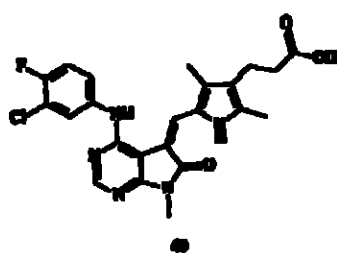
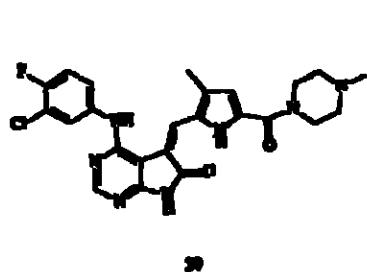
34



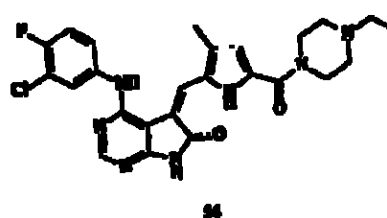
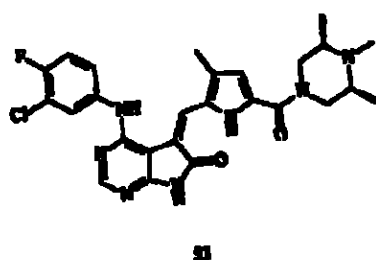
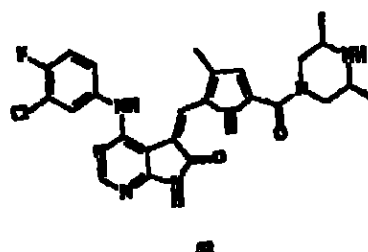
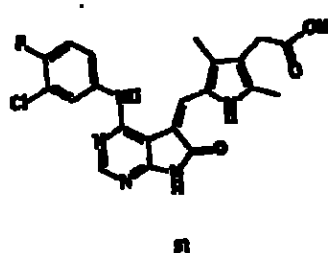
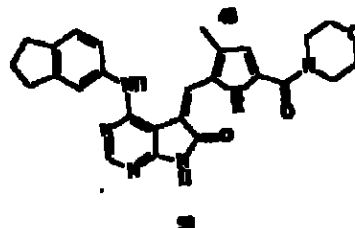
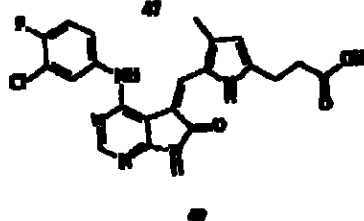
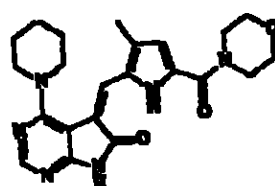
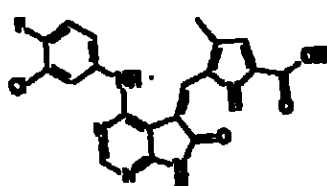
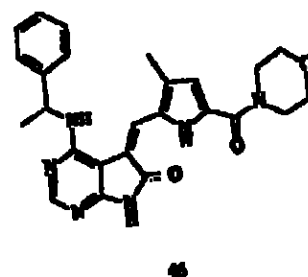
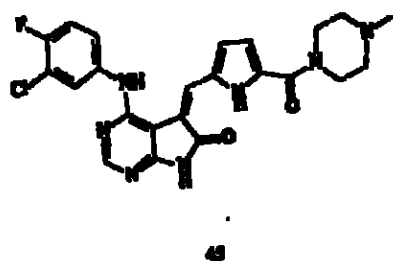
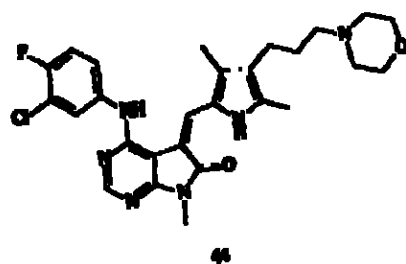
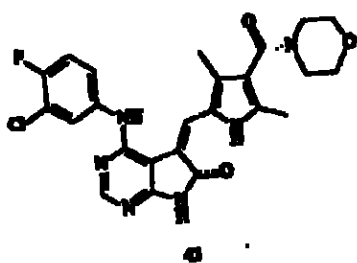
35



36



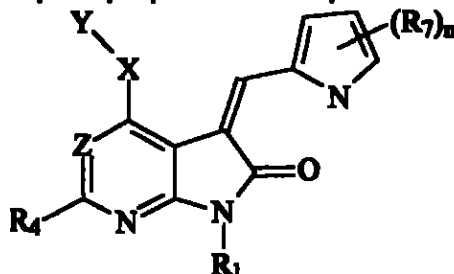
13
167
164



14
168
165

y sales aceptables para uso farmacéutico, solvatos, clatratos, y prodrogas del mismo.

12. Un método para preparar un compuesto de la Fórmula 2:



o una sal aceptable para uso farmacéutico, solvato, clatrato, o prodroga del mismo, donde:

R_1 es H o metilo;

R_4 es H, metilo, trifluorometilo, (C_1-C_4) alquilo, alcoxi, amido, amino, o anillo opcionalmente sustituido;

X es un enlace químico, etinilo, -O-, -S-, -S(O)-, -S(O₂)-, -NR₅C(O)-, o -NR₅-, donde R₅ es H, metilo, o metileno sustituido;

Y es un anillo de 5 a 10 miembros mono o bicíclico, saturado, insaturado, o aromático que comprende 0-3 heteroátomos y opcionalmente sustituido; y

Z es N o CR₈, donde R₈ es H, halógeno, nitro, ciano, alcoxi, sulfonamida, amino, o amida;

n es un entero de 0-3;

cada R₇ es independientemente H, alquilo, ácido carboxílico, amina, halógeno, nitro, ciano, X₁, X₂-(C-C₄)alquil-R₈, X₂-(C₁-C₄)alquenil-R₈, o X₂-(C₁-C₄)alquinil-R₈;

X₁ es -C(O)NR₉-, -NR₉C(O)-, -C(O)O-, C(O)R₁₁, -OC(O)-, -O-, -NR₉-, -S-, -S(O₂), o -S(O₂)NR₉;

15
169
166

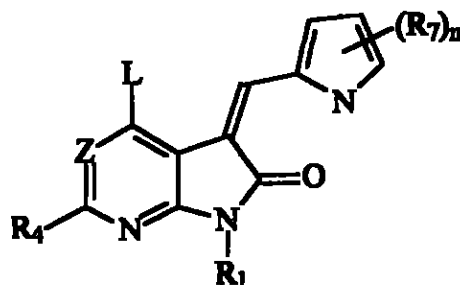
X_2 es un enlace químico, $-C(O)NR_9$, $-NR_9C(O)-$, $-C(O)O-$, $C(O)R_{11}$, $-OC(O)-$, $-O-$, $-NR_9$, $-S-$, $-S(O_2)$, o $-S(O_2)NR_9$;

R_9 se selecciona entre el grupo que consiste en hidrógeno, dialquilamino, carboxilo, hidroxilo, alcoxi, sulfonamida, urea, carbamato, diol, alquilsulfonilo, y R_{10} ;

R_9 es H o (C_1-C_3) alquilo;

R_{10} es un heterociclo saturado, insaturado, o aromático de 5 a 6 miembros opcionalmente sustituido que comprende de 1 a 4 heteroátomos; y

R_{11} es un anillo heterocíclico saturado de 5 o 6 miembros opcionalmente sustituido; que comprende hacer reaccionar un compuesto de la fórmula:



donde L es dejar un grupo con un compuesto de la Fórmula YXH bajo condiciones suficientes para formar un compuesto de la Fórmula 2.

13. El método de la reivindicación 12 en el cual L se selecciona entre el grupo que consiste en Br, Cl, SCH_3 , y $S(O)CH_3$.

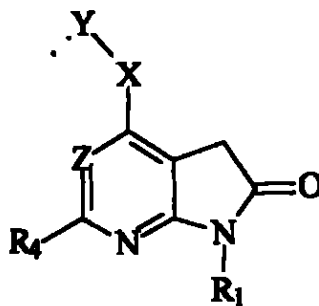
14. El método de la reivindicación 12 en el cual la reacción se lleva a cabo en un solvente polar.

15. El método de la reivindicación 14 en el cual el solvente polar se selecciona entre el grupo que consiste en alcoholes, DMF, y DMSO.

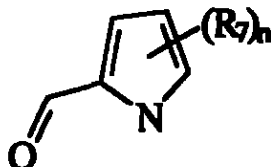
16
170
167

16. El método de la reivindicación 12 en el cual la reacción es catalizada por un catalizador seleccionado entre el grupo que consiste en AgOTf, Pd(Ph₃)₄, y *p*-TsOH.

17. Un método para preparar un compuesto de la Fórmula 2 CARACTERIZADO PORQUE que comprende hacer reaccionar un compuesto de la fórmula:



con un compuesto de la fórmula:



bajo condiciones suficientes para formar un compuesto de la Fórmula 2.

18. El método de la reivindicación 17 en el cual la reacción se lleva a cabo en un solvente polar.

19. El método de la reivindicación 18 en el cual el solvente polar se selecciona entre el grupo que consiste en alcoholes, DMF, y DMSO.

20. El método de la reivindicación 17 en el cual se cataliza con una base.

21. El método de la reivindicación 20 en el cual la base se selecciona entre el grupo que consiste en piridina y piperidina.

17
141
168

22. Una composición farmacéutica que comprende un compuesto de la Fórmula 1, o una sal aceptable para uso farmacéutico, solvato, clatrato, o prodroga del mismo, y un vehículo aceptable para uso farmacéutico.

23. Una composición farmacéutica que comprende un compuesto de la reivindicación 11 y un excipiente o vehículo aceptable para el uso farmacéutico.

24. La composición farmacéutica de la reivindicación 22 que es adecuada para administración oral, transdérmica, tópica, parenteral o mucosal.

25. Un método para regular, modular o inhibir la actividad de la proteína quinasa que comprende poner en contacto un compuesto de la Fórmula 1, o una sal aceptable para uso farmacéutico o solvato del mismo, con una proteína quinasa

26. El método de la reivindicación 25 CARACTERIZADO PORQUE la proteína quinasa es una proteína tirosina quinasa.

27. El método de la reivindicación 25 en el cual la proteína quinasa se selecciona entre el grupo que consiste en ab1, ATK, bcr-ab1, Blk, Brk, Btk, c-fms, c-kit, c-met, c-src, CDK1, CDK2, CDK4, CDK6, cRaf1, CSF1R, CSK, EGFR, ErbB2, ErbB3, ErbB4, ERK, Fak, fes, FGFR1, FGFR2, FGFR3, FGFR4, FGFR5, Fgr, FLK-4, flt-1, Fps, Frk, Fyn, GSK, Gst-Flk1, Hck, Her-2, Her-4, IGF-1R, INS-R, Jak, JNK, KDR, Lck, Lyn, MEK, p38, PANHER, PDGFR, PLK, PKC, PYK2, Raf, Rho, ros, SRC, tie1, tie2, TRK, UL97, VEGFR, Yes, y Zap70.

28. El método de la reivindicación 27 en el cual la proteína quinasa se selecciona entre el grupo que consiste en PANHER, EGFR, Her-2, Her-4, PDGFR, SRC, Lck, cdk2, p38, Raf, y Rho.

18
772
169

29. El método de la reivindicación 28 en el cual la proteína quinasa se selecciona entre el grupo que consiste en PANHER, CDK2, PDGFR, p38, y Raf.

30. El método de la reivindicación 25 en el cual la proteína quinasa está en un cultivo celular.

31. El método de la reivindicación 25 en el cual la proteína quinasa está en un mamífero.

32. Un método de tratar o prevenir una enfermedad de mamíferos caracterizada por una actividad de la proteína quinasa no regulada que comprende administrar a un mamífero que necesite dicho tratamiento o prevención una cantidad efectiva para uso terapéutico o profiláctico de un compuesto de la Fórmula 1, o una sal aceptable para uso farmacéutico, solvato, clatrato, o prodroga del mismo.

33. El método de la reivindicación 30 en el cual la enfermedad caracterizada por actividad no regulada de la proteína quinasa se selecciona entre el grupo que consiste en: trastornos proliferativos e los vasos sanguíneos; trastornos fibróticos; trastornos proliverativos de las células mesangiales; trastornos metabólicos; alergias; asma; trombosis; enfermedades del sistema nervioso; y cáncer.

34. El método de la reivindicación 31 en el cual la enfermedad caracterizada por actividad no regulada de la proteína quinasa es cáncer.

35. El método de la reivindicación 32 en el cual el cáncer se selecciona entre el grupo que consiste en cáncer de mamas, de estómago, de ovarios, de colon, de pulmón, de cerebro, de laringe, del sistema linfático, del

19
143
170

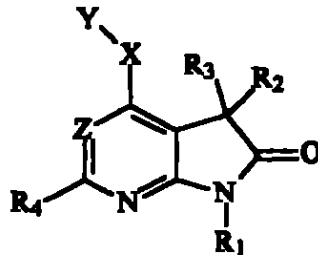
tracto genitourinario (incluyendo vejiga y próstata), de ovarios gástrico, óseo, y pancreático.

20
127
171

7-AZA-INDOLIN-2-ONAS SUSTITUIDAS EN 4 Y SU USO COMO INHIBIDORES DE PROTEÍNA QUINASA

RESUMEN

7-aza-indolin-2-onas sustituidas en 4 y su uso como inhibidores de la proteína quinasa. Las 7-aza-indolin-2-onas sustituidas en 4 específicas de la presente tienen la Fórmula 1



y sus sales aceptables para uso farmacéutico, solvatos, clatratos, y prodrogas del mismo, donde R₁, R₂, R₃, R₄, X, Y, y Z se definen en la memoria descriptiva. La invención también se refiere a composiciones farmacéuticas y formas de dosificación que comprenden los compuestos de Fórmula 1 y con métodos para su uso en el tratamiento y/o prevención de enfermedades tales como, por ejemplo, el cáncer.

A
175
172**TRADUCCIÓN****ESTADOS UNIDOS DE AMÉRICA****A quien corresponda:****DEPARTAMENTO DE COMERCIO - OFICINA DE PATENTES Y MARCAS****01 de febrero del 2001**

CERTIFICO QUE ADJUNTA A LA PRESENTE SE ENCUENTRA UNA COPIA FIEL DE LOS REGISTROS EN LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE ABAJO IDENTIFICADA QUE REUNIAN LOS REQUISITOS PARA LA ASIGNACIÓN DE UNA FECHA DE PRESENTACIÓN EN VIRTUD DE 35 USC 111.

SOLICITUD NUMERO: 60/171.288**FECHA DE DEPÓSITO: 21 de diciembre de 1999****.En nombre del COMISARIO DE PATENTES Y MARCAS.****(Firmado) W. Montgomery Certificaciones.**

(La presente certificación se encuentra unida en forma inviolable a sus adjuntos y lleva una oblea dorada que dice): OFICINA DE PATENTES Y MARCAS - ESTADOS UNIDOS DE NORTEAMÉRICA.

Carátula para Presentación Provisional de Patente

Esta solicitud es para presentación de una SOLICITUD DE PATENTE PROVISIONAL bajo el CRF 37, art 1.53 (c)

Nro. de expediente: 7683-156-888**INVENTOR/ES SOLICITANTE/S**

Apellido, Nombre e Inicial	Residencia (Ciudad y Estado o País Extranjero)
Liang, Congxin	Sunnyvale, CA

22
176
123

Sun,li	Foster City, CA
Wei, Chung C	Foster City, CA
Tang, Peng C.	Moraga, CA
McMahon, Gerald	Kenwood, CA
Hurth, Klas P.	San Francisco, CA
Cui, Jingrong	Foster City, CA

**Título de la Invención : 7-AZA-INDOLIN-2-ONAS SUSTITUÍDAS EN 4 Y SU
USO COMO INHIBIDORES DE PROTEÍNA QUINASA**

Dirección Postal: Pennie & Edmonds LLP

1667 K Street, N.W.

Washington, D.C. 20006

(202) 496-4400

Rogamos dirigir toda la correspondencia relacionada con esta Solicitud a:

Adjuntos:

Memoria descriptiva: 88 páginas

Dibujos: páginas

Forma de pago:

**Se autoriza al Comisario a cobrar los aranceles de presentación a través de la
cuenta número : 16-1150. Arancel : \$150,00**

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

Atentamente, firmado Laura A. Coruzzi

Nro. de registro: 30.742

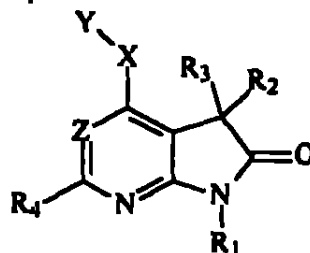
Fecha: 21 de diciembre de 1999

Por: Max Bachrach, nro. de registro: P-45.479, Pennie & Edmonds LLP

23
177
124

REIVINDICACIONES

1. Un compuesto que tiene la Fórmula 1



o una sal aceptable para uso farmacéutico, solvato, clatrato, o prodroga del mismo, donde:

R₁ es H o metilo;

cada uno de R₂ y R₃ es independientemente H, halógeno, (C₁-C₃)alquilo, o (C₁-C₃)alcoxi; o R₂ y R₃ tomados juntos de un metilindeno opcionalmente sustituido o un anillo de 3 a 7 miembros que comprende opcionalmente 0-3 heteroátomos;

R₄ es H, metilo, trifluorometilo, (C₁-C₄)alquilo, alcoxi, amido, amino, o arilo opcionalmente sustituido;

X es un enlace químico, etinilo, -O-, -S-, -S(O)-, -S(O₂)-, -NR₅C(O)-, o -NR₅-, donde R₅ es H, metilo, o metileno sustituido;

Y es un anillo mono o bicíclico de 5 a 10 miembros, saturado, insaturado o aromático que comprende 0-3 heteroátomos y opcionalmente sustituido; y

Z es N o CR₆, donde R₆ es H, halógeno, nitro, ciano, alcoxi, sulfonamida, amino, o amida.

2. El compuesto de la reivindicación 1 en el cual X es un enlace químico, -O-, -S-, o -NR₅-.

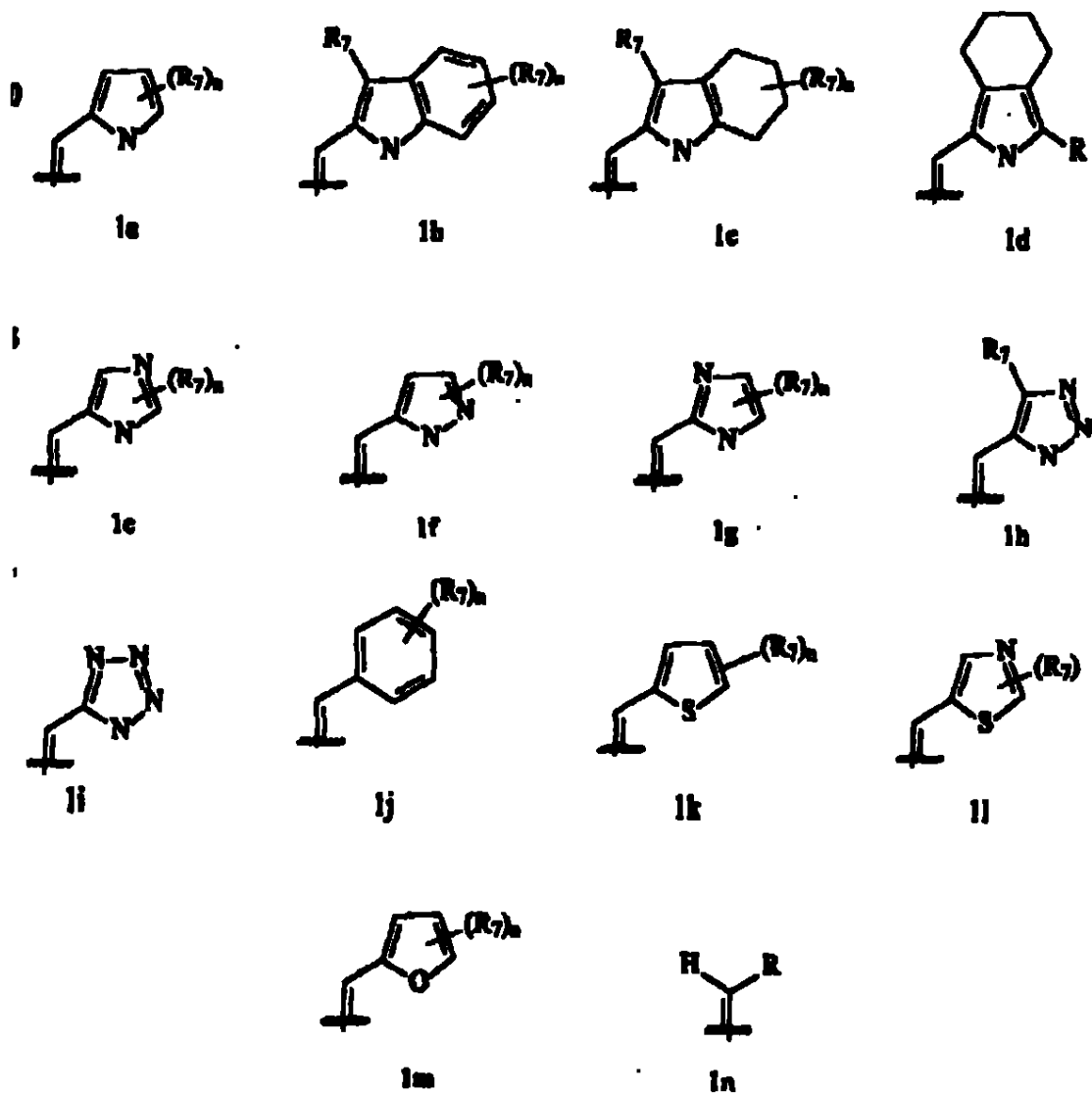
3. El compuesto de la reivindicación 1 en el cual Y se selecciona entre el grupo que consiste en fenilo, indolilo, indolinilo, 1H-indazolilo, 2,3-

24
178
125

dihidro-1H-indazolilo, 1H-bencimidazolilo, 2,3-dihidro-1H-bencimidazolilo, benzotriazolilo, piridilo, pirimidilo, 4-substituted piperazin-1-ilo, morfolino, piperidinilo, pirrolidin-1-ilo, furanilo, tiofenilo, pirrolilo, pirazolilo, imidazolilo, piridopirrolilo, piridazopirrolilo, pirimidopirrolilo, pirazopirrolilo, piridofuranilo, y derivados del mismo.

4. El compuesto de la reivindicación 1 en el cual Z es N o CH.
5. El compuesto de la reivindicación 1 en el cual R₂ y R₃ son ambos H, halógeno, o metilo.
6. El compuesto de la reivindicación 1 en el cual R₂ y R₃ se toman juntos para formar un anillo seleccionado entre el grupo que consiste en 1,3-dioxolano, 1,3-dioxano, ciclopropilo, ciclobutilo, ciclopentilo, y ciclohexilo.
7. El compuesto de la reivindicación 1 en el cual R₂ y R₃ se toman juntos para formar un metilindeno opcionalmente sustituido seleccionado entre aquellos de las fórmulas 1a-1n:

15
179
176



donde:

n es un entero de 0-3;

cada R_7 es independientemente H, alquilo, ácido carboxílico, amina, halógeno, nitro, ciano, X_1 , X_2 -(C-C₄)alquil- R_8 , X_2 -(C₁-C₄)alquénil- R_8 , o X_2 -(C₁-C₄)alquínil- R_8 ;

26
180
177

X_1 es $-C(O)NR_g$, $-NR_gC(O)-$, $-C(O)O-$, $C(O)R_{11}$, $-OC(O)-$, $-O-$, $-NR_g$, $-S-$, $-S(O_2)$, o $-S(O_2)NR_g$;

X_2 es un enlace químico, $-C(O)NR_g$, $-NR_gC(O)-$, $-C(O)O-$, $C(O)R_{11}$, $-OC(O)-$, $-O-$, $-NR_g$, $-S-$, $-S(O_2)$, o $-S(O_2)NR_g$;

R_g se selecciona entre el grupo que consiste en hidrógeno, dialquilamino, carboxilo, hidroxilo, alcoxi, sulfonamida, urea, carbamato, diol, alquilsulfonilo, y R_{10} ;

R_g es H o (C_1-C_3) alquilo;

R_{10} es un heterociclo saturado, insaturado, o aromático de 5 o 6 miembros opcionalmente sustituido que comprende de 1 a 4 heteroátomos; y

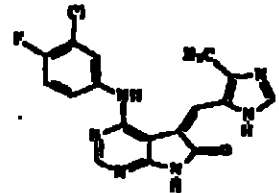
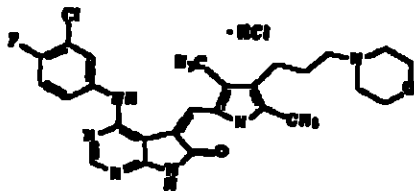
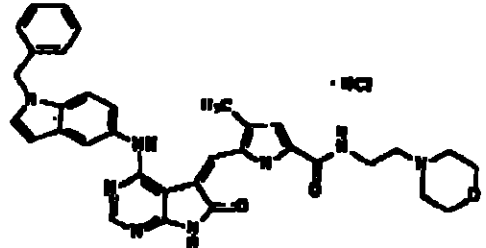
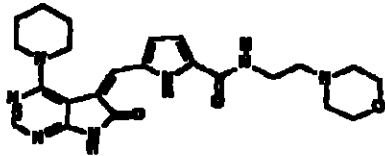
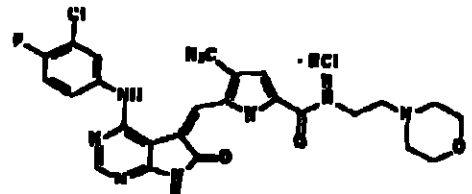
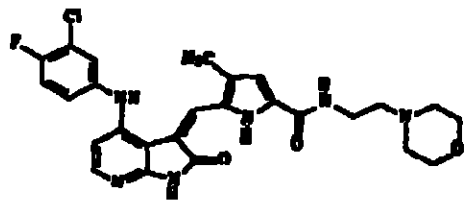
R_{11} es un anillo heterocíclico saturado de 5 a 6 miembros opcionalmente sustituido.

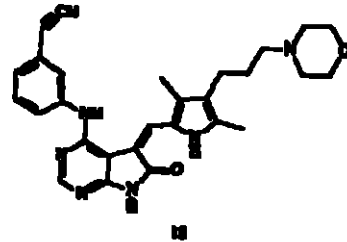
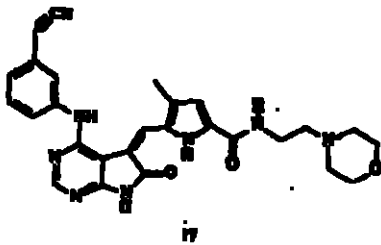
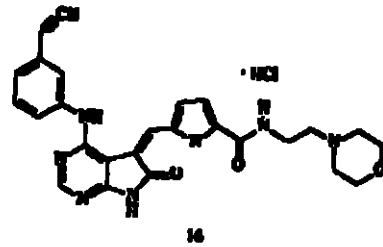
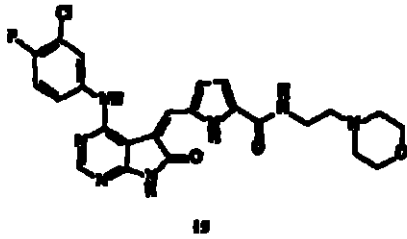
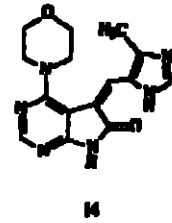
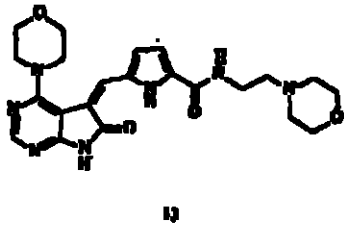
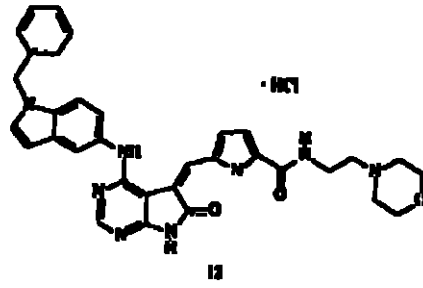
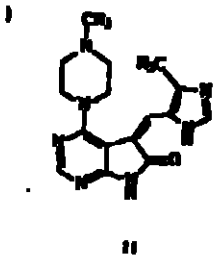
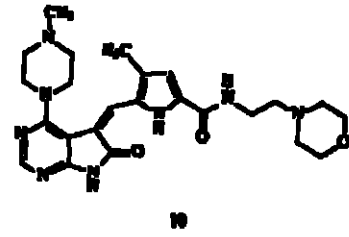
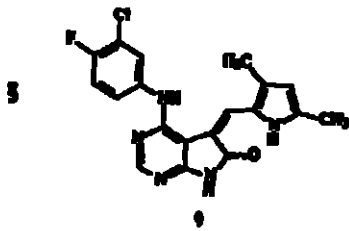
8. El compuesto de la reivindicación 7 en el cual R_7 es $X_2-(C_1-C_4)$ alquil- R_g , $X_2-(C_1-C_4)$ alquenil- R_g , o $X_2-(C_1-C_4)$ alquinil- R_g , y R_g se selecciona entre el grupo que consiste en alquilsulfonilo, alcoxi, carboxilo, morfolino, 1-alquil-piperazin-4-ilo, pirrolidinilo, piperidinilo, piridilo, imidazol, triazol, tetrazol, y tiazol.

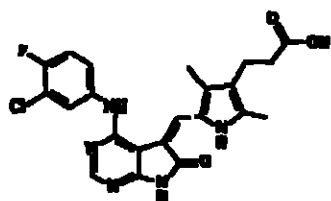
9. El compuesto de la reivindicación 1 donde R_4 es H, metilo, o trifluorometilo.

10. El compuesto de la reivindicación 1 donde si Z es CH, R_1 es CH_3 o R_3 y R_2 no forma un metilindeno opcionalmente sustituido.

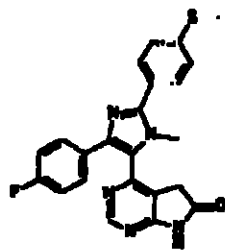
11. Un compuesto que se selecciona del grupo que consiste en:

12
181
128

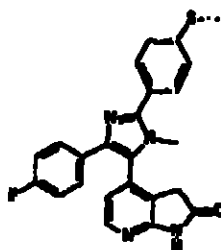
29
282
179

29.
183
180

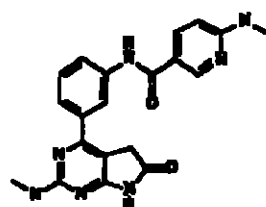
19



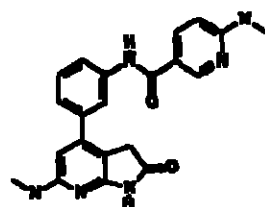
20



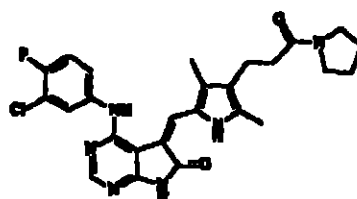
21



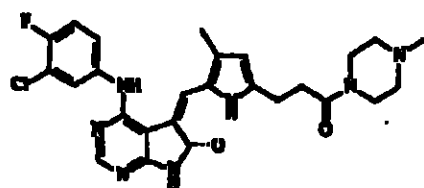
22



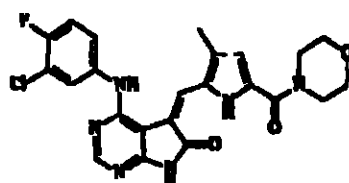
23



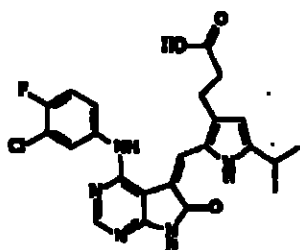
24



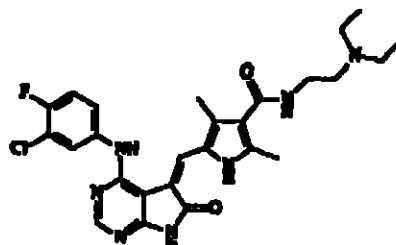
25



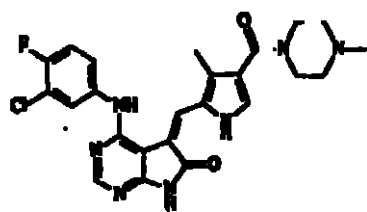
26



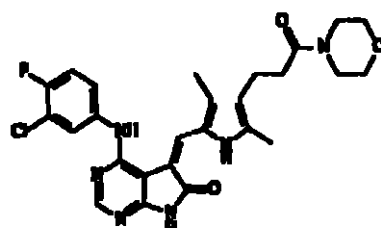
27



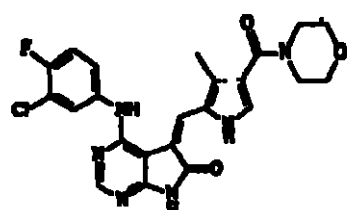
28

230
187
181

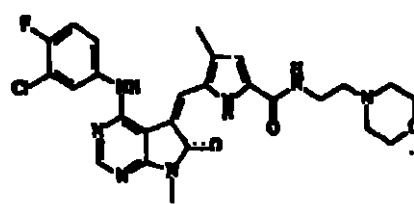
29



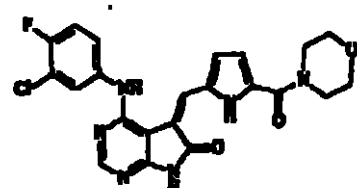
30



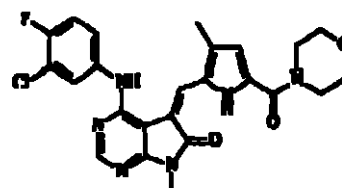
31



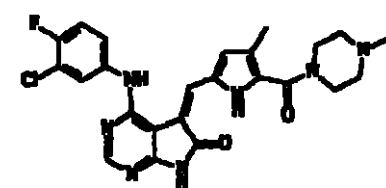
32



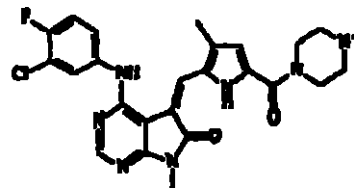
33



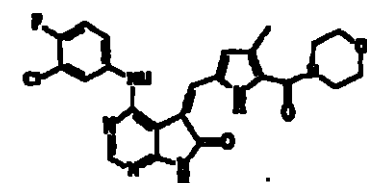
34



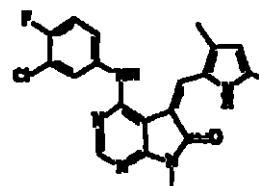
35



36

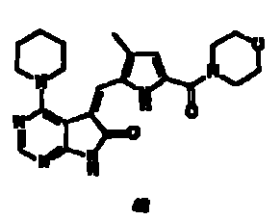
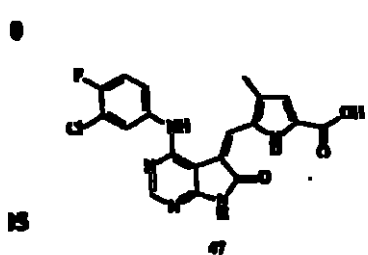
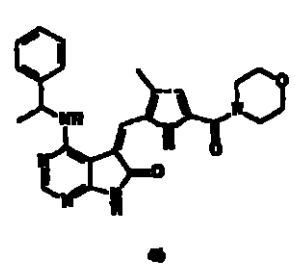
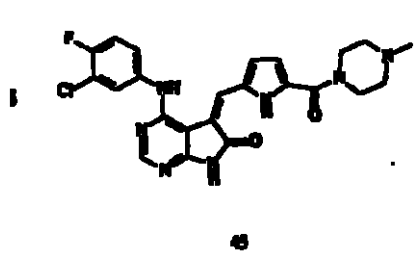
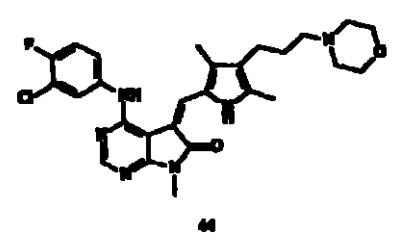
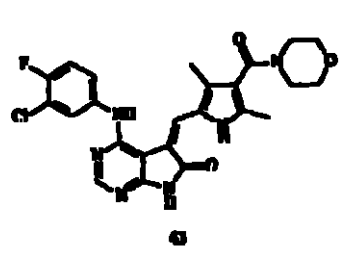
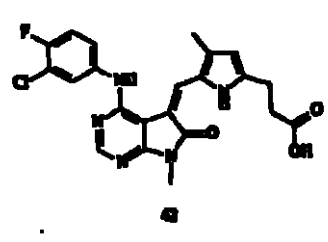
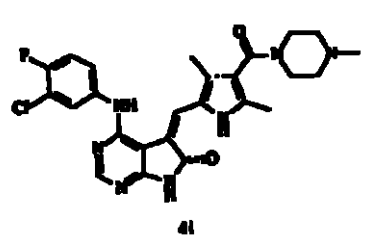
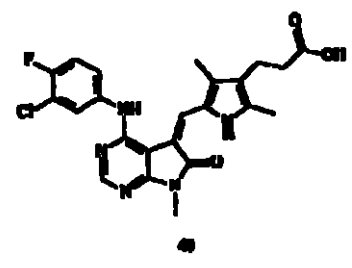
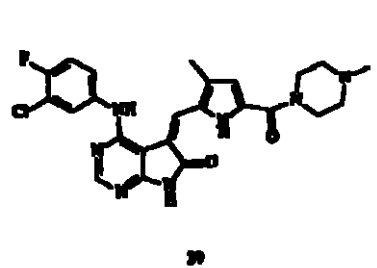


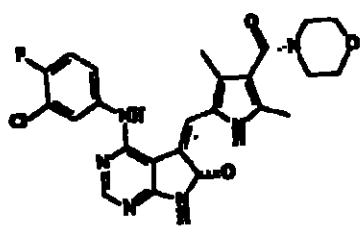
37



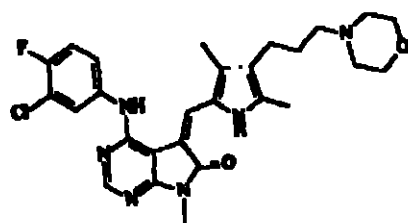
38

21
185
182

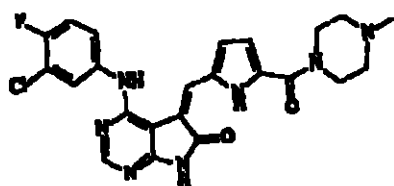


32
100
183

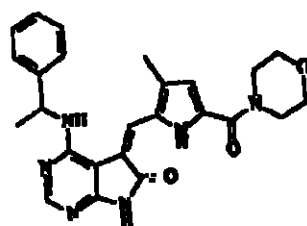
43



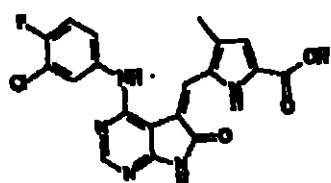
44



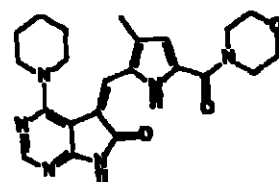
45



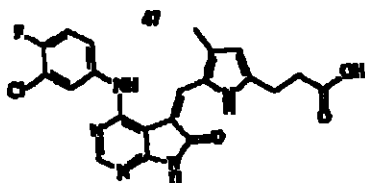
46



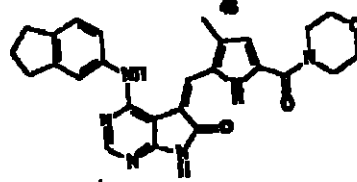
47



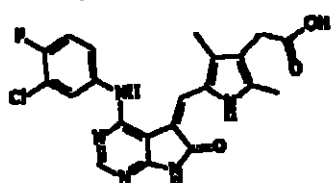
48



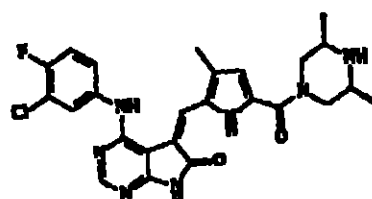
49



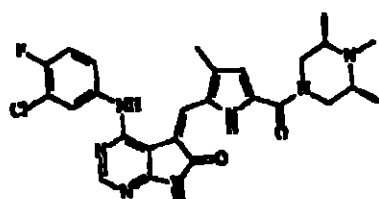
50



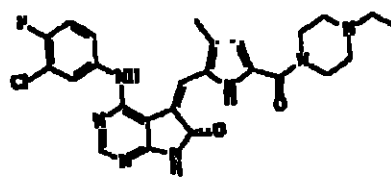
51



52



53

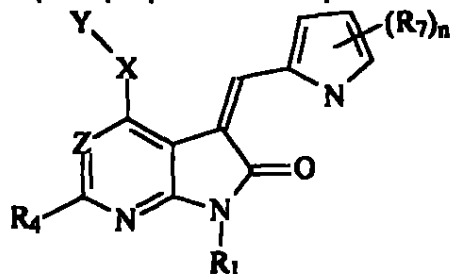


54

23
187
124

y sales aceptables para uso farmacéutico, solvatos, clatratos, y prodrogas del mismo.

12. Un método para preparar un compuesto de la Fórmula 2:



o una sal aceptable para uso farmacéutico, solvato, clatrato, o prodroga del mismo, donde:

R_1 es H o metilo;

R_4 es H, metilo, trifluorometilo, (C_1-C_4) alquilo, alcoxi, amido, amino, o arilo opcionalmente sustituido;

X es un enlace químico, etinilo, -O-, -S-, -S(O)-, -S(O₂)-, -NR₅C(O)-, o -NR₅-, donde R_5 es H, metilo, o metileno sustituido;

Y es un anillo de 5 a 10 miembros mono o bicíclico, saturado, insaturado, o aromático que comprende 0-3 heteroátomos y opcionalmente sustituido; y

Z es N o CR₆, donde R_6 es H, halógeno, nitro, ciano, alcoxi, sulfonamida, amino, o amida;

n es un entero de 0-3;

cada R_7 es independientemente H, alquilo, ácido carboxílico, amina, halógeno, nitro, ciano, X_1 , $X_2-(C-C_4)$ alquil-R₈, $X_2-(C_1-C_4)$ alquenil-R₈, o $X_2-(C_1-C_4)$ alquinil-R₈;

X_1 es -C(O)NR₉-, -NR₉C(O)-, -C(O)O-, C(O)R₁₁-, -OC(O)-, -O-, -NR₉-, -S-, -S(O₂), o -S(O₂)NR₉-;

2A
188
185

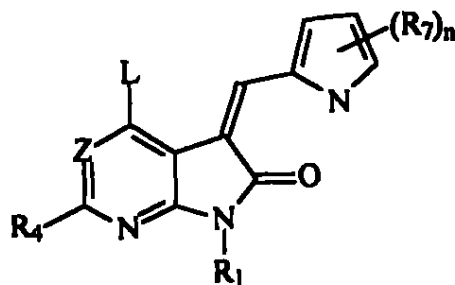
X_2 es un enlace químico, $-C(O)NR_9$ -, $-NR_9C(O)-$, $-C(O)O-$, $C(O)R_{11}$ -, $OC(O)-$, $-O-$, $-NR_9$ -, $-S-$, $-S(O_2)$, o $-S(O_2)NR_9$;

R_8 se selecciona entre el grupo que consiste en hidrógeno, dialquilamino, carboxilo, hidroxilo, alcoxi, sulfonamida, urea, carbamato, diol, alquilsulfonilo, y R_{10} ;

R_9 es H o (C_1-C_3) alquilo;

R_{10} es un heterociclo saturado, insaturado, o aromático de 5 a 6 miembros opcionalmente sustituido que comprende de 1 a 4 heteroátomos; y

R_{11} es un anillo heterocíclico saturado de 5 o 6 miembros opcionalmente sustituido; que comprende hacer reaccionar un compuesto de la fórmula:



donde L es dejar un grupo con un compuesto de la Fórmula YXH bajo condiciones suficientes para formar un compuesto de la Fórmula 2.

13. El método de la reivindicación 12 en el cual L se selecciona entre el grupo que consiste en Br, Cl, SCH_3 , y $S(O)CH_3$.

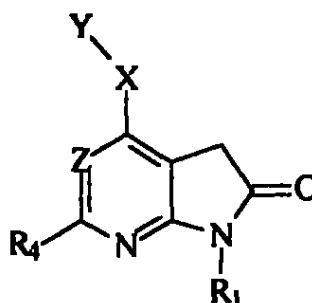
14. El método de la reivindicación 12 en el cual la reacción se lleva a cabo en un solvente polar.

15. El método de la reivindicación 14 en el cual el solvente polar se selecciona entre el grupo que consiste en alcoholes, DMF, y DMSO.

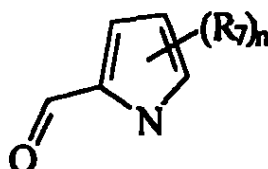
35
189
186

16. El método de la reivindicación 12 en el cual la reacción es catalizada por un catalizador seleccionado entre el grupo que consiste en AgOTf, Pd(Ph₃)₄, y *p*-TsOH.

17. Un método para preparar un compuesto de la Fórmula 2 CARACTERIZADO PORQUE que comprende hacer reaccionar un compuesto de la fórmula:



con un compuesto de la fórmula:



bajo condiciones suficientes para formar un compuesto de la Fórmula 2.

18. El método de la reivindicación 17 en el cual la reacción se lleva a cabo en un solvente polar.

19. El método de la reivindicación 18 en el cual el solvente polar se selecciona entre el grupo que consiste en alcoholes, DMF, y DMSO.

20. El método de la reivindicación 17 en el cual se cataliza con una base.

21. El método de la reivindicación 20 en el cual la base se selecciona entre el grupo que consiste en piridina y piperidina.

28
190
187

22. Una composición farmacéutica que comprende un compuesto de la Fórmula 1, o una sal aceptable para uso farmacéutico, solvato, clatrato, o prodroga del mismo, y un vehículo aceptable para uso farmacéutico.

23. Una composición farmacéutica que comprende un compuesto de la reivindicación 11 y un excipiente o vehículo aceptable para el uso farmacéutico.

24. La composición farmacéutica de la reivindicación 22 que es adecuada para administración oral, transdérmica, tópica, parenteral o mucosal.

25. Un método para regular, modular o inhibir la actividad de la proteína quinasa que comprende poner en contacto un compuesto de la Fórmula 1, o una sal aceptable para uso farmacéutico o solvato del mismo, con una proteína quinasa

26. El método de la reivindicación 25 CARACTERIZADO PORQUE la proteína quinasa es una proteína tirosina quinasa.

27. El método de la reivindicación 25 en el cual la proteína quinasa se selecciona entre el grupo que consiste en ab1, ATK, bcr-ab1, Blk, Brk, Btk, c-fms, c-kit, c-met, c-src, CDK1, CDK2, CDK4, CDK6, cRaf1, CSF1R, CSK, EGFR, ErbB2, ErbB3, ErbB4, ERK, Fak, fes, FGFR1, FGFR2, FGFR3, FGFR4, FGFR5, Fgr, FLK-4, flt-1, Fps, Frk, Fyn, GSK, Gst-Flk1, Hck, Her-2, Her-4, IGF-1R, INS-R, Jak, JNK, KDR, Lck, Lyn, MEK, p38, PANHER, PDGFR, PLK, PKC, PYK2, Raf, Rho, ros, SRC, tie1, tie2, TRK, UL97, VEGFR, Yes, y Zap70.

28. El método de la reivindicación 27 en el cual la proteína quinasa se selecciona entre el grupo que consiste en PANHER, EGFR, Her-2, Her-4, PDGFR, SRC, Lck, cdk2, p38, Raf, y Rho.

32
121
188

29. El método de la reivindicación 28 en el cual la proteína quinasa se selecciona entre el grupo que consiste en PANHER, CDK2, PDGFR, p38, y Raf.

30. El método de la reivindicación 25 en el cual la proteína quinasa está en un cultivo celular.

31. El método de la reivindicación 25 en el cual la proteína quinasa está en un mamífero.

32. Un método de tratar o prevenir una enfermedad de mamíferos caracterizada por una actividad de la proteína quinasa no regulada que comprende administrar a un mamífero que necesite dicho tratamiento o prevención una cantidad efectiva para uso terapéutico o profiláctico de un compuesto de la Fórmula 1, o una sal aceptable para uso farmacéutico, solvato, clatrato, o prodroga del mismo.

33. El método de la reivindicación 30 en el cual la enfermedad caracterizada por actividad no regulada de la proteína quinasa se selecciona entre el grupo que consiste en: trastornos proliferativos e los vasos sanguíneos; trastornos fibróticos; trastornos proliverativos de las células mesangiales; trastornos metabólicos; alergias; asma; trombosis; enfermedades del sistema nervioso; y cáncer.

34. El método de la reivindicación 31 en el cual la enfermedad caracterizada por actividad no regulada de la proteína quinasa es cáncer.

35. El método de la reivindicación 32 en el cual el cáncer se selecciona entre el grupo que consiste en cáncer de mamas, de estómago, de ovarios, de colon, de pulmón, de cerebro, de laringe, del sistema linfático, del

30
192
189

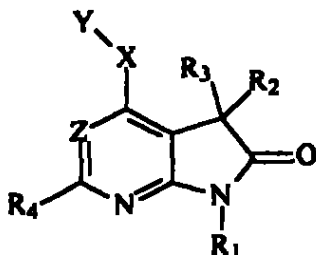
tracto genitourinario (incluyendo vejiga y próstata), de ovarios gástrico, óseo, y pancreático.

38
123
100

7-AZA-INDOLIN-2-ONAS SUSTITUIDAS EN 4 Y SU USO COMO INHIBIDORES DE PROTEÍNA QUINASA

RESUMEN

7-aza-indolin-2-onas sustituidas en 4 y su uso como inhibidores de la proteína quinasa. Las 7-aza-indolin-2-onas sustituidas en 4 específicas de la presente tienen la Formula 1



y sus sales aceptables para uso farmacéutico, solvatos, clatratos, y prodrogas del mismo, donde R₁, R₂, R₃, R₄, X, Y, y Z se definen en la memoria descriptiva. La invención también se refiere a composiciones farmacéuticas y formas de dosificación que comprenden los compuestos de Formula 1 y con métodos para su uso en el tratamiento y/o prevención de enfermedades tales como, por ejemplo, el cáncer.

P. 376. 191
44
125

PA 357980



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

February 01, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK 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: 60/171,288

FILING DATE: December 21, 1999



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

W. Montgomery
W. MONTGOMERY
Certifying Officer

COVER SHEET FOR PROVISIONAL APPLICATION FOR PATENT

Assistant Commissioner for Patents
Box PROVISIONAL PATENT APPLICATION
Washington, DC 20231

JCE53 U.S. PTO
60/171288
12/31/99

12/21/99
This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(e).

Docket Number		7623-156-888	Type a plus sign (+) inside this box -	+
INVENTOR(s) APPLICANT(s)				
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)	
LIANG SUN WEI TANG McMAHON HURTH CUI	Congxin LI Chang Feng Gerald Klaus Jingrong	C C F.	Sunnyvale, CA Foster City, CA Foster City, CA Menlo Park, CA Knoxwood, CA San Francisco, CA Foster City, CA	
TITLE OF THE INVENTION (280 character limit)				
4-SUBSTITUTED 7-AZA-INDOLIN-3-ONES AND THEIR USE AS PROTEIN KINASE INHIBITORS				
CORRESPONDENCE ADDRESS				
PENNIE & EDMONDS LLP 1667 K Street, N.W. Washington, D.C. 20006 (202) 496-4488				
ENCLOSED APPLICATION PARTS (check all that apply)				
☒ Specification	Number of Pages	88	☐ Small Entity Statement	
☐ Drawing(s)	Number of Sheets		☐ Other (specify)	
METHOD OF PAYMENT (check one)				
☐ A check or money order is enclosed to cover the Provisional filing fee			ESTIMATED PROVISIONAL FILING FEE AMOUNT	
☒ The Commissioner is hereby authorized to charge the required filing fee to Deposit Account Number 16-1150.			☒ \$150 ☐ \$ 75	

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 _____

Respectfully submitted,

Signature Laura A. Corvizi
Laura A. Corvizi

REGISTRATION NO
(if appropriate)

30,742

Date December 21, 1999

By: Max Bachrach
Max Bachrach
PENNIE & EDMONDS LLP
Reg. No P-45,479

☒ Additional inventors are being named on separately numbered sheets attached hereto.

Total number of cover sheet pages 1

PROVISIONAL APPLICATION FILING ONLY

DCI - 241037J

4-SUBSTITUTED 7-AZA-INDOLIN-2-ONES AND THEIR USE AS PROTEIN KINASE INHIBITORS

1. FIELD OF THE INVENTION

5 This invention relates to protein kinase inhibitors, pharmaceutical compositions and dosage forms comprising them, and methods of their use for the treatment and prevention of diseases such as, but not limited to, cancer.

2. BACKGROUND OF THE INVENTION

10 Cellular signal transduction is a mechanism whereby external stimuli that regulate cellular processes are relayed from receptors at the surface of a cell to its interior. One of the key biochemical mechanisms of signal transduction involves the reversible phosphorylation of proteins. The phosphorylation state of a protein, which can affect its conformation, enzymatic activity, and cellular location, is modified through the reciprocal

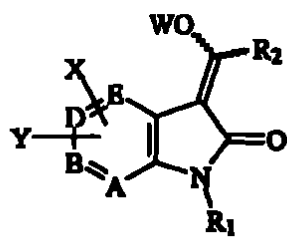
15 actions of protein kinases ("PKs") and protein phosphatases. The regulation, or lack of regulation, of protein kinases can thus have a dramatic effect on cellular behavior.

During cellular signal transduction, the function of each receptor kinase is determined by its pattern of expression, ligand availability, and the array of downstream signal transduction pathways that are activated by it. One example of a pathway includes a cascade of Growth Factor receptor tyrosine kinases ("RTKs"), such as EGF-R, PDGF-R, VEGF-R, IGF1-R, and the Insulin receptor, that deliver signals via phosphorylation to other kinases, such as Src tyrosine kinase and Raf, Mek, and Erk serine/threonine kinases. See, e.g., Davis, B. D., et al., *Microbiology* 838-841 (4th ed., 1990); and Brott, B.K., et al., *Cell Growth Differ.* 4(11):921-929 (1993). Each of these kinases play related, but functionally distinct, roles. The loss of regulation of the Growth Factor signaling pathway is a frequent occurrence in disease states such as cancer.

Aberrant expression of, or mutations in, protein kinases have been shown to lead to either uncontrolled cell proliferation (for example, malignant tumour growth) or to defects in key developmental processes. Protein kinases have been implicated as targets in central nervous system disorders (such as Alzheimer's), inflammatory disorders (such as psoriasis), bone diseases (such as osteoporosis), atherosclerosis, restenosis, thrombosis, metabolic disorders (such as diabetes), and infectious diseases (such as viral and fungal infections).

Because the regulation and/or inhibition of protein kinases can aid in the treatment and/or prevention of a variety of diseases, significant research has been directed at
35 discovering compounds that affect protein kinase activity. This research is similar to that which may have led to discovery of the compounds disclosed by PCT application WO 91/09598 and U.S. patent no. 5,811,432, which are of the formula

194
 144
 148

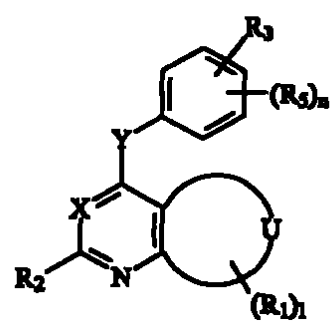


5

wherein one of A, B, D, and E is nitrogen and the others are carbon; X and Y can be, for example, halogen or hydroxy; R₁ is (C₁-C₆) alkyl or an amide; R₂ is (C₁-C₆) alkyl, preferably (C₂-C₆) alkyl; and W is, for example, hydrogen or (C₂-C₁₀) alkanoyl. These compounds, which are not reported to be kinase inhibitors, are allegedly inhibitors of prostaglandin H₂ synthase, 5-lipoxygenase, and interleukin-1 biosynthesis, and allegedly are anti-inflammatory and analgesic agents.

Examples of compounds that are allegedly protein kinase inhibitors are disclosed by PCT application WO 97/13771. These compounds are of the formula

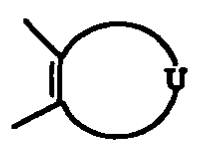
15



20

wherein X is nitrogen or CH; R₂, Y, R₃, and R₅ are each selected from a large number of moieties; the group

25



represents, for example, a 5-membered heterocyclic ring; and each R_i independently represents a 5- or 6-membered heterocyclic ring.

30

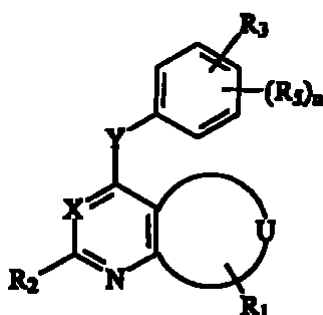
PCT application WO 98/02437 discloses compounds similar in structure to those described above, i.e.:

35

1211221-1211221

195
A5
197

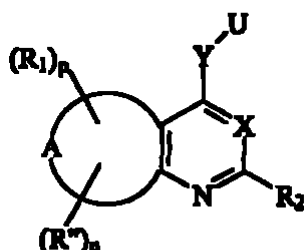
5



As above, R_1 represents a 5- or 6-membered heterocyclic ring. These compounds are also
10 allegedly protein tyrosine kinase inhibitors.

PCT application WO 98/02438 discloses compounds of the formula:

15



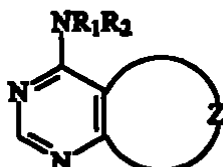
wherein X is nitrogen or CH ; R_1 , R_2 , and Y are each selected from a large number of
20 moieties; U is a 5- to 10-membered mono or bicyclic ring system; the group



25 represents, for example, a 5-membered heterocyclic ring; and R' represents a phenyl group or a 5- or 6-membered heterocyclic ring. These compounds are also allegedly protein tyrosine kinase inhibitors.

PCT application WO 98/23613 discloses compounds of the formula:

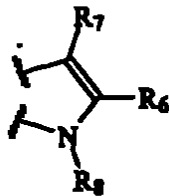
30



wherein Z can be a group of the formula

35

196
16
200

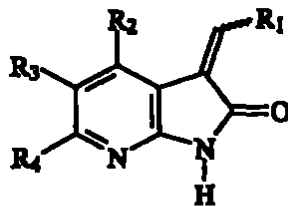


5

and R₆ is H, halogen, cyano, alkyl, or substituted alkyl. These compounds can allegedly be used in the treatment of hyperproliferative diseases such as cancer.

PCT application WO 99/21859 discloses compounds of the formula:

10

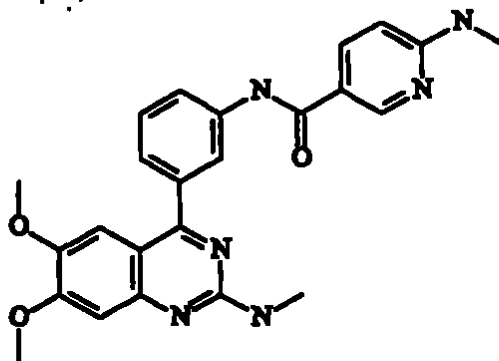


15

wherein the R groups are variously defined. These compounds are reportedly useful as protein kinase inhibitors.

PCT application WO 99/37622 discloses a compound of the formula:

20



25

which is allegedly useful as a PDE4 and TNF- α antagonist.

30

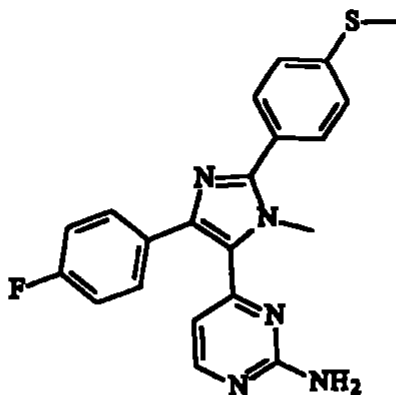
U.S. patent no. 5,916,891 discloses a compound of the formula:

35

0011333-133133

197
47
206

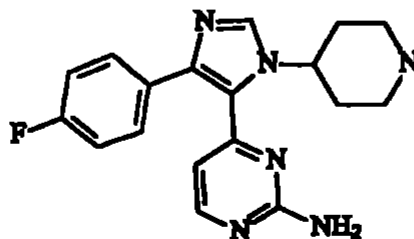
5



10

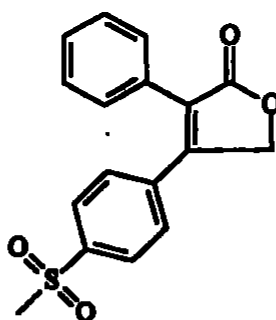
which is a derivative of the compound of formula:

15



which is allegedly a p38/Raf inhibitor. Both of these compounds share structural features
20 with rofecoxib, which is sold by Merck under the tradename Vioxx[®] and which has the
formula:

25



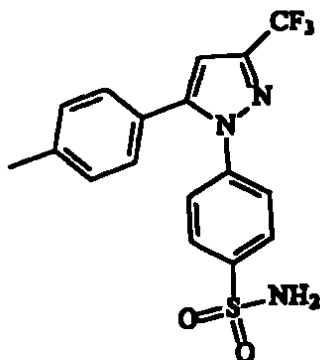
30

and celecoxib, which is sold by Monsanto and which has the formula:

35

198
48
202

5



10 Final examples of compounds that are allegedly useful as protein kinase inhibitors are disclosed in PCT applications WO 87/04928 and WO 96/16964.

Despite the large number of compounds that reportedly inhibit protein kinase activity, a need still exists for compounds that can be used in the treatment and/or prevention of cancer and other diseases in humans. This is due, in part, to bioavailability, toxicity, and other problems which render many of the known protein kinase inhibitors unsuited for clinical development.

This invention is therefore directed in part to compounds which modulate protein kinase ("PK") signal transduction by affecting the enzymatic activity of tyrosine kinases and thereby interfering with the signals transduced by them. More particularly, the present invention is directed to compounds which modulate the RTK, cellular tyrosine kinase ("CTK") and/or serine/threonine kinase ("STK") mediated signal transduction pathways as a therapeutic approach to treat many kinds of solid tumors, including but not limited to carcinoma, sarcomas including Kaposi's sarcoma, leukemia, erythroblastoma, glioblastoma, meningioma, astrocytoma, melanoma and myoblastoma. Other specific indications related to these include, but are not limited to, brain cancers, bladder cancers, ovarian cancers, gastric cancers, pancreas cancers, colon cancers, blood cancers, lung cancers, bone cancers and leukemias.

Further examples, without limitation, of the types of disorders related to unregulated PK activity that the compounds described herein may be useful in preventing, treating and/or studying, are cell proliferative disorders, fibrotic disorders and metabolic disorders. Cell proliferative disorders, which may be prevented, treated or further studied by the present invention include cancers, blood vessel proliferative disorders and mesangial cell proliferative disorders.

Blood vessel proliferative disorders refer to angiogenic and vasculogenic disorders generally resulting in abnormal proliferation of blood vessels. The formation and spreading of blood vessels, or vasculogenesis and angiogenesis, respectively, play important roles in a variety of physiological processes such as embryonic development, corpus luteum formation, wound healing and organ regeneration. They also play a pivotal role in cancer.

05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
09
08
07
06
05
04
03
02
01
00
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61

development. Other examples of blood vessel proliferation disorders include arthritis, where new capillary blood vessels invade the joint and destroy cartilage, and ocular diseases, like diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness. Conversely, disorders related to the shrinkage, contraction or

5 closing of blood vessels, such as restenosis, are also implicated.

Fibrotic disorders refer to the abnormal formation of extracellular matrices. Examples of fibrotic disorders include hepatic cirrhosis and mesangial cell proliferative disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. Hepatic cirrhosis can cause diseases such as cirrhosis of the liver. An increased extracellular matrix resulting in a hepatic scar can also be caused by viral infection such as hepatitis. Lipocytes appear to play a major role in hepatic cirrhosis. Other fibrotic disorders implicated include atherosclerosis. Mesangial cell proliferative disorders refer to disorders brought about by abnormal proliferation of mesangial cells. Mesangial proliferative disorders include various human renal diseases, such as glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes, transplant rejection, and glomerulopathies. The PDGF-R has been implicated in the maintenance of mesangial cell proliferation. Floege et al., *Kidney International* 43:47S-54S (1993).

As noted previously, PKs have been associated with such cell proliferative disorders. For example, some members of the RTK family have been associated with the development of cancer. Some of these receptors, like the EGFR (Tuzi et al., *Br. J. Cancer* 63:227-233 (1991); Torp et al., *APMTS* 100:713-719(1992)) HER2/neu (Slamon et al., *Science* 244:707-712 (1989)) and PDGFR (Kumabe et al., *Oncogene*, 7:627-633 (1992)) are over-expressed in many tumors and/or are persistently activated by autocrine loops. In fact, in the most common and severe cancers these receptor over-expressions have been demonstrated Akbasak and Suner-Akbasak et al., *J. Neurol. Sci.*, 111:119-133 (1992); Dickson et al., *Cancer Treatment Res.* 61:249-273 (1992); Korc et al., *J. Clin. Invest.* 90:1352-1360 (1992)) and autocrine loops (Lee and Donoghue, *J. Cell. Biol.*, 118:1057-1070 (1992); Korc et al., *supra*; Akbasak and Suner-Akbasak et al., *supra*). For example, EGFR has been associated with squamous cell carcinoma, astrocytoma, glioblastoma, head and neck cancer, lung cancer and bladder cancer. HER2 has been associated with breast, ovarian, gastric, lung, pancreas and bladder cancer. PDGFR has been associated with glioblastoma, lung, ovarian, melanoma and prostate. The RTK c-met has been generally associated with hepatocarcinogenesis and thus hepatocellular carcinoma. C-met has been linked to malignant tumor formation. More specifically, the RTK c-met has been associated with, among other cancers, colorectal, thyroid, pancreatic and gastric carcinoma, leukemia and lymphoma. Additionally, over-expression of the c-met gene has been detected in patients with Hodgkins disease, Burkitts disease, and the lymphoma cell line.

200
\$50
200

Flk has been associated with a broad spectrum of tumors including without limitation mammary, ovarian and lung tumors as well as gliomas such as glioblastoma. IGF-IR, in addition to being implicated in nutritional support and in type-II diabetes, has also been associated with several types of cancers. For example, IGF-I has been implicated as an autocrine growth stimulator for several tumor types, e.g., human breast cancer carcinoma cells (Arteaga et al., *J. Clin. Invest.* 84:1418-1423 (1989)) and small lung tumor cells (Macauley et al., *Cancer Res.*, 50:2511-2517 (1990)). In addition, IGF-I, integrally involved in the normal growth and differentiation of the nervous system, appears to be an autocrine stimulator of human gliomas. Sandberg-Nordqvist et al., *Cancer Res.* 53:2475-2478 (1993). The importance of the IGF-IR and its ligands in cell proliferation is further supported by the fact that many cell types in culture (fibroblasts, epithelial cells, smooth muscle cells, T-lymphocytes, myeloid cells, chondrocytes, osteoblasts, the stem cells of the bone marrow) are stimulated to grow by IGF-I. Goldring and Goldring, *Eukaryotic Gene Expression*, 1:301-326 (1991). In a series of recent publications, Baserga even suggests that IGF-IR plays a central role in the mechanisms of transformation and, as such, could be a preferred target for therapeutic interventions for a broad spectrum of human malignancies. Baserga, *Cancer Res.*, 55:249-252 (1995); Baserga, *Cell*, 79:927-930 (1994); Coppola et al., *Mol. Cell. Biol.*, 14:4588-4595 (1994). STKs have been implicated in many types of cancer including notably breast cancer. Cance, et al., *Int. J. Cancer*, 54:571-77 (1993).

The association between abnormal PK activity and disease are not restricted to cancer, however. For example, RTKs have been associated with diseases such as psoriasis, diabetes mellitus, endometriosis, angiogenesis, atheromatous plaque development, Alzheimer's disease, epidermal hyperproliferation and neurodegenerative diseases, age-related macular degeneration, hemangiomas. For example, EGFR is indicated in corneal and dermal wound healing. Defects in the Insulin-R and the IGF-1R have been indicated in type-II diabetes mellitus. A more complete correlation between specific RTKs and their therapeutic indications is set forth in Plowman et al., *DN&P* 7:334-339 (1994).

As noted previously, not only RTKs but CTKs as well including, but not limited to, src, abl, fps, yes, fyn, lyn, lck, blk, hek, fgr and yrk (reviewed by Bolen et al., *FASEB J.*, 6:3403-3409 (1992)) are involved in the proliferative and metabolic signal transduction pathway and thus would be expected, and in fact have been shown, to be involved in many PTK-mediated disorders to which the present invention is directed. For example, mutated src (v-src) has been demonstrated as an oncoprotein (pp60^{v-src}) in chicken. Moreover, its cellular homolog, the proto-oncogene pp60^{c-src} transmits oncogenic signals of many receptors. For example, over-expression of EGFR or HER2/neu in tumors leads to the constitutive activation of pp60^{c-src}, which is characteristic for the malignant cell but absent from the normal cell. On the other hand, mice deficient in the expression of c-src exhibit an osteopetrotic phenotype, indicating a key participation of c-src in osteoclast function and a

201
51
03

possible involvement in related disorders. Similarly, Zap70 is implicated in T-cell signaling.

PKs have been implicated in other diseases and disorders. For example, STKs have been associated with inflammation, autoimmune disease, immunoresponses, and hyperproliferation disorders such as retinosis, fibrosis, psoriasis, osteoarthritis and rheumatoid arthritis. PKs have also been implicated in embryo implantation and the compounds of this invention may provide an effective method of preventing embryo implantation. Finally, both RTKs and CTKs are currently suspected as being involved in hyperimmune disorders.

10

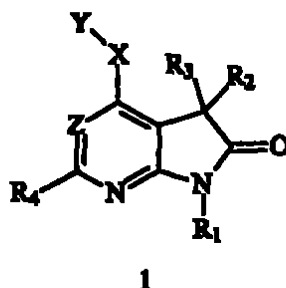
3. SUMMARY OF THE INVENTION

This invention encompasses novel 4-substituted 7-aza-indolin-2-ones, pharmaceutical compositions and dosage forms comprising them, methods of their use as protein kinase inhibitors, and methods of their use for the treatment and/or prevention of disease.

15

A first embodiment of the invention encompasses a compound of Formula 1

20



25 or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof, wherein:

R_1 is H or methyl;

each of R_2 and R_3 is independently H, halogen, (C_1-C_2) alkyl, or (C_1-C_2) alkoxy; or R_2 and R_3 taken together form an optionally substituted methyldiene or a 3- to 7-membered ring optionally comprising 0-3 heteroatoms;

30 R_4 is H, methyl, trifluoromethyl, (C_1-C_2) alkyl, alkoxy, amido, amino, or optionally substituted aryl;

X is a chemical bond, ethynyl, -O-, -S-, -S(O)-, -S(O)₂-, -NR₅C(O)-, or -NR₅-, wherein R_5 is H, methyl, or substituted methylene;

35 Y is a 5- to 10-membered mono or bicyclic, saturated, unsaturated, or aromatic ring comprising 0-3 heteroatoms and optionally substituted; and

Z is N or CR₆, wherein R_6 is H, halogen, nitro, cyano, alkoxy, sulfonamido, amino, or amide.

Preferred compounds of Formula 1 are those wherein X is a chemical bond, -O-, -S-,

501712BB-122177

202
52
206

or -NR₂-.

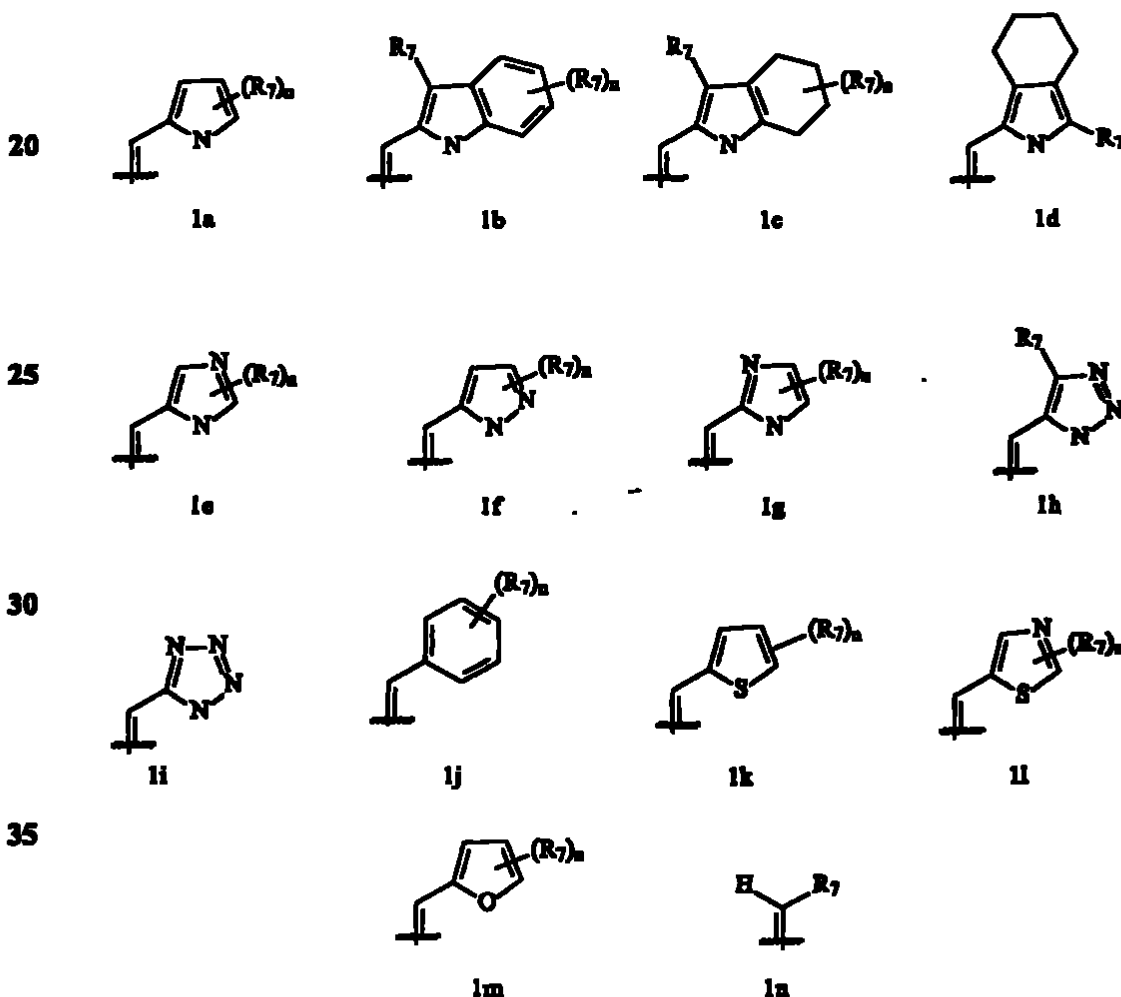
Additional preferred compounds of Formula 1 are those wherein Y is selected from the group consisting of phenyl, indolyl, indoliny, 1H-indazolyl, 2,3-dihydro-1H-indazolyl, 1H-benzimidazolyl, 2,3-dihydro-1H-benzimidazolyl, benzotriazolyl, pyridyl, pyrimidyl, 4-substituted piperazin-1-yl, morpholino, piperidinyl, pyrrolidin-1-yl, furanyl, thiophenyl, pyrrolyl, pyrazolyl, imidazolyl, pyridopyrrolyl, pyridazopyrrolyl, pyrimidopyrrolyl, pyrazopyrrolyl, pyridofuranyl, and derivatives thereof.

Additional preferred compounds of Formula 1 are those wherein Z is N or CH.

Additional preferred compounds of Formula 1 are those wherein R₂ and R₃ are both H, halogen, or methyl.

Additional preferred compounds of Formula 1 are those wherein R₂ and R₃ are taken together to form a ring selected from the group consisting of 1,3-dioxolane, 1,3-dioxane, cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

Additional preferred compounds of Formula 1 are those wherein R₂ and R₃ are taken together to form an optionally substituted methyldene selected from those of Formulas 1a-1n:



50171238-122177

203
67
267

wherein:

n is an integer of 0-3;

each R_7 is independently H, alkyl, carboxylic acid, amine, halogen, nitro, cyano, X_1 , X_2 -(C₁-C₄)alkyl- R_8 , X_2 -(C₁-C₄)alkenyl- R_8 , or X_2 -(C₁-C₄)alkynyl- R_8 ;

5 X_1 is -C(O)NR₉-, -NR₉C(O)-, -C(O)O-, C(O)R₁₁-, -OC(O)-, -O-, -NR₉-, -S-, -S(O₂), or -S(O₂)NR₉-;

X_2 is a chemical bond, -C(O)NR₉-, -NR₉C(O)-, -C(O)O-, C(O)R₁₁-, -OC(O)-, -O-, -NR₉-, -S-, -S(O₂), or -S(O₂)NR₉-;

10 R_8 is selected from the group consisting of hydrogen, dialkylamino, carboxyl, hydroxyl, alkoxy, sulfonamide, urea, carbamate, diol, alkylsulphonyl, and R_{10} ;

R_9 is H or (C₁-C₃)alkyl;

R_{10} is an optionally substituted 5- or 6-membered saturated, unsaturated, or aromatic heterocycle comprising from 1 to 4 heteroatoms; and

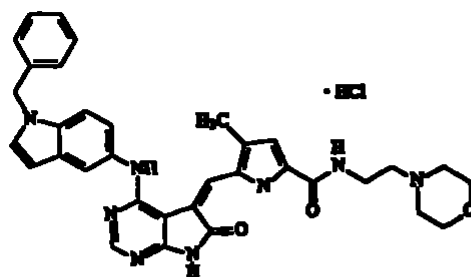
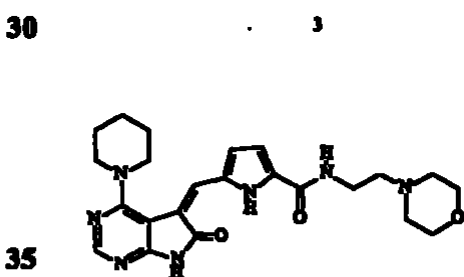
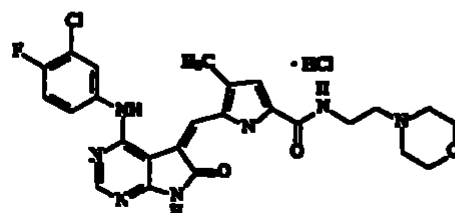
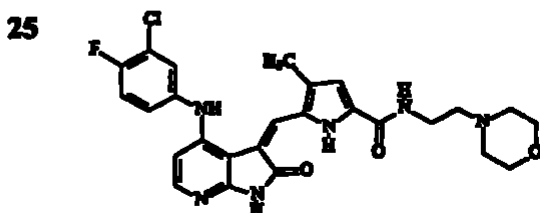
R_{11} is an optionally substituted 5- or 6-membered saturated heterocyclic ring.

15 In more preferred compounds of the invention, R_7 is X_1 -(C₁-C₄)alkyl- R_8 , X_2 -(C₁-C₄)alkenyl- R_8 , or X_2 -(C₁-C₄)alkynyl- R_8 , and R_8 is selected from the group consisting of alkylsulfonyl, alkoxy, carboxyl, morpholino, 1-alkyl-piperazin-4-yl, pyrrolidinyl, piperidinyl, pyridyl, imidazolo, triazolo, tetrazolo, and thiazolo.

Additional preferred compounds of the invention are those of Formula 1 wherein R_4 is H, methyl, or trifluoromethyl.

Additional preferred compounds of the invention are those of Formula 1 wherein if Z is CH, R_1 is CH₃ or R_2 , and R_2 do not form an optionally substituted methyldene.

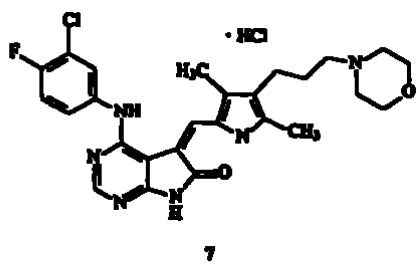
Specific preferred compounds of the invention are those of Formulas 3-54:



0171288-172175

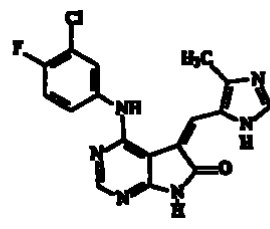
204
57
208

5

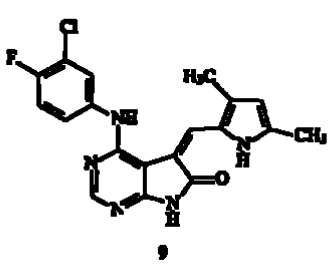


7

8

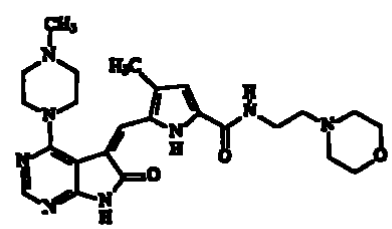


10

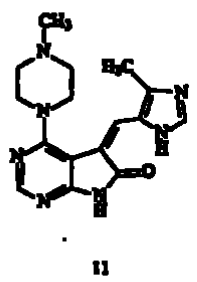


9

10

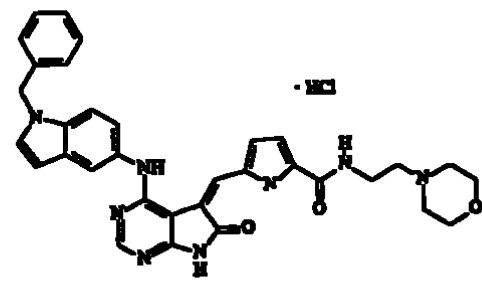


15

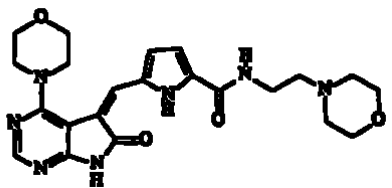


11

12

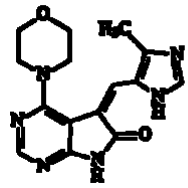


25

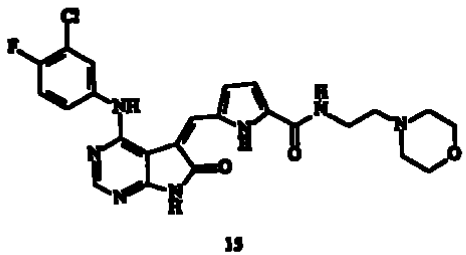


13

14

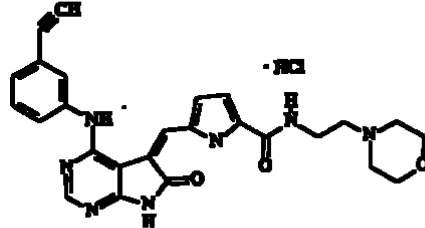


30



15

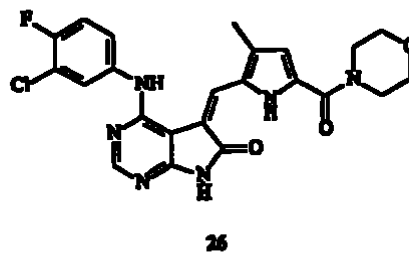
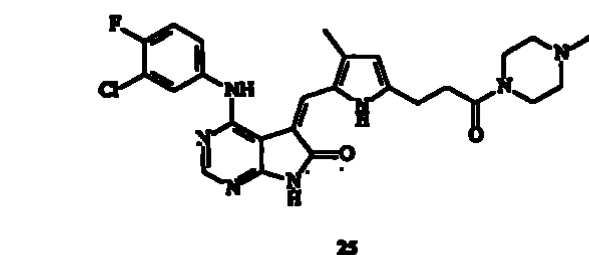
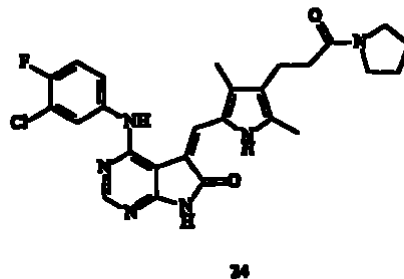
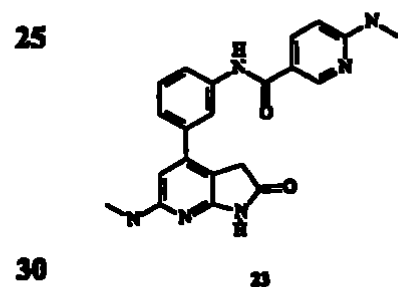
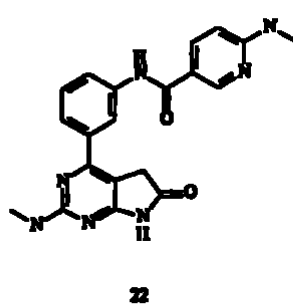
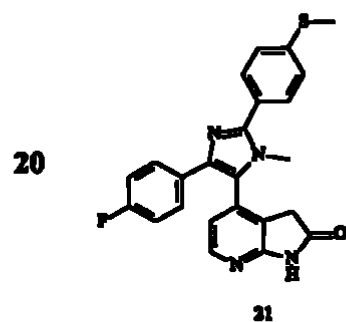
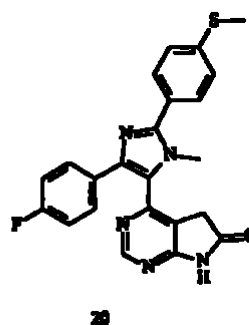
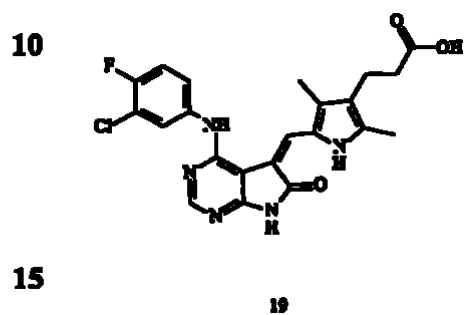
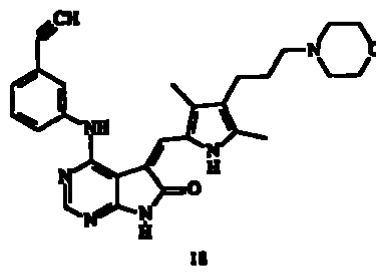
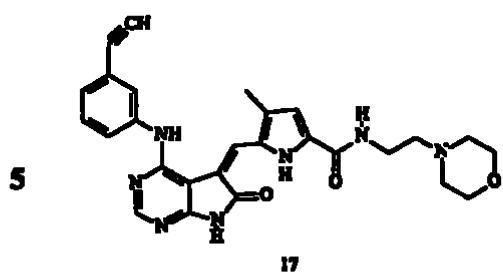
16



35

50171288-122197

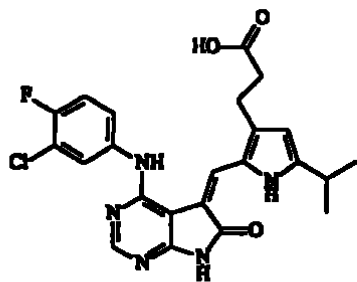
205
52
209



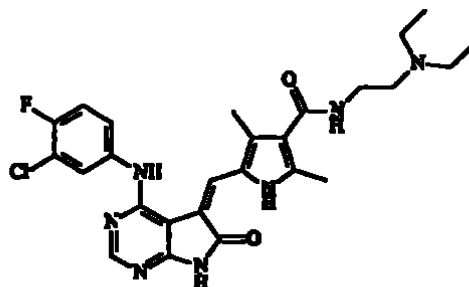
50171288.122177

206
56
210

5

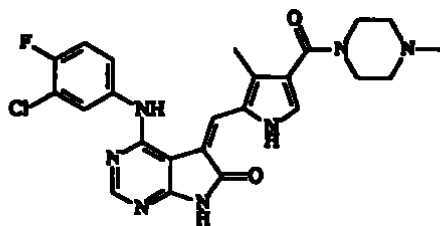


27

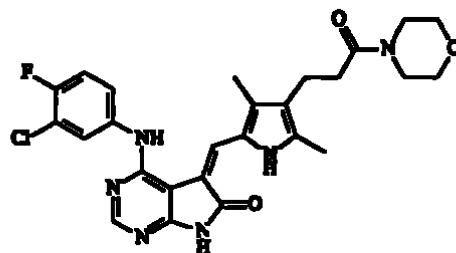


28

10

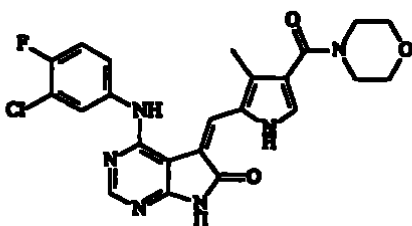


29

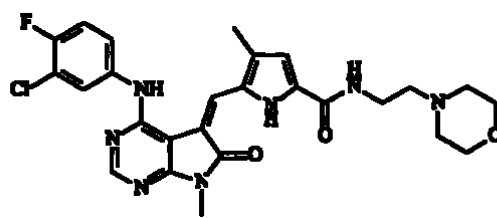


30

15

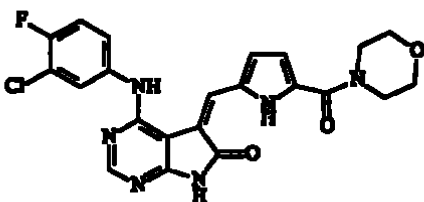


31

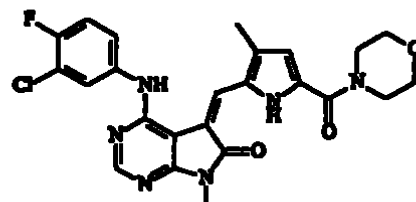


32

20



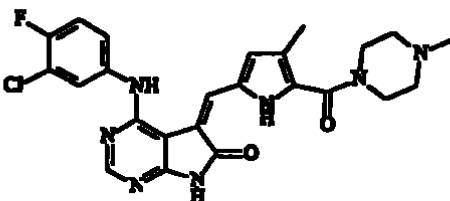
33



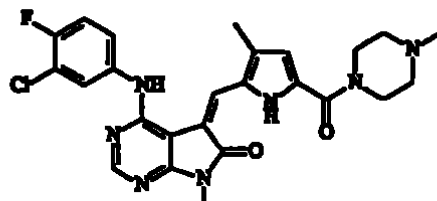
34

25

30



35



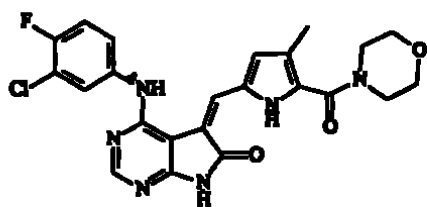
36

35

50171288-122177

207
57
24

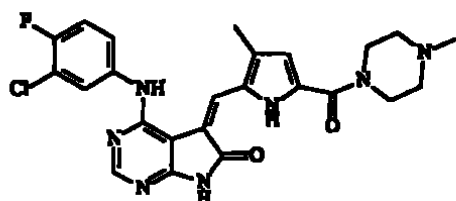
5



37

38

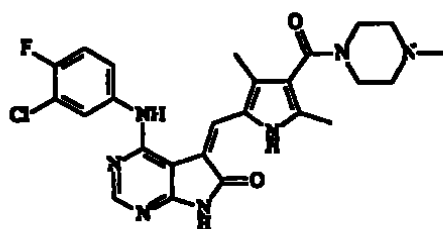
10



39

40

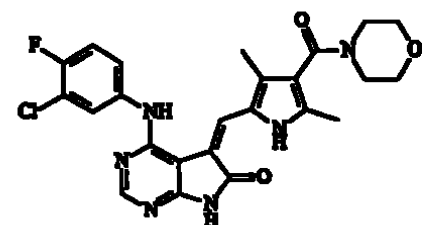
15



41

42

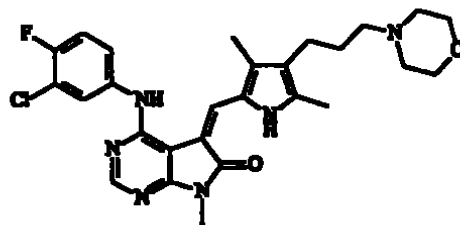
20



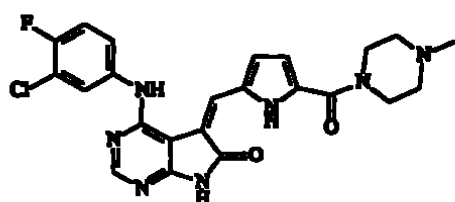
43

44

25



30



45

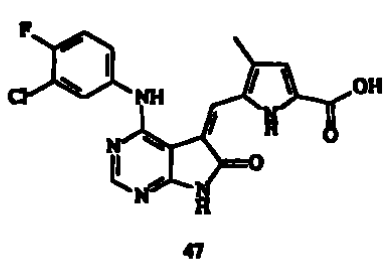
46

35

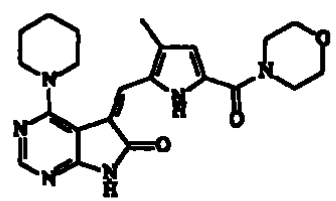
50171288.122173

28/80
ZK

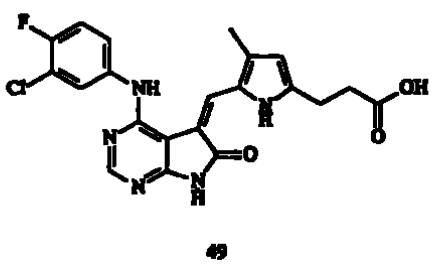
5



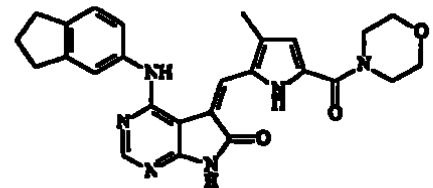
48



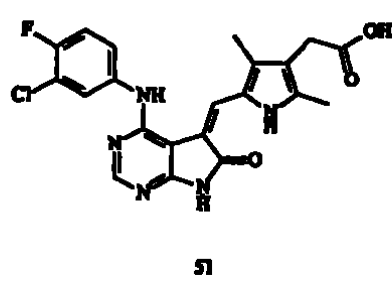
10



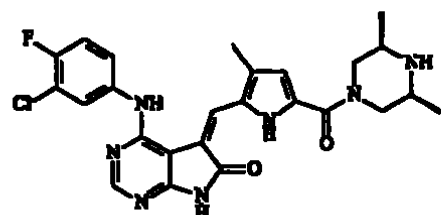
50



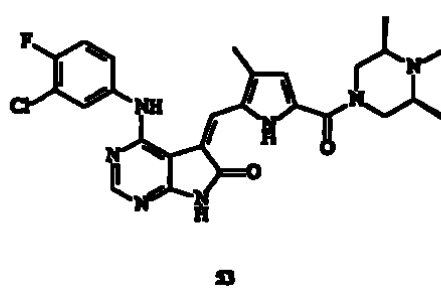
15



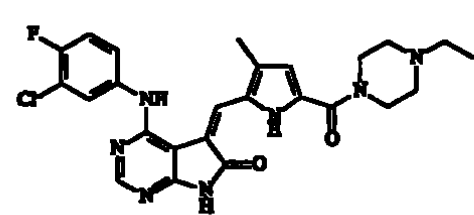
52



20



54



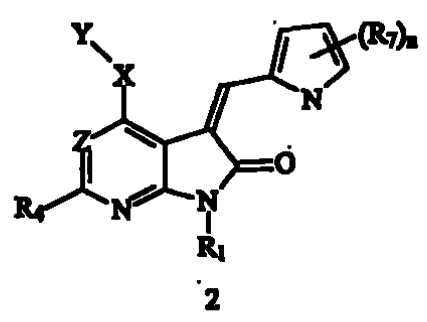
25

and pharmaceutically acceptable salts, solvates, clathrates, and prodrugs thereof. Preferred pharmaceutically acceptable salts of the compounds of the invention are hydrochloride salts.

30

A second embodiment of the invention encompasses a method of preparing a compound of Formula 2:

35

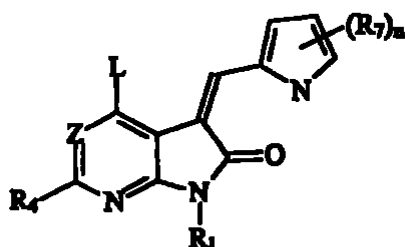


60171288-122197

wherein X, Y, Z, R₁, R₄, R₇, and n are defined above, which comprises reacting a compound of the formula:

209
59
213

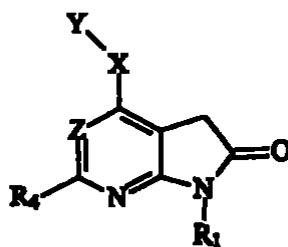
5



- 10 wherein L is a leaving group with a compound of formula YXH under conditions sufficient to form a compound of Formula 2. Preferred leaving groups include, but are not limited to, Br, Cl, SCH₃, and S(O)CH₃. In a preferred method of this embodiment, the reaction is performed in a solvent. Preferred solvents are polar. More preferred solvents include, but are not limited to, alcohols, DMF, DMSO, and mixtures thereof. In another preferred method of this embodiment, the reaction is catalyzed by a catalyst such as, but are not limited to, AgOTf, Pd(Ph₃)₄, and *p*-TsOH.

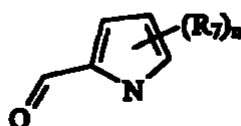
A third embodiment of the invention encompasses a method of preparing a compound of Formula 2 which comprises reacting a compound of the formula:

20



25

with a compound of the formula:



30

- under conditions sufficient to form a compound of Formula 2. In a preferred method of this embodiment, the reaction is performed in a solvent. Preferred solvents are polar. More preferred solvents include, but are not limited to, alcohols, DMF, DMSO, and mixtures thereof. In another preferred method of this embodiment, the reaction is catalyzed by a base. Preferred bases include, but are not limited to, pyridine and piperidine.

- 35 A fourth embodiment of the invention encompasses a pharmaceutical composition comprising a compound of Formula 1, or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof, and a pharmaceutically acceptable carrier. This embodiment

210 60
21A

further encompasses dosage forms suitable for oral, transdermal, topical, parenteral (e.g., subcutaneous, intrathecal, intramuscular, and intravenous), or mucosal (e.g., rectal, vaginal, and nasal) administration. Therefore, solid, lyophilized, injectable and transdermal topical formulations are contemplated herein.

5 A fifth embodiment of the invention encompasses a method of regulating, modulating, or inhibiting protein kinase activity which comprises contacting a compound of Formula 1, or a pharmaceutically acceptable salt or solvate thereof, with a protein kinase. In a preferred method of this embodiment, the protein kinase is a protein tyrosine kinase. Preferably the contact is made in cell culture (*in vitro*) or in a human, animal or bird (*in*
10 *vivo*).

In another preferred method of this embodiment, the protein kinase is selected from the group consisting of abl, ATK, bcr-abl, Blk, Brk, Btk, c-fms, c-kit, c-met, c-src, CDK1, CDK2, CDK4, CDK6, cRaf1, CSF1R, CSK, EGFR, ErbB2, ErbB3, ErbB4, ERK, Fak, fes, FGFR1, FGFR2, FGFR3, FGFR4, FGFR5, Fgr, FLK-4, flt-1, Fps, Frk, Fyn, GSK,
15 Gst-Fik1, Hck, Her-2, Her-4, IGF-1R, INS-R, Jak, JNK, KDR, Lck, Lyn, MEK, p38, PANHER, PDGFR, PLK, PKC, PYK2, Raf, Rho, ros, SRC, tie₁, tie₂, TRK, UL97, VEGFR, Yes, and Zap70.

In a more preferred method of this embodiment, the protein kinase is selected from the group consisting of PANHER, EGFR, Her-2, Her-4, PDGFR, SRC, Lck, cdk2, p38,
20 Raf, and Rho.

In an even more preferred method of this embodiment, the protein kinase is selected from the group consisting of PANHER, CDK2, PDGFR, p38, and Raf.

A sixth embodiment of the invention encompasses a method of treating or preventing a disease characterized by unregulated protein kinase activity which comprises
25 administering to a patient (e.g., a mammal, preferably a human) in need of such treatment or prevention a therapeutically or prophylactically effective amount of a compound of Formula 1, or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof.

In a preferred method of this embodiment, the disease characterized by unregulated protein kinase activity is selected from the group consisting of: blood vessel proliferative
30 disorders such as, but not limited to, arthritis and restenosis; fibrotic disorders such as, but not limited to, hepatic cirrhosis and atherosclerosis; mesangial cell proliferative disorders such as, but not limited to, glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes, organ transplant rejection, and glomerulopathies; metabolic disorders such as, but not limited to, psoriasis, diabetes
35 mellitus, chronic wounds, inflammation, and neurodegenerative diseases; auto-immune diseases; allergies; asthma; thrombosis; nervous system diseases; and cancer.

In a more preferred method of this embodiment, the disease characterized by unregulated protein kinase activity is cancer. Examples of cancers include, but are not

50171288-100100

limited to, breast, stomach, ovary, colon, lung (including non-small cell), brain, larynx, lymphatic system, genitourinary tract (including bladder and prostate), ovarian, gastric, bone, and pancreatic cancer.

21
61
215

5

3.1. DEFINITIONS

As used herein, the term "halogen" includes fluorine, chlorine, bromine, and iodine.

As used herein, the term "alkyl" includes saturated monovalent hydrocarbon radicals having straight, cyclic, or branched moieties, and combinations thereof.

As used herein, the term "alkenyl" includes monovalent hydrocarbon radicals
10 having straight, cyclic, or branched moieties, and combinations thereof which comprise at least one carbon-carbon double bond.

As used herein, the term "alkynyl" includes saturated monovalent hydrocarbon radicals having straight, cyclic, or branched moieties, and combinations thereof which comprise at least one carbon-carbon triple bond.

As used herein to describe a compound or moiety, the term "derivative" means a
15 compound or moiety wherein the degree of saturation of at least one bond has been changed (e.g., a single bond has been changed to a double or triple bond) or wherein at least one hydrogen atom is replaced with a different atom or a chemical moiety. Examples of different atoms and chemical moieties include, but are not limited to, halogen, oxygen,
20 nitrogen, sulfur, hydroxy, methoxy, alkyl, amine, amide, ketone, and aldehyde.

As used herein to describe a compound or moiety, the term "substituted" means a
compound or moiety wherein at least one hydrogen atom is replaced with a different atom or a chemical moiety. Examples of different atoms and chemical moieties include, but are not limited to, halogen, oxygen, nitrogen, sulfur, hydroxy, methoxy, alkyl, amine, amide,
25 ketone, and aldehyde.

As used herein, the term "heteroatom" means an atom selected from the group consisting of O, S, and N.

As used herein, the term "methylidene" means an optionally substituted carbon-carbon double bond.

As used herein, the term "prodrug" means a derivative of a compound that can
30 hydrolyze, oxidize, or otherwise react under biological conditions (*in vitro* or *in vivo*) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives of compounds of Formula 1 that comprise biohydrolyzable moieties such as amides, esters, carbamates, carbonates, or ureides. Such biohydrolyzable moieties may be linked, for
35 example, to a peptide.

As used herein, the terms "biohydrolyzable carbamate," "biohydrolyzable carbonate," and "biohydrolyzable ureide" mean a carbamate, carbonate, or ureide, respectively, of a compound that either: 1) does not interfere with the biological activity of

212
62
216

the compound but can confer upon that compound advantageous properties *in vivo*, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted *in vivo* to the biologically active compound. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein, the term "biohydrolyzable ester" means an ester of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties *in vivo*, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted *in vivo* to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters.

As used herein, the term "biohydrolyzable amide" means an amide of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties *in vivo*, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted *in vivo* to the biologically active compound. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, α -amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides.

As used herein, the term "disease characterized by unregulated protein kinase activity" means a disease or condition caused or aggravated by abnormal kinase activity. Examples of such diseases or conditions include, but are not limited to, uncontrolled cell proliferation (e.g., malignant tumour growth) or to defects in key developmental processes. Specific examples include, but are not limited to, central nervous system disorders, inflammatory disorders, bone diseases, atherosclerosis, restenosis, thrombosis, metabolic disorders, and infectious diseases.

As used herein, the term "treat" includes the amelioration, reduction, or eradication of the symptoms of disease.

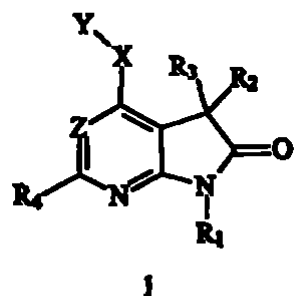
4. DETAILED DESCRIPTION OF THE INVENTION

This invention encompasses compounds that affect the activity of one or more protein kinases. The invention further encompasses compounds that are useful in methods of regulating, modulating, and/or inhibiting protein kinases of both the receptor and non-receptor types. The invention further provides methods of treating and preventing diseases and disorders that are related to unregulated protein kinase activity in birds and animals, and particularly in mammals such as humans.

Compounds of the invention are those of Formula 1

23 68
27

5



1

and pharmaceutically acceptable salts, solvates, clathrates, and prodrugs thereof, wherein
 10 R_1 , R_2 , R_3 , R_4 , X, Y, and Z are defined herein. It should be appreciated that certain compounds of the invention may have one or more chiral centers or axes, thus the invention further encompasses racemic and optically pure enantiomers of compounds of Formula 1. The invention also encompasses crystalline and amorphous forms as well as lyophilized, non-lyophilized, and sterile compositions of compounds of Formula 1.

15 Pharmaceutically acceptable salts of compounds of Formula 1 include salts of acidic or basic moieties. The compounds of the invention that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. Acids that can be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts that contain pharmacologically
 20 acceptable anions such as, but not limited to, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, and
 25 pamoate [i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)]. Compounds of the invention that comprise a basic moiety, such as an amino group, can form pharmaceutically acceptable salts with various amino acids in addition to the acids mentioned above.

Compounds of the invention that are acidic in nature are capable of forming base addition salts with various pharmacologically acceptable cations. Examples of such salts
 30 include, but are not limited to, the alkali metal or alkaline earth metal salts, e.g., calcium, magnesium, sodium, and potassium salts.

4.1. SYNTHESIS

Compounds of the invention can be readily prepared from
 35 4-chloro-1,3-dihydro-pyrrolo[2,3-b]pyridin-2-one or 4-chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidine-6-one or other commercially or readily accessible starting material. A preferred method of preparing 4-chloro-1,3-dihydro-pyrrolo[2,3-b]pyridin-2-one is shown in Scheme 1:

00171288-122177

214
 6A
 268

5

10

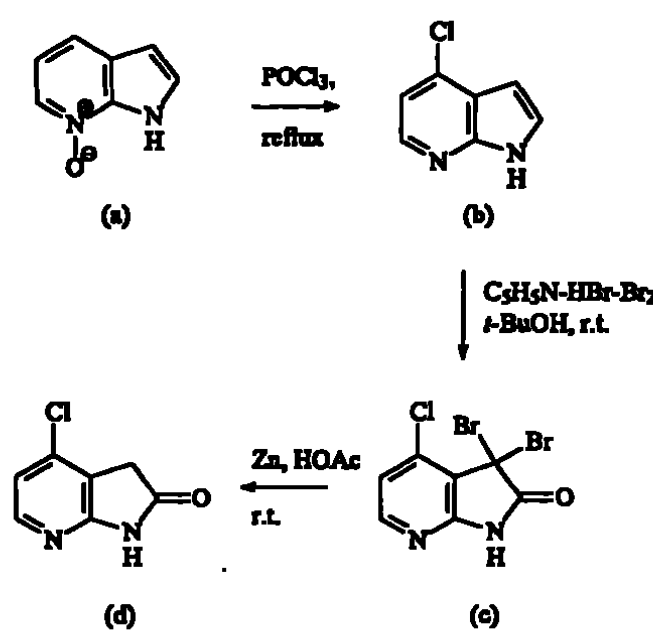
15

20

25

30

35



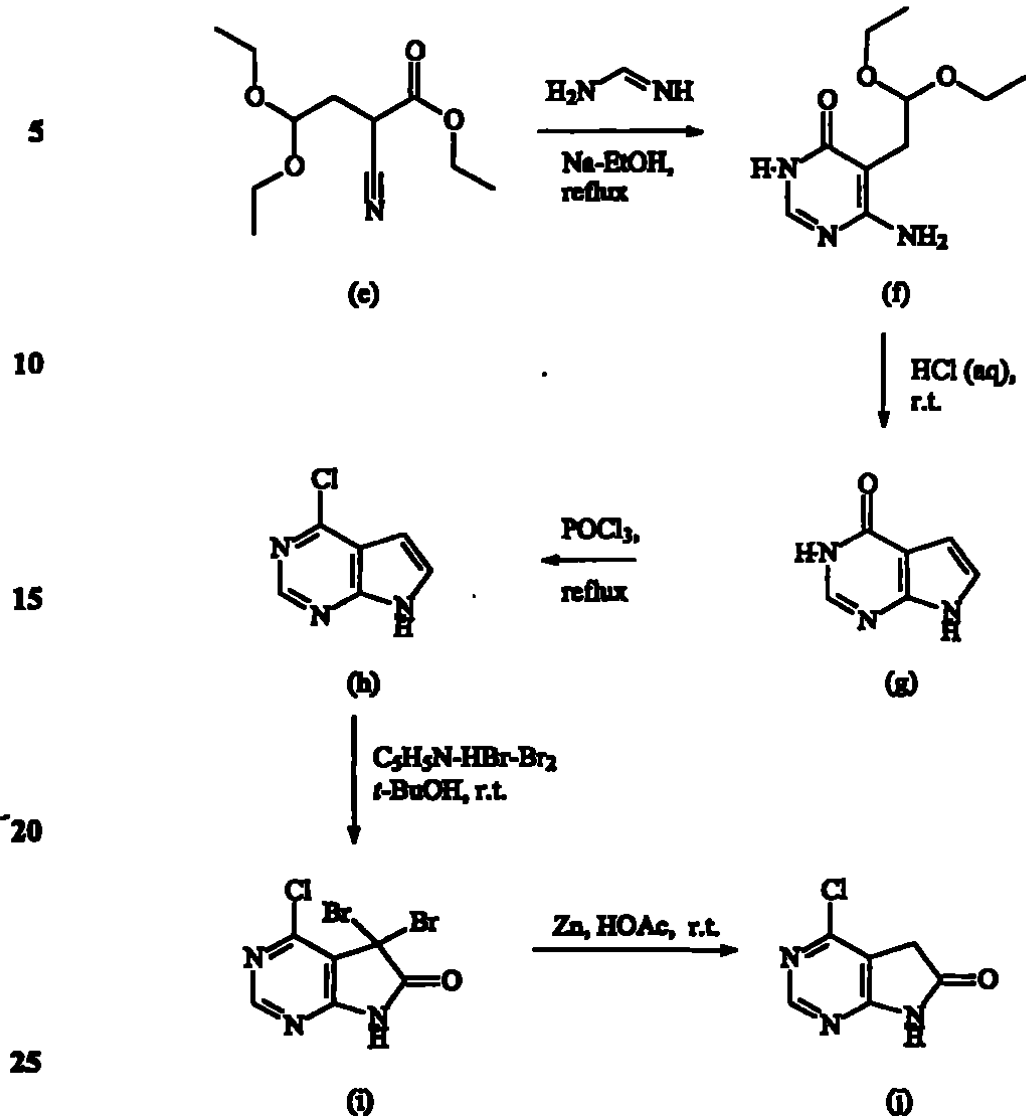
Scheme 1

According to Scheme 1, compound (a) is chlorinated to provide compound (b). Although a variety of reaction conditions known to those skilled in the art can be used to chlorinate compound (a), the use of POCl_3 is preferred. Compound (b) is then converted to compound (c) using $\text{C}_5\text{H}_5\text{N-HBr-Br}_2$ in *t*-butanol. This reaction is preferably run at room temperature for about four hours, although those skilled in the art will recognize that the solvent and the reaction temperature and time can be varied to maximize the yield of compound (c). Finally, 4-chloro-1,3-dihydro-pyrrolo[2,3-b]pyridin-2-one (compound (d)) is formed by replacing the bromine atoms bound to compound (c) with hydrogen atoms. This is readily accomplished using zinc in acetic acid, although other methods known to those skilled in the art can also be used.

A preferred method of preparing 4-chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidine-6-one is described in Scheme 2:

50171288-100000

215
65
204



Scheme 2

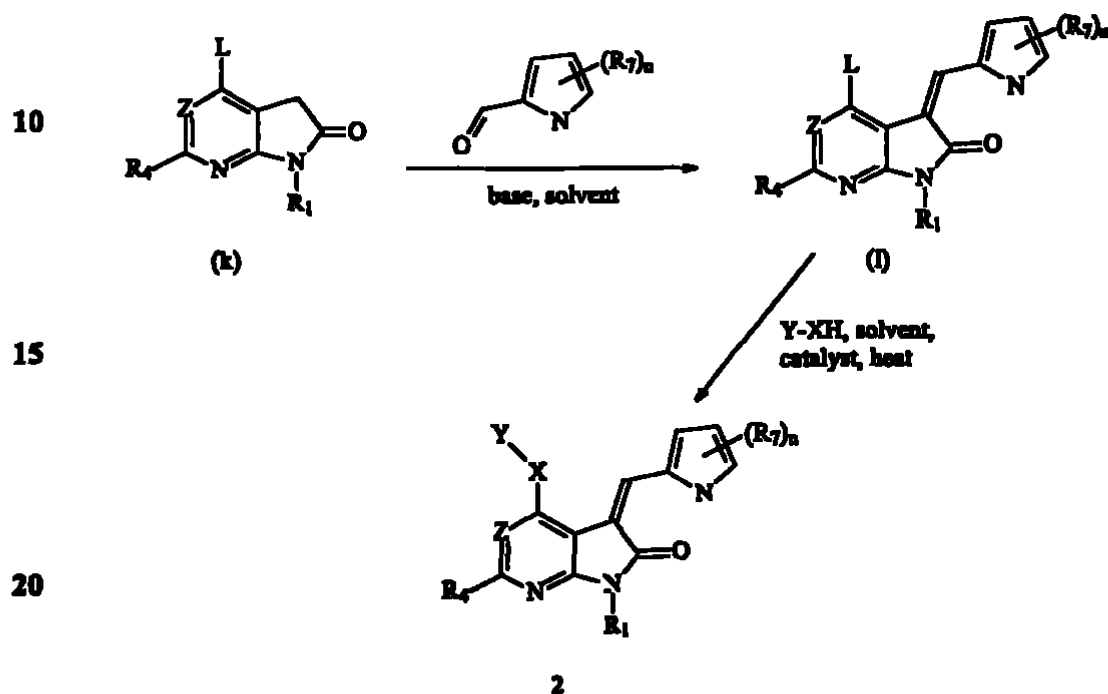
30 According to Scheme 2, compound (e) is reacted with formamidine hydrochloride under basic conditions to provide compound (f). Compound (f) is then cyclized under acidic conditions, preferably using 1N aqueous HCl, to provide compound (g). The keto moiety of compound (g) is next replaced with a chlorine using, for example, POCl_3 . The resulting compound (h) is then converted to compound (i) using $\text{C}_2\text{H}_5\text{N}-\text{HBr}-\text{Br}_2$ in *t*-butanol. This

35 reaction is preferably run at room temperature for about four hours, although those skilled in the art will recognize that the solvent and the reaction temperature and time can be varied to maximize the yield of compound (i). Finally, 4-chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidine-6-one (compound (j)) is formed by replacing the bromine atoms

216
66
220

bound to compound (i) with hydrogen atoms. This is readily accomplished using zinc in acetic acid, although other methods known to those skilled in the art can also be used.

Compounds of the invention are readily prepared from 4-chloro-1,3-dihydro-pyrrolo[2,3-b]pyridin-2-one and 4-chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one. A preferred method is shown in Scheme 3, wherein each of X, Y, Z, R₁, R₂, R₇, L, and n are defined herein:

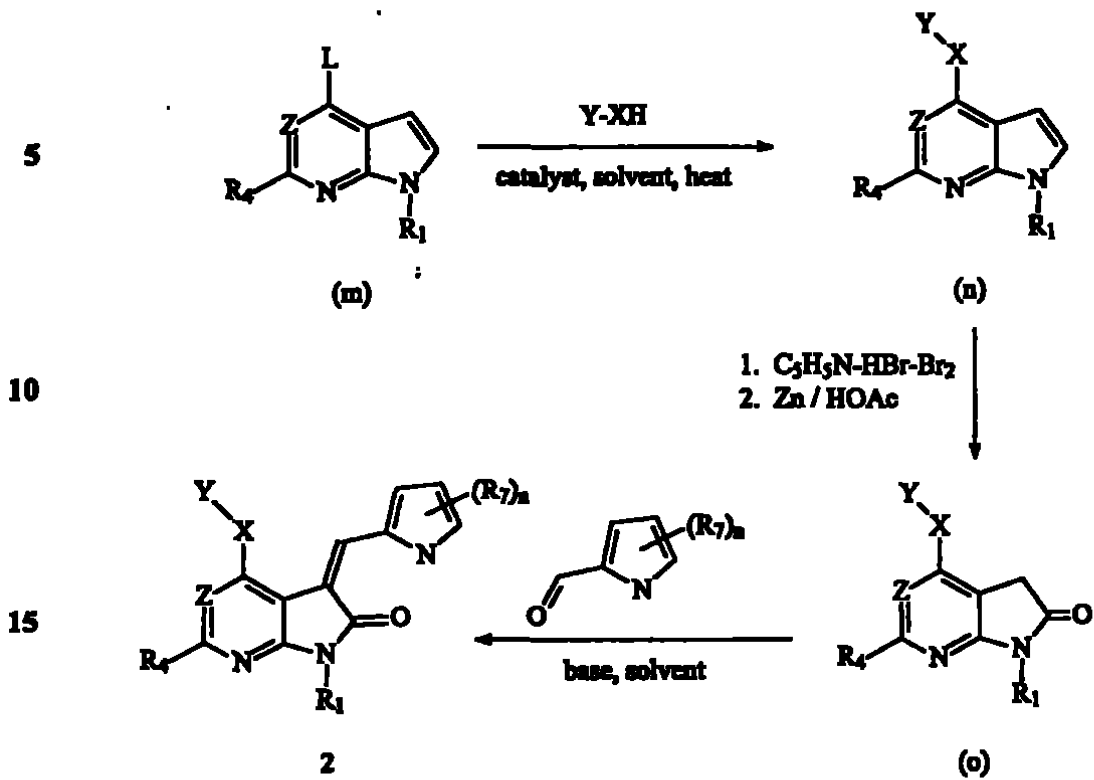


Scheme 3

According to Scheme 3, the starting material (k) is coupled with a derivative of pyrrole under suitable reaction conditions to yield compound (I). The leaving group L is then replaced with the moiety XY to provide compound 2.

Another preferred method of preparing compounds of the invention is shown in Scheme 4:

217
67
221



Scheme 4

According to Scheme 4, compound (m) is coupled with XY to form compound (n). Compound (n) is then converted to compound (o) using C₃H₅N-HBr-Br₂ in *t*-butanol. This reaction is preferably run at room temperature for about four hours, although those skilled in the art will recognize that the solvent and the reaction temperature and time can be varied to maximize the yield of compound (o). Finally, compound (o) is coupled with a derivative of pyrrole under suitable reaction conditions to yield compound 2.

4.2. BIOLOGICAL ACTIVITY

The ability of a compound of the invention to affect the activity of a protein kinase can be readily determined using methods well known to those skilled in the art. For example, a compound can be contacted (*in vitro* or *in vivo*) with cells that express a kinase of interest, after which: (a) phenotypic changes in the cell culture can be scored as compared to control cells that were not exposed to the compound; or (b) cell lysates can be prepared to assess phosphorylated proteins.

This latter approach is illustrated by several methodologies. A common technique involves incubating cells with a ligand and radioactive phosphate, lysing the cells, separating cellular components using an SDS-polyacrylamide gel (SDS-PAGE) technique, in either one or two dimensions, and then detecting the presence of phosphorylated proteins

218
68
222

- by exposing X-ray film. A similar technique involves separating cellular components using SDS-PAGE, transferring the separated components to a solid support such as a sheet of nitrocellulose, and then detecting the presence of phosphorylated tyrosines using an antiphosphotyrosine antibody (anti-PY). The anti-PY can be detected by labeling it with a radioactive substance, which then requires scanning the labeled nitrocellulose with a piece of specialized equipment designed to detect radioactivity or exposure of X-ray film. Alternatively, the anti-PY can be labeled with an enzyme, such as horseradish peroxidase, and detected by subsequent addition of a colourimetric substrate for the enzyme. A further alternative involves detecting the anti-PY by reacting it with a second antibody that recognizes the anti-PY and is labeled with either a radioactive moiety or an enzyme as previously described. Examples of these and similar techniques are described in Hansen *et al.*, *Electrophoresis* 14:112-126 (1993); Campbell *et al.*, *J. Biol. Chem.* 268:7427-7434 (1993); Donato *et al.*, *Cell Growth and Diff.* 3:258-268 (1992); and Katagiri *et al.*, *J. Immunol.* 150:585-593 (1993).
- Other, ELISA-type, assays that can be used to determine the biological activity of a compound of the invention are disclosed by Peraldi *et al.*, *J. Biochem.* 285:71-78 (1992); Schraag *et al.*, *Analytical Biochemistry* 211:233-239 (1993); Cleavland, *Analytical Biochemistry* 190:249-253 (1990); Farley, *Analytical Biochemistry* 203:151-157 (1992); and Lázaro, *Analytical Biochemistry* 192:257-261 (1991).
- A preferred method of determining the ability of a compound of the invention to affect protein kinase activity is disclosed by U.S. patent application no. 08/234,440, which is incorporated herein by reference. According to this method, a target cell that expresses a kinase and is phosphorylated or dephosphorylated during signal transduction is exposed to a compound of the invention. The target cell is thereafter lysed to release cellular contents, which include the protein substrate. The substrate is isolated by contacting the cell lysate with a substrate-specific antibody immobilized on a solid support and subsequently washing away other cellular components. An immunoassay is performed on the isolated substrate to detect the presence or absence of phosphotyrosine residues on the substrate as compared to lysates of control target cells that were not exposed to the compound of interest. Other preferred methods of measuring the biological effects of compounds of the invention are described below in Examples 20-24.

4.3. PHARMACEUTICAL COMPOSITIONS AND METHODS OF TREATMENT

- Compounds of the invention (herein also referred to as "active ingredients" or "active compounds") can be used to regulate, modulate, or inhibit protein kinase activity. They can thus be used in the treatment and/or prevention of a disease or disorder in birds and animals. Preferred patients are mammals, and particularly humans. Examples of diseases and disorders that can be treated or prevented by methods of the invention include,

219
65
223

but are not limited to, blood vessel proliferative disorders such as, but not limited to, arthritis and restenosis; fibrotic disorders such as, but not limited to, hepatic cirrhosis and atherosclerosis; mesangial cell proliferative disorders such as, but not limited to, glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic
5 microangiopathy syndromes, organ transplant rejection, and glomerulopathies; metabolic disorders such as, but not limited to, psoriasis, diabetes mellitus, chronic wounds, inflammation, and neurodegenerative diseases; auto-immune diseases; allergies; asthma; thrombosis; nervous system diseases; and cancer. Examples of cancers include, but are not limited to, breast, stomach, ovary, colon, lung (including non-small cell lung cancer), brain,
10 larynx, lymphatic system, genitourinary tract (including bladder and prostate), ovarian, gastric, bone, and pancreatic cancer.

4.3.1. ROUTES OF ADMINISTRATION AND DOSAGE FORMS

Compounds of the invention can be administered to a patient by any suitable route,
15 including, but not limited to, oral, transdermal, topical, parenteral (e.g., subcutaneous, intrathecal, intramuscular, and intravenous), and mucosal (e.g., rectal, vaginal, and nasal) routes. Dosage forms encompassed by the invention include, but are not limited to, tablets, caplets, capsules, troches, dispersions, suspensions, suppositories, solutions, creams, patches, solutions, lyophilized solids suitable for reconstitution into solutions, and aerosols
20 (e.g., in the form of minipumps).

A compound of the invention can be administered in a local rather than systemic manner by, for example, injection of the compound directly into a solid tumor, often in a depot or sustained release formulation. A compound can further be administered in a targeted drug delivery system, for example, in a liposome coated with tumor-specific
25 antibody. The liposome will be targeted to, and taken up selectively by, the tumor.

Pharmaceutical compositions of the invention can be manufactured in a manner well known to those skilled in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Pharmaceutical compositions can thus be formulated using one or
30 more physiologically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into pharmaceutically acceptable preparations. Proper formulation is dependent upon the route of administration chosen.

For injection, an active ingredient can be formulated in an aqueous solution, preferably in a physiologically compatible buffer such as Hanks's solution, Ringer's
35 solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, an active ingredient can be combined with one or more of

the many pharmaceutically acceptable carriers well known in the art. Such carriers enable compounds of the invention to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, and suspensions for oral ingestion by a patient. Pharmaceutical preparations for oral use can be obtained by admixing a compound of the invention with a solid excipient, optionally grinding the resulting mixture, processing the mixture into granules, and adding optional suitable auxiliaries to obtain tablets or dragee cores. Suitable excipients include, for example, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents can be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores, which can be lactose-free, are provided with suitable coatings to provide tablets of the invention. For this purpose, concentrated sugar solutions can be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations that can be orally administered include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain an active ingredient in admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compound can be dissolved or suspended in any suitable liquid, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. Stabilizers can also be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, dosage forms of the invention can take the form of tablets
30 or lozenges formulated in conventional manner.

For administration by inhalation, active ingredients of the invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, or carbon dioxide. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator can be formulated to contain a powder mix of the compound and a suitable powder base such as lactose or starch.

221
X
223

Compounds of the invention can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions
5 in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic
10 solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions can contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension can also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the
15 preparation of highly concentrated solutions.

Active ingredients of the invention can also be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

Compounds of the invention can also be formulated in rectal compositions such as suppositories or retention enemas containing, for example, conventional suppository bases
20 such as cocoa butter or other glycerides. The compounds disclosed herein can further be formulated as depot preparations. Such long acting formulations can be administered by implantation (e.g., subcutaneous or intramuscular) or by intramuscular injection. Thus, for example, an active ingredient can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as a
25 sparingly soluble derivative, for example as a sparingly soluble salt.

A preferred pharmaceutical carrier for compounds of the invention that are hydrophobic is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The cosolvent system can be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the
30 nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:D5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system can be varied
35 considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components can be varied: for example, other low-toxicity nonpolar surfactants can be used instead of polysorbate 80; the fraction size of polyethylene glycol can be varied; other biocompatible polymers can replace polyethylene glycol, e.g., -

222
72
226

polyvinyl pyrrolidone; and other sugars or polysaccharides can substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds can be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also
5 can be employed, although usually at the cost of greater toxicity. Additionally, compounds of the invention can be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. A variety of sustained-release materials are well known by those skilled in the art. Sustained-release capsules can, depending on their chemical nature, release the compounds for a few weeks
10 up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization can be employed.

Pharmaceutical compositions also can comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives,
15 gelatin, and polymers such as polyethylene glycols.

In addition to the common dosage forms set out herein, the compounds of the invention can be administered by controlled release means and/or delivery devices including, but not limited to, Alzet® osmotic pumps which are available from Alza Corporation, Palo Alto, CA. Suitable delivery devices are described in U.S. Patent Nos.
20 3,536,809; 3,598,123; 3,845,770; 3,916,899; 3,944,064; and 4,008,719, the disclosures of which are incorporated herein by reference.

Compositions of the invention can, if desired, be presented in a pack or dispenser device which can contain one or more unit dosage forms containing the active ingredient. The pack can, for example, comprise metal or plastic foil, such as a blister pack. The pack
25 or dispenser device can be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier can also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated on the label can include treatment of a tumor, such as a glioma or glioblastoma, and inhibition of angiogenesis.

30 Pharmaceutical compositions suitable for use in this invention include compositions wherein the active ingredient(s) is contained in an effective amount to achieve its intended purpose. Determination of effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

35

4.3.2. DOSAGES

For any compound used in the methods of the invention, a therapeutically or prophylactically effective dose can be initially estimated from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration-

001171288-1121171

223
23
227

range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the kinase activity). Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective amount refers to that amount of a compound that results in amelioration of symptoms or a prolongation of survival in a patient. A prophylactically effective amount refers to that amount of a compound which is sufficient to prevent or slow the onset of a disease or condition. Toxicity and therapeutic efficacy of compounds of the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals wherein the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population) is determined. The dose ratio between toxic and therapeutic or prophylactic effects is the therapeutic index and it can be expressed as the ratio of LD_{50} to ED_{50} . Compounds that exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration, and dosage can be chosen by the individual physician in view of the patient's condition. See, e.g., Fingl *et al.*, *The Pharmacological Basis of Therapeutics* 1 (1975).

Dosage amount and interval can be adjusted individually to provide plasma levels of the active ingredient that are sufficient to maintain the kinase modulating effects, or minimal effective concentration (MEC). The MEC will vary for each compound, but can be estimated from *in vitro* data; e.g., the concentration necessary to achieve a 50-90% inhibition of the kinase using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration, but high performance liquid chromatography (HPLC) assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using the MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

Usual patient dosages for systemic administration range from 1 to 2000 mg/day, commonly from 1 to 250 mg/day, and typically from 10 to 150 mg/day. Stated in terms of patient body weight, usual dosages range from 0.02 to 25 mg/kg/day, commonly from 0.02 to 3 mg/kg/day, typically from 0.2 to 1.5 mg/kg/day. Stated in terms of patient body surface areas, usual dosages range from 0.5 to 1200 $mg/m^2/day$, commonly from 0.5 to 150 $mg/m^2/day$, typically from 5 to 100 $mg/m^2/day$. Usual average plasma levels should be maintained within 50 to 5000 $\mu g/ml$, commonly 50 to 1000 $\mu g/ml$, and typically 100 to 500

224
74
228

5 $\mu\text{g/ml}$, although in cases of local administration or selective uptake the effective local concentration of the drug can not be related to plasma concentration. As those skilled in the art will recognized, however, these dosages and plasma levels will vary with the patient, the disease treated (e.g., different cancers may require different dosages), the route of administration, and the particular active ingredient used. The dose, and perhaps the dosage frequency, will also vary according to the age, body weight, and response of the individual patient. It is thus recommended that infants, children, and patients over 65 years, and those with impaired renal, or hepatic function, initially receive low doses, and that they be titrated based on individual clinical response(s) and blood level(s).

10 Desirable blood levels can be maintained by a continuous infusion of the compound as ascertained by plasma levels measured by HPLC. It should be noted that the attending physician would know how and when to terminate, interrupt, or adjust therapy to lower dosage due to toxicity, or to bone marrow, liver, or kidney dysfunction. The attending physician would also know to adjust treatment to higher levels if the clinical response is not

15 adequate (precluding toxicity).

Further advantages of the invention can be understood from the following non-limiting Examples.

5. EXAMPLES

5.1. EXAMPLE 1

SYNTHESIS OF 4-CHLORO-1,3-DIHYDRO-PYRROLO[2,3-b]PYRIDIN-2-ONE

20 1H-Pyrrolo[2,3-b]pyridine 7-oxide (1.86 g, 13.9 mmol, literature reference: *J. Org. Chem.* 45(20):4045-8 (1980)) was dissolved in 10 mL of phosphorus oxychloride. The reaction mixture was refluxed for 6 hours, cooled to room temperature and concentrated.

25 The residue was extracted with ethyl acetate/water. The organic layer was washed with brine to pH 6, dried over anhydrous sodium sulfate, concentrated and dried in a vacuum oven overnight to give 0.88 g (42%) of 4-chloro-1H-pyrrolo[2,3-b]pyridine as a tan solid. ^1H NMR (360 MHz, DMSO- d_6) δ 11.99 (s, br, 1H, NH), 8.16 (d, $J = 5.15$ Hz, 1H), 7.57 (t, br, $J = 3.38$ Hz, 1H), 7.17 (d, $J = 5.15$ Hz, 1H), 6.49 (dd, $J = 1.80, 3.38$ Hz, 1H).

30 MS m/e 153 [M^+].

To a stirred solution of 4-chloro-1H-pyrrolo[2,3-b]pyridine (0.4 g, 2.6 mmol) in t-butanol (25 mL) was added pyridinium bromide perbromide (PBPB, 2.5 g, 7.8 mmol) portionwise. The reaction mixture was stirred at room temperature for 3 hours and added another 0.39 g (3.1 mmol) of PBPB. The resultant reaction mixture was further stirred for 1

35 hour and extracted with ethyl acetate/water. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated. Trituration of the crude product with dichloromethane gave 0.733 g (86%) of 3,3-dibromo-4-chloro-1,3-dihydro-pyrrolo[2,3-b]pyridin-2-one as a tan solid. ^1H NMR (360 MHz, DMSO- d_6) δ 12.23 (s, br, -

00171238.122177

225
X
229

NH), 8.20 (d, $J = 5.66$ Hz, 1H), and 7.30 (d, $J = 5.66$ Hz, 1H). MS m/e 326 [M^+].

A mixture of 3,3-dibromo-4-chloro-1,3-dihydro-pyrrolo[2,3-*b*]pyridin-2-one (0.745 g, 2.3 mmol), zinc dust (1.49 g, 23 mmol), acetic acid (10 mL) and methanol (10 mL) was stirred at room temperature for 2 hours. The reaction mixture was then diluted with brine and extracted with ethyl acetate. The organic layer was further washed with brine, dried over anhydrous sodium sulfate, concentrated and dried in a vacuum oven to give 0.3 g (78%) of 4-chloro-1,3-dihydro-pyrrolo[2,3-*b*]pyridin-2-one as a tan solid. ^1H NMR (360 MHz, DMSO- d_6) δ 11.21 (s, br, NH), 8.01 (d, $J = 5.66$ Hz, 1H), 7.23 (d, $J = 5.66$ Hz, 1H), and 3.59 (s, 2H, CH_2). MS m/e 168 [M^+].

10

5.2. EXAMPLE 2

SYNTHESIS OF 4-CHLORO-5,7-DIHYDRO-PYRROLO[2,3-*d*]PYRIMIDIN-6-ONE

To a solution of 45.2 mmol of sodium ethoxide (made in situ from sodium and absolute ethanol) in 40 mL of absolute ethanol was added formamidine hydrochloride (1.74 g, 21.5 mmol) and 2-cyano-4,4-diethoxy-butyric acid ethyl ester (2.47 g, 11 mmol, literature reference: Davoll, J., *J. Chem. Soc.* 131-138 (1960)). The mixture was refluxed for 6 hours, cooled to room temperature and filtered. The solid was washed with hot acetonitrile. The filtrate was neutralized with acetic acid to pH 6.5 and then evaporated to half of the volume. Ethyl acetate (50 mL) was added to the resultant filtrate. The precipitate was filtered, washed with EtOAc to give 1.6 g (66%) of 6-amino-5-(2,2-diethoxy-ethyl)-3*H*-pyrimidin-4-one as a white solid which was used without further purification. ^1H NMR (300 MHz, DMSO- d_6) δ 11.46 (s, br, 1H, NH), 7.68 (s, 1H), 6.07 (s, br, 2H, NH_2), 4.54 (t, $J = 6.0$ Hz, 1H, $CH(OCH_2CH_3)_2$), 3.53-5.62 (m, 2H, $CH(OCH_2CH_3)_2$), 3.31-3.49 (m, 2H, $CH(OCH_2CH_3)_2$), 2.50 (m, 2H, CH_2), and 1.05 (t, $J = 6.93$ Hz, 6H, $CH(OCH_2CH_3)_2$).

6-Amino-5-(2,2-diethoxy-ethyl)-3*H*-pyrimidin-4-one (150 mg, 0.7 mmol) was dissolved in 5.0 mL of 1N HCl solution. The mixture was stirred at room temperature for 1 hour and the precipitate was filtered, washed with minimal amount of water and dried in a vacuum oven overnight to give 60 mg (64%) of 3,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-4-one as a white solid. ^1H NMR (360 MHz, DMSO- d_6) δ 11.83 (s, 1H, NH), 1.75 (s, 1H, NH), 7.80 (s, 1H), 7.01 (dd, $J = 2.65, 3.21$ Hz, 1H), and 6.42 (dd, $J = 2.09, 3.21$ Hz, 1H). MS m/e 134 [$M+1$] $^+$.

3,7-Dihydro-pyrrolo[2,3-*d*]pyrimidin-4-one (60 mg, 0.45 mmol) was reacted with 3.0 mL of phosphorus oxychloride to give 49 mg (70%) of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine as an off-white solid. ^1H NMR (300 MHz, DMSO- d_6) δ 12.57 (s, br, 1H, NH), 8.58 (s, 1H), 7.68 (dd, $J = 2.53, 3.36$ Hz, 1H), and 6.59 (dd, $J = 1.86, 3.36$ Hz, 1H). MS m/e 153 [$M-1$] $^+$.

4-Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (245 mg, 1.65 mmol) was oxidized using

226
26
230

PBPB to give 230 mg (42%) of 5,5-dibromo-4-chloro-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one as a white solid. It was then reduced using zinc dust to give 80 mg (42%) of 4-chloro-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one as a light tan solid.

5

5.3. EXAMPLE 3

SYNTHESIS OF 5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-2- OXO-1,2-DIHYDRO-PYRROLO[2,3-*B*]PYRIDIN-3-YLIDENEMETHYL]- 4-METHYL-1*H*-PYRROLE-2-CARBOXYLIC ACID (2-MORPHOLIN-4-YL-ETHYL)-AMIDE (FORMULA 3)

- 10 To ice-cold 3 mL (39.2 mmol) of *N,N*-dimethylformamide (DMF) was added phosphorus oxychloride (0.67 mL, 7.18 mmol) dropwise and the resultant mixture was stirred for 30 minutes. A solution of 1 g (6.53 mmol) of 4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester in 3 mL of DMF was added to the reaction. After 1 hour, the reaction was warmed to room temperature for another 2.5 hours. The reaction mixture was diluted with
- 15 water (100 mL) and basified to pH = 11 with 1*N* sodium hydroxide solution. The precipitate was removed by filtration, rinsing with water and dried to afford 0.8 g (68%) of 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester as a white solid. ¹H NMR (360 MHz, DMSO-*d*₆) δ 12.6 (br s, 1H, NH), 9.78 (s, 1H, CHO), 6.68 (s, 1H), 4.26 (q, *J* = 7.0 Hz, 2H, OCH₂CH₃), 2.28 (s, 3H, CH₃), 1.28 (t, *J* = 7.0 Hz, 3H, OCH₂CH₃). MS 181 [M⁺].
- 20 To a solution of 0.8 g (4.4 mmol) of 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester in 35 mL of water and 15 mL of ethanol was added 0.5 g (8.9 mmol) of potassium hydroxide. The reaction mixture was heated to 100 °C for 1 hour, cooled to room temperature, and evaporated ethanol. The water layer was acidified to pH = 3 using 2*N* hydrogen chloride solution. The precipitate was filtered and washed with water to afford
- 25 0.67 g (68%) of 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid as a tan solid. ¹H NMR (360 MHz, DMSO-*d*₆) δ 12.92 (br s, 1H, CO₂H), 12.48 (br s, 1H, NH), 9.76 (s, 1H, CHO), 6.63 (s, 1H), 2.28 (s, 3H, CH₃). MS *m/z* 152 [M-1].

- To a suspension of 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (2 g, 13 mmol) in acetonitrile was added 4-(2-aminoethyl)morpholine (1.9 mL, 14 mmol), EDC (2.7 g, 14 mmol), HOBt (1.9 g, 14 mmol) and triethylamine (3.6 mL, 26 mmol) sequentially. The
- 30 reaction mixture was stirred at room temperature for 12 hours and concentrated at reduced pressure. The residue was dissolved in 300 mL of 1*N* hydrogen chloride solution. The aqueous layer was washed with 150 mL of ethyl acetate twice and basified with sodium bicarbonate. The product was then extracted with 250 mL of dichloromethane twice, dried
- 35 over MgSO₄, and concentrated to afford 1.8 g (53%) of 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide as a yellowish solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.19 (br s, 1H, NH), 9.72 (s, 1H, CHO), 8.29 (br t, *J* = 6.6 Hz, 1H, CONH), 6.65 (d, *J* = 1.7 Hz, 1H), 3.5 (m, 4H), 3.3 (m, 2H, CONHCH₂), 2.4 (m, 6H), 2.28 (s, 3H, -

227
27
231

CH₃). MS *m/z* 265 [M⁺].

A reaction mixture of 4-chloro-1,3-dihydro-pyrrolo[2,3-*b*]pyridin-2-one (85 mg, 0.5 mmol), 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (125 mg, 0.5 mmol), 0.1 mL of piperidine and 10 mL of ethanol was stirred at room temperature overnight. The yellow precipitate was filtered after cooled with an ice bath, washed with cold ethanol and dried to give 185 mg (92%) of 5-(4-chloro-2-oxo-1,2-dihydro-pyrrolo[2,3-*b*]pyridin-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide.

A mixture of 5-(4-chloro-2-oxo-1,2-dihydro-pyrrolo[2,3-*b*]pyridin-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (10 mg, 0.024 mmol), 3-chloro-4-fluoro-phenylamine (50 mg, 0.34 mmol), *p*-toluene sulfonic acid monohydrate (5 mg, 0.026 mmol) and 1 mL of 2-methoxyethyl ether was heated to 180-190 °C in a sealed tube for 6 hours, cooled to room temperature and concentrated. The residue was then purified using preparative TLC plates eluting with 10% methanol in dichloromethane to give 5-[4-(3-chloro-4-fluoro-phenylamino)-2-oxo-1,2-dihydro-pyrrolo[2,3-*b*]pyridin-3-ylidenemethyl]-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide as an orange solid (yield is about 28%).

5.4. EXAMPLE 4

20 SYNTHESIS OF 5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-*D*]PYRIMIDIN-5-YLIDENEMETHYL]-4-METHYL-1*H*-PYRROLE-2-CARBOXYLIC ACID (2-MORPHOLIN-4-YL-ETHYL)-AMIDE HYDROCHLORIDE (FORMULA 4)

4-Chloro-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (80 mg, 0.47 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (124 mg, 0.47 mmol) at room temperature to give 50 mg (26%) of 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-5-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide as a yellow solid.

A mixture of 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-5-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (44 mg, 0.11 mmol), 3-chloro-4-fluoro-phenylamine (154 mg, 1.1 mmol) and *p*-toluenesulfonic acid monohydrate (10.1 mg, 0.053 mmol), 1-methyl-2-pyrrolidinone (2.5 mL) and 2-methoxyethyl ether (2.5 mL) was heated to 190 °C for 16 hours and concentrated. The residue was purified by reverse phase HPLC, then dissolved in 2N HCl and acetonitrile and freeze-dried to give 24 mg (39%) of 5-[4-(3-chloro-4-fluoro-phenylamino)-6-oxo-6,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-5-ylidenemethyl]-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide hydrochloride as a red solid. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.46 (s, 1H, NH), 11.79 (s, 1H, NH), 10.74 (br s, 1H, HCl), 9.33 (s, 1H), 8.83

228
29
232

(t, $J = 5.5$ Hz, 1H, NH), 8.33 (s, 1H, H-vinyl), 7.74 (dd, $J = 2.39, 6.68$ Hz, 1H), 7.38-7.47 (m, 2H), 7.35 (t, $J = 9.03$, 1H), 6.94 (s, br, 1H), 3.96 (dt, $J = 5.74, 11.37$ Hz, 2H, CH₂), 3.80 (t, $J = 12.10$ Hz, 2H, CH₂), 3.68 (dd, $J = 5.99 \& 11.61$ Hz, 2H, CH₂), 3.52 (d, br, $J = 12.44$ Hz, 2H, CH₂), 3.29-3.31 (m, 2H, CH₂), 3.10-3.13 (m, 2H, CH₂), and 2.23 (s, 3H, CH₃). MS 526 [M⁺].

5.5. EXAMPLE 5

SYNTHESIS OF 5-(6-OXO-4-PIPERIDIN-1-YL-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL)-1H-PYRROLE-2-CARBOXYLIC ACID (2-MORPHOLIN-4-YL-ETHYL)-AMIDE (FORMULA 5)

To a mixture of 4-chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (85 mg, 0.5 mmol) and 5-formyl-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (125 mg, 0.5 mmol) in ethanol (4 mL) was added 0.2 mL of piperidine. The mixture was stirred at room temperature for 20 hours. The yellow precipitate was filtered after cooled with an ice bath, washed with cold ethanol and dried to give 114 mg (50.6%) of the title compound. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.23 (br s, 1H, NH), 11.68 (br s, 1H, NH), 8.39 (m, 1H, CONHCH₂), 8.32 (s, 1H, H-vinyl), 7.11 (s, 1H), 6.91-6.98 (m, 2H), 3.56 (m, 4H, 2xCH₂), 3.38 (m, 6H, 3xCH₂), 2.39-2.47 (m, 6H, 3xCH₂), 1.65 (m, 6H, 3xCH₂). MS 452.2 [M⁺+1].

5.6. EXAMPLE 6

SYNTHESIS OF 5-[4-(1-BENZYL-1H-INDOL-5-YLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-4-METHYL-1H-PYRROLE-2-CARBOXYLIC ACID (2-MORPHOLIN-4-YL-ETHYL)-AMIDE HYDROCHLORIDE (FORMULA 6)

The title compound (8% yield) was prepared from 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-d]pyrimidin-5-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide and 1-benzyl-1H-indol-5-ylamine according to the procedure described for Example 12. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.44 (br s, 1H, NH), 11.79 (br s, 1H, NH), 10.97 (br s, 1H, HCl), 9.28 (br s, 1H), 8.85 (br s, 1H, CONHCH₂), 8.25 (s, 1H, H-vinyl), 7.1-7.5 (m, 10H), 6.89 (br s, 1H), 6.43 (s, 1H), 5.40 (s, 2H), 3.96 (m, 2H, CH₂), 3.84 (m, 2H, CH₂), 3.68 (m, 2H, CH₂), 3.52 (m, 2H, CH₂), 3.29 (m, 2H, CH₂), 3.12 (m, 2H, CH₂), 2.53 (s, 3H, CH₃).

229
29
233

5.7. EXAMPLE 7

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3,5-DIMETHYL-4-(3-MORPHOLIN-4-YL-PROPYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE

5

HYDROCHLORIDE (FORMULA 7)

To a mixture of 4-chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (85 mg, 0.5 mmol) and 3,5-dimethyl-4-(3-morpholin-4-yl-propyl)-1H-pyrrole-2-carbaldehyde (125 mg, 0.5 mmol) in ethanol (4 mL) was added 0.1 to 0.2 mL of piperidine. The mixture was stirred at room temperature for 20 hours. The yellow precipitate was filtered after cooled with an ice bath, washed with cold ethanol, and dried to give 89 mg (45%) of 4-chloro-5-[3,5-dimethyl-4-(3-morpholin-4-yl-propyl)-1H-pyrrol-2-ylmethylene]-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one.

The title compound (10% yield) was prepared from 4-chloro-5-[3,5-dimethyl-4-(3-morpholin-4-yl-propyl)-1H-pyrrol-2-ylmethylene]-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one and 3-chloro-4-fluoro-phenylamine according to the procedure described for Example 12.

5.8. EXAMPLE 8

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-(5-METHYL-3H-IMIDAZOL-4-YLMETHYLENE)-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 8)

20

4-Chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (1 eq.) was condensed with 5-methyl-3H-imidazole-4-carbaldehyde (1 eq.) at room temperature to give (76.9%) of 4-chloro-5-(5-methyl-3H-imidazol-4-ylmethylene)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one.

25

The title compound (23% yield) was prepared from 4-chloro-5-(5-methyl-3H-imidazol-4-ylmethylene)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one and 3-chloro-4-fluoro-phenylamine according to the procedure described for Example 12 without the conversion to the HCl salt. ¹H NMR (360 MHz, DMSO-d₆) δ 13.45 (br s, 1H, NH), 11.76 (br s, 1H, NH), 9.19 (s, 1H), 8.31 (s, 1H, H-vinyl), 7.92 (s, 1H), 7.73 (m, 1H), 7.45 (m, 2H), 7.36 (m, 1H), 2.33 (s, 3H, CH₃). MS 371.4 [M⁺+1].

5.9. EXAMPLE 9

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-(3,5-DIMETHYL-1H-PYRROL-2-YLMETHYLENE)-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 9)

35

4-Chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (1 eq.) was condensed with 3,5-dimethyl-1H-pyrrole-2-carbaldehyde (1 eq.) at room temperature to give (44.5%) of

4-chloro-5-(3,5-dimethyl-1*H*-pyrrol-2-ylmethylene)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one.

The title compound (57% yield) was prepared from 4-chloro-5-(3,5-dimethyl-1*H*-pyrrol-2-ylmethylene)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one and 3-chloro-4-fluoro-phenylamine according to the procedure described for Example 12 without the conversion to the HCl salt. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.10 (br s, 1H, NH), 11.60 (br s, 1H, NH), 9.10 (s, 1H), 8.27 (s, 1H, H-vinyl), 7.70 (m, 1H), 7.3-7.4 (m, 3H), 6.04 (br s, 1H), 2.33 (s, 3H, CH₃), 2.16 (s, 3H, CH₃). MS 384.3 [M⁺+1].

10

5.10. EXAMPLE 10

SYNTHESIS OF 4-METHYL-5-[4-(4-METHYL-PIPERAZIN-1-YL)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-*D*]PYRIMIDIN-5-YLIDENEMETHYL]-1*H*-PYRROLE-2-CARBOXYLIC ACID

(2-MORPHOLIN-4-YL-ETHYL)-AMIDE (FORMULA 10)

The title compound (47% yield) was prepared from 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-5-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide and 1-methylpiperazine according to the procedure described for Example 14. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.37 (br s, 1H, NH), 11.83 (br s, 1H, NH), 10.56 (v br s, 1H), 8.45 (v br s, 1H), 8.38 (s, 1H, H-vinyl), 7.09 (s, 1H), 6.87 (s, 1H), 3.4-3.6 (m, 10H, 5xCH₂), 2.85 (m, 4H, 2xCH₂), 2.4-2.6 (m, 6H, 3xCH₂), 2.49 (under DMSO, 3H, CH₃), 2.31 (s, 3H, CH₃). MS 481.3 [M⁺+1].

20

5.11. EXAMPLE 11

SYNTHESIS OF 5-(5-METHYL-3*H*-IMIDAZOL-4-YLMETHYLENE)-4-(4-METHYL-PIPERAZIN-1-YL)-5,7-DIHYDRO-PYRROLO[2,3-*D*]PYRIMIDIN-6-ONE (FORMULA 11)

The title compound (49% yield) was prepared from 4-chloro-5-(5-methyl-3*H*-imidazol-4-ylmethylene)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one and 1-methylpiperazine according to the procedure described for Example 14. MS-EI 325 [M⁺].

30

5.12. EXAMPLE 12

SYNTHESIS OF 5-[4-(1-BENZYL-1*H*-INDOL-5-YLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-*D*]PYRIMIDIN-5-YLIDENEMETHYL]-1*H*-PYRROLE-2-CARBOXYLIC ACID

(2-MORPHOLIN-4-YL-ETHYL)-AMIDE HYDROCHLORIDE (FORMULA 12)

A mixture of 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-5-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide, 1-benzyl-1*H*-indol-5-ylamine and *p*-toluenesulfonic acid (5 mg) in 2 mL of

231
231
231

1-methyl-2-pyrroline and 2-methoxyethyl ether (1:3) was heated at 170-185°C for 7 to 15 hours. The reaction mixture was evaporated to dryness and purified by reversed phase HPLC, then dissolved in 2N HCl and acetonitrile and freeze-dried to give 13.5 mg (42%) of the title compound. MS 589.3 [M+1].

5

5.13. EXAMPLE 13

SYNTHESIS OF 5-(4-MORPHOLIN-4-YL-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL)-1H-PYRROLE-2-CARBOXYLIC ACID (2-MORPHOLIN-4-YL-ETHYL)-AMIDE (FORMULA 13)

10 The title compound (35% yield) was prepared from 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-d]pyrimidin-5-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide and morpholine according to the procedure described for Example 14. MS-EI 453 [M⁺].

15

5.14. EXAMPLE 14

SYNTHESIS OF 5-(5-METHYL-3H-IMIDAZOL-4-YLMETHYLENE)-4-MORPHOLIN-4-YL-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 14)

A mixture of 4-chloro-5-(5-methyl-3H-imidazol-4-ylmethylene)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (26 mg, 0.1 mmol) and morpholine (100 mg, 1.15 mmol) in ethanol (1.5 mL) was heated in a sealed tube at 110°C for 3 hours. The reaction solution was evaporated to dryness and treated with cold ethanol. The yellow precipitate was filtered to give 22 mg (70.5%) of the title compound. MS-EI 312 [M⁺].

25

5.15. EXAMPLE 15

SYNTHESIS OF 5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-1H-PYRROLE-2-CARBOXYLIC ACID (2-MORPHOLIN-4-YL-ETHYL)-AMIDE (FORMULA 15)

30 4-Chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (1 eq.) was condensed with 5-formyl-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (1 eq.) and triethylamine in ethanol at room temperature to give (92.5%) of 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-d]pyrimidin-5-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide.

35 The title compound (16% yield) was prepared from 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-d]pyrimidin-5-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide and 3-chloro-4-fluoro-phenylamine according to the procedure described for Example 12 without the conversion to HCl salt. MS 512.3 [M+1].

232
82
236

5.16. EXAMPLE 16

SYNTHESIS OF 5-[4-(3-ETHYNYL-PHENYLAMINO)-
6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-
1H-PYRROLE-2-CARBOXYLIC ACID

5 (2-MORPHOLIN-4-YL-ETHYL)-AMIDE HYDROCHLORIDE (FORMULA 16)

The title compound (20% yield) was prepared from 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-d]pyrimidin-5-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide and 3-ethynylaniline according to the procedure described for Example 12. MS 484.3 [M⁺+1].

10

5.17. EXAMPLE 17

SYNTHESIS OF 5-[4-(3-ETHYNYL-PHENYLAMINO)-6-OXO-
6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-4-METHYL-
1H-PYRROLE-2-CARBOXYLIC ACID

15 (2-MORPHOLIN-4-YL-ETHYL)-AMIDE (FORMULA 17)

The title compound (15% yield) was prepared from 5-(4-chloro-6-oxo-6,7-dihydro-pyrrolo[2,3-d]pyrimidin-5-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide and 3-ethynylaniline according to the procedure described for Example 12 without the conversion to HCl salt. MS 498.2 [M⁺+1].

20

5.18. EXAMPLE 18

SYNTHESIS OF 5-[3,5-DIMETHYL-4-(3-MORPHOLIN-4-
YL-PROPYL)-1H-PYRROL-2-YLMETHYLENE]-4-(3-ETHYNYL-
PHENYLAMINO)-5,7-DIHYDRO-

25 PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 18)

The title compound (7% yield) was prepared from 4-chloro-5-[3,5-dimethyl-4-(3-morpholin-4-yl-propyl)-1H-pyrrol-2-ylmethylene]-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one and 3-ethynylaniline according to the procedure described for Example 12 without the conversion to HCl salt. MS 483.3 [M⁺+1].

30

5.19. EXAMPLE 19

SYNTHESIS OF 3-[5-[4-(3-CHLORO-4-FLUORO-
PHENYLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-
5-YLIDENEMETHYL]-2,4-DIMETHYL-1H-PYRROL-3-YL]-
35 PROPIONIC ACID (FORMULA 19)

To a solution of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (767.9 mg, 5 mmol) and 3-chloro-4-fluoro-phenylamine (873.4 mg, 6 mmol) in 25 mL of dry DMF under nitrogen was added silver trifluoromethanesulfonate (1.54 g, 6 mmol). The mixture was stirred at

233
932
237

95°C for 15 hours. The cooled reaction was diluted with ethyl acetate (70 mL) and filtered through celite, washed thoroughly with ethyl acetate. The filtrate was washed with brine (5x). The brine was extracted with ethyl acetate which was then washed with brine. The combined ethyl acetate was dried (magnesium sulfate), concentrated and purified to give 1.22 g (93%) of (3-chloro-4-fluoro-phenyl)-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amine as an off-white solid. m.p. 274-275 °C. ¹H NMR (360 MHz, DMSO-*d*₆) δ 11.78 (br s, 1H, NH), 9.41 (s, 1H), 8.31 (s, 1H), 8.28 (m, 1H), 7.79 (m, 1H), 7.37 (t, 1H), 7.25 (m, 1H), 6.76 (m, 1H). MS 263.4 [M⁺+1].

To a stirred solution of (3-chloro-4-fluoro-phenyl)-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amine (26.3 mg, 0.1 mmol) in one mL of t-butanol was added portionwise 64 mg (0.2 mmol) of pyridinium bromide perbromide (PBPB). The reaction mixture was stirred at room temperature for 4 hours. The reaction was diluted with ethyl acetate, washed with water, aqueous sodium sulfate, brine, dried and concentrated to give 5,5-dibromo-4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (used in the next step without any purification).

A mixture of 5,5-dibromo-4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (from above), zinc dust in 2 mL of acetic acid was stirred at room temperature for one hour. The reaction was diluted with ethyl acetate and filtered. The filtrate was washed with water, sodium bicarbonate and brine, dried, concentrated and purified to give 11.2 mg (combined yield of 40% for the 2 steps) of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one as an off-white solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.08 (br s, 1H, NH), 9.02 (s, 1H), 8.33 (s, 1H), 8.02 (m, 1H), 7.58 (m, 1H), 7.35 (t, 1H), 3.45 (s, 2H, CH₂). MS 279.3 [M⁺+1].

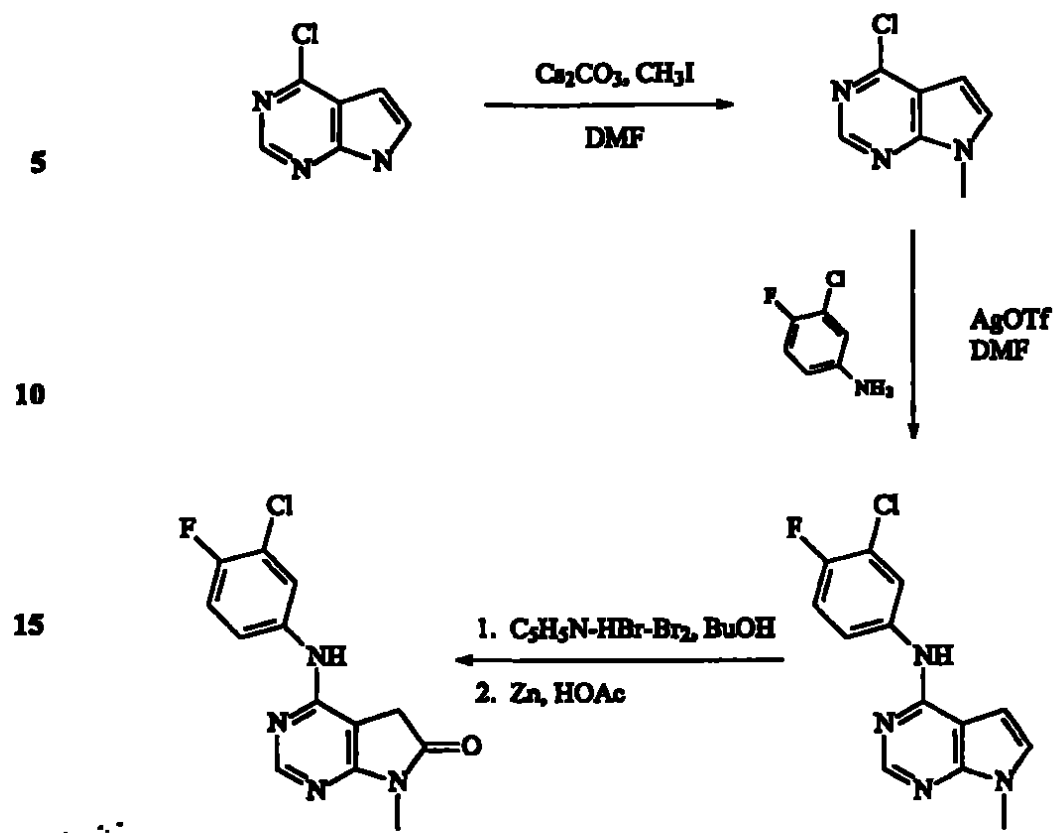
A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (6.3 mg, 0.0226 mmol), 3-(5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)-propionic acid (4.9 mg, 0.0249 mmol) and piperidine (4 drops) in ethanol (1 mL) was stirred at room temperature for 24 hours. The reaction was concentrated and purified (reversed phase HPLC) to give 5.2 mg (51%) of the title compound. MS 456.4 [M⁺+1].

5.20. EXAMPLE 20

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-7-METHYL-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE

In this specific method of the invention, the title compound is prepared according to Scheme 5:

234
 94
 238



Scheme 5

According to Scheme 5, cesium carbonate (968 mg, 3 mmol) followed by methyl iodide (1 g, 7.1 mmol) was added to a solution of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (121 mg, 0.79 mmol) in DMF (5 mL). The mixture was stirred at room temperature for 6 hours. The reaction was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried, concentrated and column chromatographed to give 116 mg (88%) of 4-chloro-7-methyl-7H-pyrrolo[2,3-d]pyrimidine as a white solid. ¹H NMR (360 MHz, DMSO-*d*₆) 8.62 (s, 1H), 7.71 (s, 1H), 6.62 (s, 1H), 3.84 (s, 3H, CH₃).

A mixture of 4-chloro-7-methyl-7H-pyrrolo[2,3-d]pyrimidine (398.8 mg, 2.4 mmol) and 3-chloro-4-fluorophenylamine (378 mg, 2.6 mmol) in DMF (6 mL) was stirred at room temperature for 2 minutes. To it was added silver triflate (672 mg, 2.6 mmol), the mixture was then stirred at 90°C for 2 hours. The reaction was then diluted with ethyl acetate and filtered off the precipitate, washing the precipitate with 10% ammonia solution. The filtrate was extracted with ethyl acetate, dried and concentrated. The residue was triturated with ethyl acetate and filtered to give 549.1 mg (83%) of (3-chloro-4-fluorophenyl)-(7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amine as an off-white solid. ¹H NMR (300 MHz, DMSO-*d*₆) 9.48 (s, 1H, NH), 8.35 (s, 1H), 8.29 (dd, *J* = 2.7 & 6.8 Hz, 1H), 7.75-7.80 (m,

235
95
239

1H), 7.37 (t, $J = 9.3$ Hz, 1H), 7.30 (d, $J = 3.1$ Hz, 1H), 6.77 (d, $J = 3.1$ Hz, 1H), 3.75 (s, 3H, CH₃). MS 277 [M⁺+1].

PBPB (2.8 g, 7.8 mmol) was added portionwise to the (3-chloro-4-fluoro-phenyl)-(7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amine (1.08 g, 3.9 mmol) suspended in *tert*-butanol (20 mL) and acetic acid (10 mL). After stirring at room temperature for 18 hours, to the mixture was added zinc dust (760 mg, 11.7 mmol) portionwise and stirring was continued for overnight. The precipitate was filtered off and the filtrate was concentrated. The residue was triturated with little water and the precipitate was collected by vacuum filtration, washed with little ethyl acetate and dried to give 1.12 g (98%) of 4-(3-chloro-4-fluoro-phenylamino)-7-methyl-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one as a tan colored solid. ¹H NMR (DMSO-*d*₆) 9.07 (s, 1H, NH), 8.42 (s, 1H), 8.01 (d, $J = 2.6$ & 7.2 Hz, 1H), 7.56 (m, 1H), 7.34 (t, $J = 9.1$ Hz, 1H), 3.48 (s, 2H, CH₂), 3.07 (s, 3H, CH₃).

5.21. EXAMPLE 21

15 SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3,5-DIMETHYL-4-(3-OXO-3-PYRROLIDIN-1-YL-PROPYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 24)

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (70 mg, 0.25 mmol), 3,5-dimethyl-4-(3-oxo-3-pyrrolidin-1-yl-propyl)-1H-pyrrole-2-carbaldehyde (64.6 mg, 0.26 mmol) and piperidine (3 drops) in ethanol was stirred at room temperature under nitrogen for 2 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 65 mg (51%) of the title compound. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.18 (br s, 1H, NH), 11.61 (br s, 1H, NH), 9.13 (br s, 1H), 8.26 (s, 1H), 7.70 (dd, $J = 2.4$ & 6.9 Hz, 1H), 7.31-7.42 (m, 3H), 3.25 (m, 4H, 2xCH₂), 2.63 (t, $J = 7.5$ Hz, 2H, CH₂), 2.35 (m, 2H, CH₂), 2.31 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 1.76 (m, 4H, 2xCH₂). MS 509 [M⁺+1].

5.22. EXAMPLE 22

30 SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3-METHYL-5-[3-(4-METHYL-PIPERAZIN-1-YL)-3-OXO-PROPYL]-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 25)

To a solution of 3-(5-formyl-4-methyl-1H-pyrrol-2-yl)-propionic acid (271.8 mg, 1.5 mmol) in DMF (4mL) was added HOBt (243.2 mg, 1.8 mmol), EDC (345.1 mg, 1.8 mmol) and then 1-methylpiperazine (0.2 mL, 1.8 mmol). The mixture was stirred at room temperature under nitrogen for overnight. The reaction was poured into sat. sodium bicarbonate (50 mL) and extracted with ethyl acetate. The combined organic layer was dried, concentrated and column chromatographed (MeOH/DCM) to give 325.2 mg (82%) of

236
95
E40

3-methyl-5-[3-(4-methyl-piperazin-1-yl)-3-oxo-propyl]-1*H*-pyrrole-2-carbaldehyde as a white solid.

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (70 mg, 0.25 mmol), 3-methyl-5-[3-(4-methyl-piperazin-1-yl)-3-oxo-propyl]-1*H*-pyrrole-2-carbaldehyde (79 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred in an oil bath at 65°C for 2 hours. The reaction was cooled at room temperature for overnight. The precipitate was collected by vacuum filtration, washed with ethanol (5x) and dried to give 88.9 mg (68%) of the title compound. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.15 (s, 1H, NH), 11.55 (br s, 1H, NH), 9.08 (s, 1H), 8.27 (s, 1H), 7.69 (dd, *J* = 2.5 & 6.9 Hz, 1H), 7.30-7.40 (m, 3H), 6.08 (d, *J* = 2.23 Hz, 1H), 3.43 (m, 4H, 2xCH₂), 2.89 (t, *J* = 7.4 Hz, 2H, CH₂), 2.68 (t, *J* = 7.4 Hz, 2H, CH₂), 2.24 (m, 4H, 2xCH₂), 2.16 (s, 6H, 2xCH₃). MS 524 [M⁺+1].

5.23 EXAMPLE 23

15 SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3-METHYL-5-(MORPHOLINE-4-CARBONYL)-1*H*-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-DIPYRIMIDIN-6-ONE (FORMULA 26)

To a suspension of 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (4 g, 26 mmol) in DMF (80 mL) was added morpholine (1.9 mL, 31 mmol), followed by EDAC (5.9 g, 31 mmol), HOBt (4.2 g, 31 mmol) and triethylamine (8 mL, 62 mmol). The reaction mixture was stirred at room temperature for 2 days. The precipitate was filtered off, washing with hexane and the filtrate was then concentrated, diluted with water and extracted with ethyl acetate (2x100 mL). The aqueous layer was adjusted to pH = 9 using sodium carbonate and extracted again. The combined organic layer was concentrated and re-suspended in ethyl acetate. The resulting precipitate was collected by vacuum filtration to give 3 g of solid. The filtrate was purified by column chromatographed to give 1.2 g of the solid. Total yield collected was 4.2 g (73%) of 3-methyl-5-(morpholine-4-carbonyl)-1*H*-pyrrole-2-carbaldehyde as a light yellow solid. ¹H NMR (30 MHz, DMSO-*d*₆) δ 12.24 (br s, 1H, NH), 9.71 (s, 1H, CHO), 6.39 (s, 1H), 3.59 (br s, 8H, 4xCH₂), 2.29 (s, 3H, CH₃).

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (93 mg, 0.33 mmol), 3-methyl-5-(morpholine-4-carbonyl)-1*H*-pyrrole-2-carbaldehyde (73 mg, 0.33 mmol), and piperidine (1 drop) in ethanol (2 mL) was stirred at room temperature for 3 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 110.3 mg (69%) of the title compound. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.43 (br s, 1H, NH), 11.74 (br s, 1H, NH), 9.23 (br s, 1H), 8.33 (s, 1H), 7.73 (dd, *J* = 2.5 & 6.7 Hz, 1H), 7.44 (m, 1H), 7.32-7.37 (m, 2H), 6.61 (d, *J* = 2.28 Hz, 1H), 3.69 (m, 4H, 2xCH₂), 3.65 (m, 4H, 2xCH₂), 2.22 (s, 3H, CH₃). MS 483 [M⁺].

237
Q7
241

5.24. EXAMPLE 24

SYNTHESIS OF 3-(2-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-5-ISOPROPYL-1H-PYRROL-3-YL)-PROPIONIC ACID (FORMULA 27)

5 A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (70 mg, 0.25 mmol), 3-(2-formyl-5-isopropyl-1H-pyrrol-3-yl)-propionic acid (54.4 mg, 0.26 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for 2 days. The precipitate was collected by vacuum filtration, washed with water, 1N HCl, water and finally ethanol, dried in vacuum oven to give 83 mg (71%) of the
10 title compound. ¹H NMR (300 MHz, DMSO-d₆) δ 13.44 (br s, 1H, NH), 12.15 (br s, 1H, COOH), 11.69 (br s, 1H, NH), 9.02 (br s, 1H), 8.29 (s, 1H), 7.77 (dd, J = 2.5 & 6.4 Hz, 1H), 7.47 (s, 1H), 7.43 (m, 1H), 7.33 (t, J = 8.9 Hz, 1H), 6.14 (d, J = 2 Hz, 1H), 3.01 (m, 1H, CH(CH₃)₂), 2.83 (t, J = 7.2 Hz, 2H, CH₂), 2.48 (m, 2H, CH₂), 1.21.27 (d, J = 6.8 Hz, 6H, CH(CH₃)₂). MS 469 [M⁺+1].

5.25 EXAMPLE 25

SYNTHESIS OF 5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-2,4-DIMETHYL-1H-PYRROLE-3-CARBOXYLIC ACID (2-DIETHYLAMINO-ETHYL)-AMIDE (FORMULA 28)

15 A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (66 mg, 0.24 mmol), 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide (63.7 mg, 0.24 mmol) and piperidine (1 drop) in ethanol (2 mL) was stirred at room temperature for 3 days. The precipitate was collected by vacuum
20 filtration, washed with ethanol and dried to give 78.5 mg (62%) of the title compound. ¹H NMR (360 MHz, DMSO-d₆) δ 13.33 (br s, 1H, NH), 11.61 (br s, 1H, NH), 9.17 (br s, 1H), 8.29 (s, 1H), 7.69 (dd, J = 2.5 & 6.5 Hz, 1H), 7.35 (m, 4H), 3.29 (m, 2H, CH₂), 2.5 (m, 6H, N(CH₂CH₃)₂ & CH₂), 2.45 (s, 3H, CH₃), 2.25 (s, 3H, CH₃), 0.96 (t, J = 7 Hz, 6H, N(CH₂CH₃)₂). MS 526 [M⁺+1].

5.26. EXAMPLE 26

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3-METHYL-4-(4-METHYL-PIPERAZINE-1-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 29)

35 A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (70 mg, 0.25 mmol), 3-methyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (70.7 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for 4 days. The precipitate was collected by vacuum filtration,

238
99
242

washed with ethanol to give 63 mg (51%) of the title compound as a yellow solid. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.29 (br s, 1H, NH), 11.73 (br s, 1H, NH), 9.16 (br s, 1H), 8.31 (s, 1H), 7.72 (dd, *J* = 2.7 & 6.5 Hz, 1H), 7.46 (d, *J* = 2.7 Hz, 1H), 7.43 (m, 1H), 7.40 (s, 1H), 7.34 (t, *J* = 9.05 Hz, 1H), 3.49 (m, 4H, 2xCH₂), 2.29 (m, 4H, 2xCH₂), 2.19 (s, 3H, CH₃), 2.18 (s, 3H, CH₃). MS 496 [M⁺+1].

5.27. EXAMPLE 27

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3,5-DIMETHYL-4-(3-MORPHOLIN-4-YL-3-OXO-PROPYL)-1*H*-PYRROL-2-YLMETHYLENE]-5,7-

DIHYDRO-PYRROLO[2,3-*D*]PYRIMIDIN-6-ONE (FORMULA 30)

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (86 mg, 0.31 mmol), 3,5-dimethyl-4-(3-morpholin-4-yl-3-oxo-propyl)-1*H*-pyrrole-2-carbaldehyde (83 mg, 0.31 mmol) and piperidine (1 drop) in ethanol (2 mL) was stirred at room temperature for 3 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give the title compound. MS 525 [M⁺+1].

5.28. EXAMPLE 28

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3-METHYL-4-(MORPHOLINE-4-CARBONYL)-1*H*-PYRROL-2-YLMETHYLENE]-5,7-

DIHYDRO-PYRROLO[2,3-*D*]PYRIMIDIN-6-ONE (FORMULA 31)

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (70 mg, 0.25 mmol), 3-methyl-4-(morpholine-4-carbonyl)-1*H*-pyrrole-2-carbaldehyde (66.7 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for 4 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 39 mg (32%) of the title compound. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.30 (br s, 1H, NH), 11.69 (br s, 1H, NH), 9.16 (br s, 1H), 8.31 (s, 1H), 7.72 (dd, *J* = 2.8 & 6.7 Hz, 1H), 7.46 (d, *J* = 2.8 Hz, 1H), 7.43 (m, 1H), 7.41 (s, 1H), 7.35 (t, *J* = 9 Hz, 1H), 3.59 (m, 4H, 2xCH₂), 3.51 (m, 4H, 2xCH₂), 2.21 (s, 3H, CH₃). MS 483 [M⁺+1].

5.29. EXAMPLE 29

SYNTHESIS OF 5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-7-METHYL-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-*D*]PYRIMIDIN-5-YLIDENEMETHYL]-4-

METHYL-1*H*-PYRROLE-2-CARBOXYLIC ACID

(2-MORPHOLIN-4-YL-ETHYL)-AMIDE (FORMULA 32)

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-7-methyl-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (244 mg, 0.83 mmol), 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (1.2 equiv.) and piperidine (1 drop) in ethanol and DMF (2 mL) was stirred at 90°C for 2 hours. The reaction was diluted with water and the precipitate

239
98
243

was collected by vacuum filtration, washed with water, ethyl acetate and hexane and dried to give 138.5 mg (31%) of the title compound. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.39 (br s, 1H, NH), 9.29 (br s, 1H), 8.38 (m, 2H), 7.72 (m, 1H), 7.39 (s, 1H, H-vinyl), 7.34 (m, 2H), 6.81 (s, 1H), 3.56 (m, 4H, 2xCH₂), 3.35 (m, 2H, CH₂), 3.31 (s, 3H, CH₃), 2.45 (m, 2H, CH₂), 2.41 (m, 4H, 2xCH₂), 2.20 (s, 3H, CH₃). MS 540 [M⁺+1].

5.30. EXAMPLE 30

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[5-(MORPHOLINE-4-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 33)

10 A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (70 mg, 0.25 mmol), 5-(morpholine-4-carbonyl)-1H-pyrrole-2-carbaldehyde (62.5 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for 4 days. The precipitate was collected by vacuum filtration, washed
15 with ethanol and dried to give 69 mg (59%) of the title compound. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.41 (br s, 1H, NH), 11.65 (br s, 1H, NH), 8.81 (br s, 1H, NH), 8.28 (s, 1H), 7.76 (s, 1H), 7.75 (m, 1H), 7.53 (m, 1H), 7.38 (t, *J* = 9.05 Hz, 1H), 6.91 (d, *J* = 3.6 Hz, 1H), 6.76 (d, *J* = 3.6 Hz, 1H), 3.71 (m, 4H, 2xCH₂), 3.65 (m, 4H, 2xCH₂). MS 469 [M⁺+1].

20 5.31. EXAMPLE 31

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-7-METHYL-5-[3-METHYL-5-(MORPHOLINE-4-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 34)

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-7-methyl-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (116 mg, 0.4 mmol), 3-methyl-5-(morpholine-4-carbonyl)-1H-pyrrole-2-carbaldehyde (130 mg) and piperidine (1 drop) in ethanol (2 mL) was stirred at 90°C for 2
25 hours. The reaction was diluted with water and the precipitate was collected by vacuum filtration, washed with water, ethyl acetate and hexane and dried to give 141 mg (74%) of the title compound. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.42 (br s, 1H, NH), 9.29 (br s, 1H), 8.38 (s, 1H), 7.72 (m, 1H), 7.34-7.41 (m, 3H), 6.6 (s, 1H), 3.67 (m, 4H, 2xCH₂), 3.65 (m, 4H, 2xCH₂), 3.29 (s, 3H, CH₃), 2.20 (s, 3H, CH₃). MS 497 [M⁺+1].
30

5.32. EXAMPLE 32

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[4-METHYL-5-(4-METHYL-PIPERAZINE-1-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 35)

Acetic acid (360 mL) and water (300 mL) was added to a solution of ethyl 3,5-dimethyl-2-pyrrolocarboxylate (5 g, 29.9 mmol) in THF (300 mL). The mixture was cooled

240
98
244

to -10 to -5 °C for 30 minutes. To the mixture was then added ceric ammonium nitrate (CAN, 67.21 g, 122.6 mmol) over a period of 30 minutes. The mixture was stirred at -10 to -5 °C for 1.5 hours. The reaction was diluted with water (800 mL) and extracted with dichloromethane (3x). The combined extracts were washed with sodium bicarbonate

5 solution until the aqueous layer was basic, dried and concentrated. The residue was purified on a silica gel column (10% EtOAc in hexane) to give 2.4 g (44%) of 5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester as a white solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.61 (br s, 1H, NH), 9.64 (s, 1H, CHO), 6.78 (s, 1H), 4.27 (q, *J* = 7.17 Hz, 2H, OCH₂CH₃), 2.26 (s, 3H, CH₃), 1.30 (t, *J* = 7.17 Hz, 3H, OCH₂CH₃). MS 182 [*M*⁺+1].

10 Lithium hydroxide (8.73 g, 36.44 mmol) was added to the 5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (1.32 g, 7.27 mmol) in methanol (27 mL) and water (9mL). The mixture was stirred at room temperature for over the weekend. The reaction was diluted with water (10 mL), evaporated most of the methanol and washed with ether. The aqueous layer was acidified with 1N HCl (37 mL) to pH = 2, extracted with ethyl acetate.

15 Washed with brine, dried and concentrated. The residue was washed with dichloromethane and filtered to give 0.98 g (88%) of 5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid as a tan solid. ¹H NMR (360 MHz, DMSO-*d*₆) δ 12.87 (br s, 1H, COOH), 12.41 (br s, 1H, NH), 9.63 (s, 1H, CHO), 6.74 (s, 1H), 2.26 (s, 3H, CH₃).

To a solution of 5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid (306.3 mg, 2
20 mmol) in DMF 5 mL was added HOBt (324.3 mg, 2.4 mmol), EDC (460.1 mg, 2.4 mmol) and then 1-methylpiperazine (0.27 mL, 2.4 mmol). The mixture was stirred at room temperature under nitrogen for overnight. The reaction was diluted with ethyl acetate and washed with sodium bicarbonate. The aqueous layer was extracted several times with ethyl acetate. The combined organic layer was washed with brine, dried, concentrated and column
25 chromatographed (MeOH/DCM) to give 310 mg (66%) of 4-methyl-5-(4-methyl-piperazine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde as a light yellow foam solid. ¹H NMR (360 MHz, DMSO-*d*₆) δ 12.20 (br s, 1H, NH), 9.47 (s, 1H, CHO), 6.80 (s, 1H), 3.58 (m, 4H, 2xCH₂), 3.44 (m, 4H, 2xCH₂), 2.03 (s, 3H, CH₃).

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-
30 *d*]pyrimidin-6-one (70 mg, 0.25 mmol), 4-methyl-5-(4-methyl-piperazine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde (70.6 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for 4 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 89.1 mg (72%) of the title compound. ¹H NMR (360
35 MHz, DMSO-*d*₆) δ 13.13 (br s, 1H, NH), 11.74 (br s, 1H, NH), 8.77 (br s, 1H, NH), 8.27 (s, 1H), 7.75 (dd, *J* = 2.7 & 6.5 Hz, 1H), 7.71 (s, 1H), 7.52 (m, 1H), 7.38 (t, *J* = 8.85 Hz, 1H), 6.74 (s, 1H), 3.52 (m, 4H, 2xCH₂), 2.37 (m, 4H, 2xCH₂), 2.21 (s, 3H, CH₃), 2.14 (s, 3H, CH₃). MS 496 [*M*⁺+1].

241
95
243

5.33. EXAMPLE 33

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-7-METHYL-5-[3-METHYL-5-(4-METHYL-PIPERAZINE-1-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-

5 PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 36)

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-7-methyl-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (80 mg, 0.27 mmol), 3-methyl-5-(4-methyl-piperazine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde (1.2 equiv.) and piperidine (1 drop) in ethanol (2 mL) was stirred at 90°C for 2 hours. The reaction was diluted with water and the precipitate was collected by vacuum filtration, washed with water, ethyl acetate and hexane and dried to give 120.5 mg (72%) of the title compound. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.42 (br s, 1H, NH), 9.32 (br s, 1H), 8.40 (s, 1H), 7.71 (m, 1H), 7.35-7.41 (m, 3H), 6.60 (s, 1H), 3.69 (m, 4H, 2xCH₂), 3.30 (s, 3H, CH₃), 2.35 (m, 4H, 2xCH₂), 2.21 (s, 3H, CH₃), 2.20 (s, 3H, CH₃). MS 510 [M⁺+1].

15

5.34. EXAMPLE 34

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[4-METHYL-5-(MORPHOLINE-4-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 37)

20 To a solution of 5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid (306.3 mg, 2 mmol) in DMF (5 mL) was added HOBt (324.3 mg, 2.4 mmol), EDC (460.1 mg, 2.4 mmol) and then morpholine (0.21 mL, 2.4 mmol). The mixture was stirred at room temperature under nitrogen for overnight. The reaction was diluted with ethyl acetate and washed with sodium bicarbonate. The aqueous layer was extracted several times with ethyl acetate. The combined organic layer was washed with brine, dried, concentrated and column chromatographed (MeOH/DCM) to give 395 mg (89%) of 4-methyl-5-(morpholine-4-carbonyl)-1*H*-pyrrole-2-carbaldehyde as a light yellow foam solid. ¹H NMR (360 MHz, DMSO-*d*₆) δ 12.18 (br s, 1H, NH), 9.46 (s, 1H, CHO), 6.79 (s, 1H), 3.43 (m, 4H, 2xCH₂), 2.29 (m, 4H, 2xCH₂), 2.17 (s, 3H, CH₃), 2.01 (s, 3H, CH₃).

30 A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (70 mg, 0.25 mmol), 4-methyl-5-(morpholine-4-carbonyl)-1*H*-pyrrole-2-carbaldehyde (66.7 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for 4 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 105 mg (87%) of the title compound. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.16 (br s, 1H, NH), 11.74 (br s, 1H, NH), 8.78 (br s, 1H), 8.27 (s, 1H), 7.74 (dd, *J* = 2.2 & 6.5 Hz, 1H), 7.71 (s, 1H), 7.52 (m, 1H), 7.38 (t, *J* = 9.1 Hz, 1H), 6.74 (s, 1H), 3.65 (m, 4H, 2xCH₂), 3.54 (m, 4H, 2xCH₂), 2.15 (s, 3H, CH₃). MS 483 [M⁺+1].

35

242
J
26

5.35. EXAMPLE 35

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-(3,5-DIMETHYL-1H-PYRROL-2-YLMETHYLENE)-7-METHYL-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 38)

5 4-(3-Chloro-4-fluoro-phenylamino)-7-methyl-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (100 mg, 0.34 mmol) was condensed with 3,5-dimethyl-1H-pyrrole-2-carbaldehyde (50 mg, 0.41 mmol) and piperidine (2 drops) in ethanol (3 mL) at 80°C for 2 hours to give 50 mg (37%) of the title compound. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.07 (br s, 1H, NH), 9.18 (s, 1H), 8.34 (s, 1H), 7.69 (m, 1H), 7.35 (s, 1H), 7.30-7.40 (m, 2H), 6.05 (s, 1H),
10 3.32 (s, 3H, CH₃), 2.34 (s, 3H, CH₃), 2.16 (s, 3H, CH₃). MS 398 [M⁺+1].

5.36 EXAMPLE 36

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3-METHYL-5-(4-METHYL-PIPERAZINE-1-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 39)

15 To a suspension of 5-formyl-4-methyl-1H-pyrrole-2-carboxylic acid (1.53 g, 10 mmol) in DMF (20 mL) was added 1-methylpiperazine (1.2 g, 12 mmol), followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC, 2.3 g, 12 mmol), 1-hydroxybenzotriazole (HOBt, 1.6g, 12 mmol) and triethylamine (3.2 mL, 24 mmol). The
20 reaction mixture was stirred at room temperature for 2 days. The reaction was concentrated, diluted with water and extracted with ethyl acetate (5x). The combined ethyl acetate was concentrated to a volume of 20 mL and triturated with hexane (10 mL). The resulting solid was collected by vacuum filtration to give 1.6 g (68%) of 3-methyl-5-(4-methyl-piperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde as a yellow solid. ¹H NMR (300 MHz, DMSO-*d*₆)
25 δ 12.21 (s, 1H, NH), 9.7 (s, 1H, CHO), 6.35 (s, 1H), 3.58 (m, 4H 2xCH₂), 2.30 (m, 7H, 2xCH₂ & CH₃), 2.18 (s, 3H, CH₃). MS 236 [M⁺+1].

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-
d]pyrimidin-6-one (70 mg, 0.25 mmol) and 3-methyl-5-(4-methyl-piperazine-1-carbonyl)-
1H-pyrrole-2-carbaldehyde (70.6 mg, 0.30 mmol) and piperidine (3 drops) in ethanol was
30 stirred at room temperature for 4 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 124 mg (100%) of the title compound. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.41 (s, 1H, NH), 11.77 (br s, 1H, NH), 9.22 (br s, 1H), 8.32 (s, 1H), 7.72 (dd, *J* = 2.7 & 6.6 Hz, 1H), 7.42 (m, 1H), 7.32-7.37 (m, 2H), 6.58 (d, *J* = 1.8 Hz, 1H), 3.68 (m, 4H, 2xCH₂), 2.36 (m, 4H, 2xCH₂), 2.22 (s, 3H, CH₃), 2.20 (s, 3H, CH₃). MS 496
35 [M⁺+1].

243
087
247

5.37. EXAMPLE 37

SYNTHESIS OF 3-{5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-7-METHYL-6- OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-2,4- DIMETHYL-1H-PYRROL-3-YL]-PROFIONIC ACID (FORMULA 40)

5 4-(3-Chloro-4-fluoro-phenylamino)-7-methyl-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-
6-one (100 mg, 0.34 mmol) was condensed with 3-(5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)-
propionic acid (80 mg, 0.41 mmol) and piperidine (2 drops) in ethanol (3 mL) at 80°C for 2
hours to give 36 mg (23%) of the title compound. ¹H NMR (300 MHz, DMSO-d₆) δ 13.15
(br s, 1H, NH), 12.07 (br s, 1H, COOH), 9.18 (s, 1H), 8.33 (s, 1H), 7.71 (m, 1H), 7.40 (s,
10 1H), 7.30-7.39 (m, 2H), 3.32 (s, 3H, CH₃), 2.63 (m, 2H, CH₂), 2.35 (m, 2H, CH₂), 2.33 (s,
3H, CH₃), 2.13 (s, 3H, CH₃).

5.38. EXAMPLE 38

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3,5-DIMETHYL- 15 4-(4-METHYL-PIPERAZINE-1-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]- 5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 41)

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-
d]pyrimidin-6-one (70 mg, 0.25 mmol), 3,5-dimethyl-4-(4-methyl-piperazine-1-carbonyl)-
1H-pyrrole-2-carbaldehyde (74.8 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL)
20 was stirred at room temperature for 4 days. The precipitate was collected by vacuum
filtration, washed with ethanol and dried to give 102 mg (80%) of the title compound. ¹H
NMR (360 MHz, DMSO-d₆) δ 13.27 (br s, 1H, NH), 11.66 (br s, 1H, NH), 9.12 (br s, 1H),
8.29 (s, 1H), 7.71 (m, 1H), 7.40 (m, 1H), 7.31-7.36 (m, 2H), 3.43 (m, 4H, 2xCH₂), 2.30 (s,
3H, CH₃), 2.27 (m, 4H, 2xCH₂), 2.18 (s, 3H, CH₃), 2.12 (s, 3H, CH₃). MS 510 [M⁺+1].

25

5.39. EXAMPLE 39

SYNTHESIS OF 3-{5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-7-METHYL-6- OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-4- METHYL-1H-PYRROL-2-YL]-PROPIONIC ACID (FORMULA 42)

30 4-(3-Chloro-4-fluoro-phenylamino)-7-methyl-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-
6-one (100 mg, 0.34 mmol) was condensed with 3-(5-formyl-4-methyl-1H-pyrrol-2-yl)-
propionic acid (62 mg, 0.41 mmol) and piperidine (2 drops) in ethanol (3 mL) stirred at
80°C for 2 hours to give 31 mg (20%) of the title compound. ¹H NMR (360 MHz, DMSO-
d₆) δ 13.14 (br s, 1H, NH), 9.14 (s, 1H), 8.34 (s, 1H), 7.69 (m, 1H), 7.30-7.40 (m, 3H), 6.08
35 (s, 1H), 3.32 (s, 3H, CH₃), 2.89 (m, 2H, CH₂), 2.53 (m, 2H, CH₂), 2.16 (s, 3H, CH₃).

244
03A
248

5.40. EXAMPLE 40

SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3,5-DIMETHYL-4-(MORPHOLINE-4-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 43)

- 5 A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (70 mg, 0.25 mmol), 3,5-dimethyl-4-(morpholine-4-carbonyl)-1H-pyrrole-2-carbaldehyde (70.9 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for 4 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 98.5 mg (79%) of the title compound. ¹H NMR (360
- 10 MHz, DMSO-*d*₆) δ 13.28 (br s, 1H, NH), 11.68 (br s, 1H, NH), 9.12 (br s, 1H), 8.29 (s, 1H), 7.71 (m, 1H), 7.40 (m, 1H), 7.31-7.36 (m, 2H), 3.56 (m, 4H, 2xCH₂), 3.45 (m, 4H, 2xCH₂), 2.31 (s, 3H, CH₃), 2.14 (s, 3H, CH₃). MS 497 [M⁺+1].

5.41. EXAMPLE 41

15 SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3,5-DIMETHYL-4-(3-MORPHOLIN-4-YL-PROPYL)-1H-PYRROL-2-YLMETHYLENE]-7-METHYL-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 44)

- A mixture of 4-(3-chloro-4-fluoro-phenylamino)-7-methyl-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (100 mg, 0.34 mmol), 3,5-dimethyl-4-(3-morpholin-4-yl-propyl)-1H-pyrrole-2-carbaldehyde (85 mg, 0.34 mmol) and piperidine (2 drops) in ethanol (3 mL) was
- 20 stirred at 80°C for 1 hour. The reaction was concentrated and column chromatographed to give 46 mg (26%) of the title compound as a light yellow solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.14 (br s, 1H, NH), 9.17 (s, 1H), 8.33 (s, 1H), 7.68 (m, 1H), 7.35 (s, 1H, H-vinyl), 7.32 (m, 2H), 3.55 (m, 4H, 2xCH₂), 3.30 (s, 3H, CH₃), 2.40 (m, 2H, CH₂), 2.31 (s,
- 25 3H, CH₃), 2.30 (m, 4H, 2xCH₂), 2.20 (m, 2H, CH₂), 2.10 (s, 3H, CH₃), 1.55 (m, 2H, CH₂).

5.42. EXAMPLE 42

30 SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[5-(4-METHYL-PIPERAZINE-1-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 45)

- A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (70 mg, 0.25 mmol), 5-(4-methyl-piperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (66.4 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at
- 35 room temperature for 4 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 101.5 mg (84%) of the title compound. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.39 (br s, 1H, NH), 11.74 (br s, 1H, NH), 8.81 (br s, 1H), 8.28 (s, 1H), 7.76 (s, 1H), 7.75 (m, 1H), 7.53 (m, 1H), 7.39 (t, *J* = 8.9 Hz, 1H), 6.91 (m, 1H), 6.74 (m, 1H), 3.70 (m, 4H, 2xCH₂), 2.37 (m, 4H, 2xCH₂), 2.21 (s, 3H, CH₃). MS 482 [M⁺+1].

245
GJ
249

5.43. EXAMPLE 43

SYNTHESIS OF 5-[3-METHYL-5-(MORPHOLINE-4-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-4-(1-PHENYL-ETHYLAMINO)-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 46)

5 A mixture of 4-chloro-5-[3-methyl-5-(morpholine-4-carbonyl)-1H-pyrrol-2-ylmethylene]-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (37 mg, 0.1 mmol) and (R)-(+)-1-phenylethylamine (121 mg, 1 mmol) in 2-methoxyethanol (0.5 mL) was heated at 110-120°C for 1.5 hours. The reaction was diluted with water and filtered. The precipitate was washed with water, little ethanol, ethyl acetate, hexane and dried to give 38.1 mg (81%) of
10 the title compound as a yellow solid. MS 459 [M⁺+1].

5.44. EXAMPLE 44

SYNTHESIS OF 5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-4-METHYL-1H-PYRROLE-2-CARBOXYLIC ACID (FORMULA 47)

15 A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (70 mg, 0.25 mmol), 5-formyl-4-methyl-1H-pyrrole-2-carboxylic acid (45.9 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for 4 days. The precipitate was collected by vacuum filtration, washed with
20 ethanol and dried, then washed with 1N HCl (3x), water (5x) and dried to give 88.8 mg (86%) of the title compound. ¹H NMR (360 MHz, DMSO-d₆) δ 13.55 (br s, 1H, NH), 11.84 (br s, 1H, NH), 9.28 (s, 1H), 8.34 (s, 1H), 7.74 (m, 1H), 7.43 (m, 1H), 7.41 (s, 1H), 7.38 (t, J = 9.1 Hz, 1H), 6.74 (d, J = 2.15 Hz, 1H), 2.22 (s, 3H, CH₃). MS 414 [M⁺+1].

5.45. EXAMPLE 45

SYNTHESIS OF 5-[3-METHYL-5-(MORPHOLINE-4-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-4-PIPERIDIN-1-YL-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 48)

25 A mixture of 4-chloro-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (169 mg, 1 mmol), 3-methyl-5-(morpholine-4-carbonyl)-1H-pyrrole-2-carbaldehyde (222 mg, 1 mmol) and piperidine (2 drops) in ethanol (3 mL) was heated to reflux for overnight. The precipitate was collected by vacuum filtration, washed with ethyl acetate/ hexane and dried to give 332.2 mg (79%) of the title compound as a yellow solid. MS 423 [M⁺+1].
30

246 96 240

5.46. EXAMPLE 46

SYNTHESIS OF 3-[5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-4-METHYL-1H-PYRROL-2-YL]-PROPIONIC ACID (FORMULA 49)

5 Sodium *tert*-butoxide (1.37 g, 14.3 mmol) was added to the dry *tert*-butoxycarbonylmethyl)triphenylphosphonium bromide (6.56 g, 14.3 mmol) in THF (anhydrous, 30 mL) under nitrogen. The mixture was stirred at room temperature for 30 minutes. To the mixture was added 5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (2.36 g, 13 mmol) in THF (35 mL). The mixture was then stirred at room temperature

10 under nitrogen for overnight. The solid was filtered off and the filtrate was concentrated followed by column chromatograph (10% EtOAc in hexane) to give 2.95 g (81 %) of 5-(2-*tert*-butoxycarbonyl-vinyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.85 (br s, 1H, NH), 7.35 (d, *J* = 15.7 Hz, 1H), 6.73 (s, 1H), 6.41 (d, *J* = 15.7 Hz, 1H), 4.23 (q, *J* = 7.1 Hz, 2H, OCH₂), 2.22 (s, 3H, CH₃), 1.44 (s, 9H, 3xCH₃), 1.28 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃). MS 279 [M⁺].

A solution of 5-(2-*tert*-butoxycarbonyl-vinyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (2.48 g) in ethyl acetate (60 mL) and ethanol (30 mL) was hydrogenated using 1% palladium on carbon at room temperature for overnight to give 2.49 g (100%) of 5-(2-*tert*-butoxycarbonyl-ethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester as a

20 white solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.12 (br s, 1H, NH), 5.74 (d, *J* = 2.3 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H, OCH₂), 2.71 (t, *J* = 7.5 Hz, 2H, CH₂), 2.47 (t, *J* = 7.5 Hz, 2H, CH₂), 2.18 (s, 3H, CH₃), 1.36 (s, 9H, 3xCH₃), 1.28 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃). MS 282 [M⁺+1].

Lithium hydroxide (1.02 g, 42.65 mmol) was added to a suspension of 5-(2-*tert*-butoxycarbonyl-ethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (2.4 g, 8.53 mmol) in methanol (50 mL) and water (12 mL). Ethanol (4 mL) was added to the mixture (to aid solvation of the starting material) and it was stirred at room temperature for overnight. The reaction was diluted with water (20 mL), concentrated (to remove most of the alcohol) and extracted with ether. The aqueous layer was the acidified to pH = 2 using

30 1N HCl (43 mL) and extracted with ethyl acetate (2x). The combined extract was then washed with brine, dried and concentrated to give 1.89 g of 5-(2-carboxy-ethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester as a white solid.

A mixture of 5-(2-carboxy-ethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (1.8 g, 8 mmol) and lithium hydroxide (1.94 g, 8 mmol) in THF (40 mL) and water (48 mL)

35 was stirred at room temperature for 2 days then in an oil bath of 80°C for 4 hours. The reaction was cooled to room temperature, acidified to pH = 2 using concentrated HCl, extracted with ethyl acetate. The organic layer was then dried and concentrated to give 1.32 g of 5-(2-carboxy-ethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid.

A solution of 5-(2-carboxy-ethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (1.3 g, 6.59 mmol) in trifluoroacetic acid (6 mL) was stirred at room temperature for 10 minutes. It was then cooled to 0°C and to it was added triethyl orthoformate (6 mL). The mixture was stirred at 0°C for 10 minutes and at room temperature for 10 minutes. The reaction was poured into water (50 mL) and extracted with ethyl acetate. The extract was dried and concentrated to give 1.2 g of 3-(5-formyl-4-methyl-1*H*-pyrrol-2-yl)-propionic acid as a brown solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.17 (br s, 1H), 11.50 (br s, 1H), 9.44 (s, 1H, CHO), 5.85 (s, 1H), 2.74 (t, *J* = 7.4 Hz, 2H, CH₂), 2.54 (t, *J* = 7.4 Hz, 2H, CH₂), 2.23 (s, 3H, CH₃). MS 182 [*M*⁺+1].

10 A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-
d]pyrimidin-6-one (70 mg, 0.25 mmol), 3-(5-formyl-4-methyl-1H-pyrrol-2-yl)-propionic
acid (54.4 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room
temperature for 4 days. The reaction was concentrated, the residue was dissolved in water
and acidified with 1N HCl to pH=2. The resultant solid was then washed with water,
15 ethanol and dried to give 93.9 mg (85%) of the title compound. ¹H NMR (360 MHz,
DMSO-*d*₆) δ 13.18 (br s, 1H, NH), 12.19 (br s, 1H, COOH), 11.61 (br s, 1H, NH), 9.09 (s,
1H), 8.27 (s, 1H), 7.70 (m, 1H), 7.39 (m, 1H), 7.30-7.35 (m, 2H), 6.07 (s, 1H), 2.90 (t, *J*=
7.2 Hz, 2H, CH₂), 2.60 (t, *J*= 7.2 Hz, 2H, CH₂), 2.16 (s, 3H, CH₃). MS 442 [*M*⁺+1].

5.47. EXAMPLE 47

SYNTHESIS OF 4-(INDAN-4-YLAMINO)-5-[3-METHYL-5-(MORPHOLINE-4-CARBONYL)-1*H*-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,1-*b*]PYRIMIDIN-6-ONE (FORMULA 50)

A mixture of 4-chloro-5-[3-methyl-5-(morpholine-4-carbonyl)-1*H*-pyrrol-2-ylmethylene]-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (50 mg, 0.13 mmol), 5-aminoindan (178.12 mg, 1.3 mmol) in 2-methoxyethanol (0.6 mL) was heated at 110-120°C for 8 hours and extracted with ethyl acetate/ water. The organic layer was concentrated, the residue was triturated with acetone and filtered to give 29 mg of a yellow solid. The mother liquor was concentrated and column chromatographed to give 10.1 mg of the product. Total yield was 39.1 mg (64%) of the title compound as a yellow solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.42 (br s, 1H, NH), 11.74 (br s, 1H, NH), 9.11 (br s, 1H), 8.27 (s, 1H, H-vinyl), 7.25 (br s, 1H, NH), 7.04-7.15 (m, 3H), 6.57 (d, *J* = 2.2 Hz, 1H), 3.63 (m, 8H, 4xCH₂), 2.79 (m, 4H, 2xCH₂), 2.08 (s, 3H, CH₃), 1.99 (m, 2H, CH₂). MS 471 [*M*⁺+1].

248
GG
ZSL

5.48. EXAMPLE 48

SYNTHESIS OF 5-[4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-6-OXO-6,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-5-YLIDENEMETHYL]-2,4-DIMETHYL-1H-PYRROL-3-YL-ACETIC ACID (FORMULA 51)

- 5 A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (70 mg, 0.25 mmol), (5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)-acetic acid (54.3 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for 1 day. The reaction was concentrated, the residue was suspended in 1N HCl. The precipitate was filtered, washed with water, suspended in hot ethyl acetate, 10 filtered and dried to give 42 mg (38%) of the title compound. ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.21 (br s, 1H, NH), 12.17 (br s, 1H, COOH), 11.65 (s, 1H, NH), 9.13 (s, 1H), 8.26 (s, 1H), 7.71 (m, 1H), 7.3-7.4 (m, 3H), 3.37 (s, 2H, CH₂), 2.3 (s, 3H, CH₃), 2.10 (s, 3H, CH₃). MS 442 [M⁺+1].

5.49 EXAMPLE 49

- 15 SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[5-(3,5-DIMETHYL-PIPERAZINE-1-CARBONYL)-3-METHYL-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 52)

- A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one (70 mg, 0.25 mmol), 5-(3,5-dimethyl-piperazine-1-carbonyl)-3-methyl-1H-pyrrole-2-carbaldehyde (74.8 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for overnight. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 93.1 mg (73%) of the title compound as a yellow solid. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.39 (br s, 1H, NH), 11.75 (br s, 1H, NH), 20 9.23 (br s, 1H), 8.32 (s, 1H), 7.73 (dd, *J* = 2.6 & 6.7 Hz, 1H), 7.41 (m, 1H), 7.33-7.38 (m, 2H), 6.57 (d, *J* = 2 Hz, 1H), 4.22 (m, 2H, CH₂), 2.68 (m, 2H, CH₂), 2.52 (m, 2H, 2xCH), 2.22 (s, 3H, CH₃), 0.99 (d, *J* = 6 Hz, 6H, 2xCH₃), 0.98 (s, 3H, CH₃). MS 510 [M⁺+1].

5.50. EXAMPLE 50

- 30 SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[3-METHYL-5-(3,4,5-TRIMETHYL-PIPERAZINE-1-CARBONYL)-1H-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-D]PYRIMIDIN-6-ONE (FORMULA 53)

- 4-(3-Chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one was 35 condensed with 3-methyl-5-(3,4,5-trimethyl-piperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde to give the title compound. ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.41 (br s, 1H, NH), 11.78 (br s, 1H, NH), 9.23 (br s, 1H), 8.33 (s, 1H), 7.72 (dd, *J* = 2.5 & 6.5 Hz, 1H), 7.43 (m, 1H), 7.33-7.38 (m, 2H), 6.58 (m, 1H), 4.19 (m, 2H, CH₂), 2.78 (m, 2H), 2.23 (s, -

249
99
253

3H, CH₃), 2.18 (s, 3H, CH₃), 2.11 (m, 2H), 1.06 (d, *J* = 6.6 Hz, 6H, 2xCH₃). MS 524 [M⁺+1].

5.51. EXAMPLE 51

5 SYNTHESIS OF 4-(3-CHLORO-4-FLUORO-PHENYLAMINO)-5-[5-(4-ETHYL-PIPERAZINE-1-CARBONYL)-3-METHYL-1*H*-PYRROL-2-YLMETHYLENE]-5,7-DIHYDRO-PYRROLO[2,3-*D*]PYRIMIDIN-6-ONE (FORMULA 54)

A mixture of 4-(3-chloro-4-fluoro-phenylamino)-5,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-6-one (70 mg, 0.25 mmol), 5-(4-ethyl-piperazine-1-carbonyl)-3-methyl-1*H*-pyrrole-2-carbaldehyde (74.8 mg, 0.3 mmol) and piperidine (3 drops) in ethanol (2 mL) was stirred at room temperature for overnight. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 93 mg (73%) of the title compound. ¹H NMR (300 MHz, DMSO-*d*₆) δ 3.40 (br s, 1H, NH), 11.81 (br s, 1H, NH), 9.28 (br s, 1H), 8.33 (s, 1H), 7.72 (m, 1H), 7.33-7.45 (m, 3H), 6.58 (m, 1H), 3.68 (m, 4H, 2xCH₂), 2.31-2.42 (m, 6H), 2.22 (s, 3H, CH₃), 1.01 (t, *J* = 7.2 Hz, 3H, CH₂CH₃). MS 510 [M⁺+1].

5.52. EXAMPLE 52: ASSAY FOR MODULATION OF PDGFR ACTIVITY

Using the following reagents, supplies, and methods, the ability of the compounds of the invention to modulate the *in vitro* activity of the PDGFR can be readily determined in an enzyme-linked immunosorbent assay (ELISA).

Reagents and Supplies

All ELISA reactions are performed in Corning 96-well ELISA plates (Corning, Catalog # 25805-96). Monoclonal anti-PDGFR antibody (28D4C10) is stored at -20°C in 50 mL tubes prior to use. Antibody isolation methods employed herein are commonly known in the art, such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

The dilution buffer is Dulbecco's Phosphate-Buffered Saline ((PBS); Gibco, Catalog # 450-1300EB). Alternatively, PBS can be made, including magnesium, by using the following reagents in the following protocol. In order to make a one liter 10x stock solution of PBS, first add approximately 900 mL dH₂O to a 1 liter graduated cylinder to which is added 2.013 grams of KCl, 1.916 grams of KH₂PO₄ (monobasic), 80.65 grams of NaCl, and 11.50 grams of Na₂HPO₄ (dibasic). Stir the mixture, and when dissolved, bring the solution to pH 7.2 with HCl. Finally, add 1.017 grams of MgCl₂·6H₂O (anhydrous), and bring the volume to 1 liter dH₂O. Note that PBS can be left at room temperature, but 4°C is the preferred storage temp.

TBST buffer (TBS + 0.1% Triton X-100) is used at a final 1x working

250
100
254

concentration. The buffer is prepared by adding about 900mL dH₂O to a 1 liter graduated cylinder, and adding 6.057 grams of Tris and 8.766 grams of NaCl and stirring. When the reagents are dissolved, the mixture is brought to pH 7.2 with HCl. Next, 1 mL of Triton X-100 is added and the volume is brought to 1 liter with dH₂O. Alternatively one can use a
5 stock solution of TBS which is pre-made and stored at 4°C, and to which is added Triton X-100 to a final concentration of 0.1%, with stirring until the detergent is dissolved. Note that TBST can be left at room temperature, but 4°C is the preferred storage temperature.

The ELISA Blocking Buffer is prepared by weighing out 5 grams of Non-fat Instant Milk (Carnation) and pouring 100 mL of PBS (see above) into a clean beaker. Next, add
10 the Instant milk to the PBS and stir at room temperature until the milk has dissolved. One hundred mL of ELISA Blocking Buffer (also "blocking buffer") is enough for about 6 assay plates. When stored at 4°C, this solution is stable for 1 to 2 weeks.

Lysates from PDGFR-β expressing NIH3T3 cells are obtained and stored at -80°C in 1 mL aliquots. PDGFR-β expression in eukaryotic cells is well known in the art.

15 Lysates are prepared by the following method. Place carrier dishes in ice trays, and after cooling, remove the media and replace with ice cold PBS. Remove the PBS and repeat. Aspirate left-over PBS if necessary and add 4 mL of ice cold HNTG lysis buffer (described below in Example 54) with a pipettor to each dish. Let sit on ice for 5-10 minutes and then scrape the cells with a rubber policeman. Take up lysate with a pipettor and add to a 30 mL
20 centrifuge tube on ice. Vortex the tubes for 30-60 seconds and then centrifuge for 10 minutes at 7000 rpm in a J-20 rotor which has been precooled to below 0°C. Recover the supernatants and combine them in a 250 mL tube and place on ice. Perform protein determination to measure the total quantity of protein per mL of solution. Aliquot the lysate into 1 mL fractions and store at -80°C.

25 A 1x working solution of TBS buffer is prepared by adding 900 mL of dH₂O to a 1 liter graduated cylinder, and adding 6.057 grams of Tris and 8.766 grams of NaCl and stirring. When all reagents have dissolved, the solution is adjusted to pH 7.2 with HCl and the volume is brought to 1 liter dH₂O. A 10x stock solution can be made by multiplying the amounts by 10 (but keeping the final volume of 1 liter). This stock is then diluted 10 fold
30 with dH₂O and its pH adjusted to 7.2. Note that TBS can be kept at room temperature, but is best stored at 4°C.

To make 1 liter of a 1x working solution of TBS + 10% DMSO, add about 850 mL dH₂O to a 1 liter graduated cylinder and then add 6.057 grams of Tris and 8.766 grams of NaCl and stir. When all reagents have dissolved, pH the solution to pH 7.2 with HCl.
35 Next, add 100 mL of DMSO and bring the volume to 1 liter with dH₂O. Alternatively add 25 mL DMSO to 225mL TBS (see above). Note that the TBS will now only be 0.9x, but this should not effect the assay.

To make 1 mM Stock solution of 1 mM Adenosine-5'-triphosphate (ATP, from

251
101
255

Equine muscle) add 2.75 mg ATP (Sigma, Catalog # A-5394) to 5 mL of dH₂O, and vortex. Note that larger volumes of stock solution can be prepared so long as the same ratio of ATP to dH₂O is used. Also note this reagent should be made up immediately prior to use and kept on ice.

5 A stock solution of 1 M MnCl₂ is prepared by adding 19.79 grams of MnCl₂ to 100 ml dH₂O and stirring until dissolved. The solution is then filtered, sterilized and stored in 1 ml aliquots at -80°C.

The 10x Kinase Buffer is composed of the following parts:

Reagent	Stock solution	Amount per 10 mL	Working Concentration
10 Tris	1 M	250 µL	25 mM
NaCl	5 M	200 µL	100 mM
MnCl ₂	1 M	100 µL	10 mM
TX-100	100%	10 µL	0.1%
15 DTT	100 mM	50 µL	0.5 mM

Ten milliliters of kinase buffer mix is sufficient for about 12.5 assay plates. To make Kinase Buffer, dilute 1.0 mL 10x Kinase Buffer to a final volume of 8.0 mL with water.

NUNC 96-well V bottom polypropylene plates (Applied Scientific, Catalog # AS-72092) are used for mixing the test drugs and the lysates.

20 An Ethylenediamine-tetraacetic acid (EDTA) 500 mM stock solution is prepared as follows: 1) Add about 70 mL dH₂O to a 250 mL beaker; 2) Add 14.12 g of EDTA; 3) With pH probe in beaker, add 10 N NaOH dropwise (EDTA will not dissolve until pH is around 7.0), and note that as EDTA dissolves, the pH will fall so more NaOH may need to be added; 4) When all EDTA is dissolved, adjust the pH to 8.0; 5) Transfer to 100 mL graduated cylinder, bring volume to 100 mL with dH₂O. To make 200 mM working solution add 30 mL dH₂O to 20 mL 500 mM EDTA (above).

25 Rabbit polyclonal anti-phosphotyrosine serum is generated as described by *Methods of Enzymology*, 201:65-79 (1991), and is stored at -80°C in 0.1 mL aliquots. Antiserum is stable for several weeks when thawed and stored at 4°C. Goat anti-rabbit IgG peroxidase conjugate is commercially available (Biosource, Catalog #AL10404) is commercially available.

30 The developing reagent, which includes 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Sigma, Catalog # A-1888) is prepared as follows. Add about 900 mL dH₂O to a 1 liter graduated cylinder. Add 19.21 grams of Citric Acid and 35.49 grams of Na₂HPO₄ and pH to 4.0 with phosphoric acid. Add ABTS and cover with foil. Let dissolve for about 0.5 hour and filter the solution. Note that the solution must be kept in the

001712000-122173

252
102
256

dark at 4°C until ready to use.

Hydrogen peroxide 30% solution is commonly available (Fisher, Catalog # H325). This should be stored in the dark at 4°C until ready to use.

An ABTS/H₂O₂ formulation consists of 15 mL ABTS solution (above) and 2 µL H₂O₂. Prepare 5 minutes before use and leave at room temperature. Take out ABTS about 60 minutes prior to use and warm to room temperature, or warm quickly by placing tube in 37°C water bath. Add 3 µL H₂O₂ prior to use.

0.2 M HCl is prepared by mixing 98.3 mL of dH₂O with 1.7 mL of HCl. This solution is stored at room temperature.

10

Procedure

Coat Corning 96 well ELISA plates with 0.5 µg per well 28D4C10 antibody in a volume of 100 µl PBS overnight, at 4°C and remove unbound antibody, 28D4C10, from wells by inverting plate to remove liquid. Wash 1x with distilled H₂O by filling wells. Pat the plate on a paper towel to remove excess liquid.

Add 150 µL of Blocking Buffer to each well. Incubate for 30 minutes at room temperature, with shaking and wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles

Dilute lysate in PBS (10 mg lysate/100 µL PBS). Add 100 µL of diluted lysate to each well. Shake at room temperature for 60 min. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

Add 80 µL Kinase Buffer to ELISA plate containing captured PDGFR.

Dilute drugs/extracts 1:10 (unless specified otherwise) in TBS + 10% DMSO in 96-well polypropylene plates. (i.e., 10 µL compound +90 µL (TBS + 10% DMSO)). Add 10 µL diluted drugs/extracts to ELISA plate. To control wells (wells which do not receive any drug) add 10 µL of TBS +10% DMSO. Incubate for 30 minutes while shaking at room temperature.

Add 10 µL 25 µM ATP directly to all wells except negative control well which does not receive ATP. (100 µL final volume in well with 2.5 µM ATP final concentration in well.) Incubate 30 minutes while shaking.

After 30 minutes stop reaction with addition of 10 µL of 200 mM EDTA pH 8.0 for 20 mM final in well. Wash 4x with deionized water, twice with TBST.

Add 100 µL per well of anti-phosphotyrosine mab (1:10,000 dilution in TBST). Incubate 30-45 minutes at room temperature, with shaking. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

Add 100 µL per well of biosource Goat anti-rabbit IgG peroxidase conjugate (1:6,000 dilution in TBST). Incubate 30 minutes at room temperature with shaking. Wash.

253
103
257

plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

Add 100 μ L of ABTS/ H_2O_2 solution to each well. Incubate 10 to 30 minutes while shaking. Remove any bubbles. If necessary stop reaction with the addition of 100 μ L of

5 0.2M HCl per well

Read assay on Dynatech MR7000 ELISA reader. Test Filter: 410 nm; Reference Filter: 630 nm.

A preferred template for the placement of controls in this experiment is provided below:

10		1	2	3	4	5	6	7	8	9	10	11	12
	A	POS											NEG
	B	POS											NEG
	C	POS											NEG
	D	POS											NEG
15	E	NEG											POS
	F	NEG											POS
	G	NEG											POS
	H	NEG											POS

20 **5.53. EXAMPLE 53: ASSAY FOR MODULATION OF GST-FLK1 ACTIVITY**

Using the following reagents, supplies, and method, the ability of compounds of the invention to affect the *in vitro* activity of Gst-Flk1 on the poly glutamic acid, tyrosine (pEY) peptide in a high throughput screening assay.

25 **Reagents and Supplies**

Assays are performed in Corning 96-well ELISA plates (Corning, Catalog # 25805-96), using the pEY 4:1, lyophilizate (Sigma, Catalog # P0275) as a substrate for FLK1. Prepare 1 mg/mL pEY in sterile PBS and store in 1 mL aliquots at -20°C.

30 Coat 2 μ g/well of pEY in 100 μ L PBS at room temperature for 2 hours or 4°C overnight. Cover plates well to prevent evaporation. Store at 4°C for 7 to 10 days.

PBS Buffer is prepared by adding to a 1 liter graduated cylinder, about 900mL dH₂O and 0.2g of KH₂PO₄, 1.15g of Na₂HPO₄, 0.2g of KCl, and 8.0g of NaCl. When all reagents have dissolved, pH to 7.2 with HCl, and bring volume to 1 liter dH₂O. A 10x stock solution can be made by multiplying the amounts by 10 (but keeping the final volume of 1 liter). This stock is then diluted 10 fold with dH₂O and its pH adjusted to 7.2. Note that PBS can be kept at room temperature, but is best stored at 4°C.

50171388-132177

254
104
258

PBS-Tw Buffer is prepared as above, except prior to bringing the volume to 1 liter, 1 mL of tween-20 is added. Note that TBS-Tw can be left at room temperature, but 4°C is the preferred storage temp.

TBB Blocking Buffer is prepared by adding to a 1 liter graduated beaker, about 900 mL dH₂O 1.21g of Tris, 8.77g of NaCl, and 1 mL of Tween-20. When all reagents have dissolved, bring pH to 7.2 with HCl. Next, add 10g of BSA, and stir until dissolved. Bring volume to 1 liter with dH₂O and filter the solution to remove any particulate matter, before storing at 4°C. A 10x stock solution can be made by multiplying the amounts by 10 (but keeping the final volume of 1 liter). This stock is then diluted 10 fold with dH₂O. Filter the solution to remove any particulate matter, and store at 4°C. Note that when stored at 4°C, this solution is stable for about 4 to 8 weeks.

A PBS + 1% BSA solution is prepared by adding to a 1 liter graduated beaker about 990 mL PBS. Next, 10 grams of BSA is added and stir until dissolved. Bring volume to 1 liter with PBS and filter the solution to remove any particulate matter, before storing at 4°C. Note that when stored at 4°C, this solution is stable for about 4 to 8 weeks.

HEPES buffer is commercially available (GIBCO, Catalog #15630-080).

GST-Flk1cd is purified from Sf9 recombinant baculovirus transformation and stored at -80°C in 100 µL aliquots (use 5 ng (0.005 µg)/well in kinase dilution buffer, KDB). The transformation is prepared as follows. GST-fusion proteins of Flk-1(cd) is produced in the baculovirus expression system using pFBG2T as the transfer vector. This plasmid contains the GST coding sequence, amplified by PCR as a *Bam* HI/*Bgl* II fragment and cloned into the *Bam* HI site of pFastBac-1 (GIBCO-BRL). The portion of Flk-1 cDNA encoding amino acids 812-1346 is amplified by PCR as a *Not* I/*Spa* I fragment and ligated downstream of and in-frame with the GST coding sequence in pFBG2T. Recombinant viruses are produced following standard protocols (GIBCO-BRL, FastBac manual). For protein production Sf9 cells are infected following standard procedures (King, L. A. and Possee, R. D. "The Baculovirus Expression System. A Laboratory Guide," Chapman and Hall, London (1992)) and fusion proteins purified by affinity chromatography on glutathione-sepharose (Sigma).

A solution of dH₂O + 4% DMSO is prepared by mixing 10 mL of DMSO with 240 mL dH₂O and stirring.

Adenosine-5'-triphosphate (from Equine muscle) 10 mM ATP (Sigma, Catalog # A-5394) is prepared by adding 5 mL of dH₂O to 27.5 mg ATP vortexing. Note that any amount of ATP can be used provided it is kept in the same ATP to dH₂O ratio. Also note this reagent can be stored at -20°C in small aliquots to be taken out just prior to use and kept on ice. Do not freeze/thaw aliquots; discard any unused portion.

A stock solution of 1 M MnCl₂ is prepared by adding 19.79 grams of MnCl₂ to 10 mL of dH₂O and stirring. Once the MnCl₂ has dissolved, filter sterilize and store at -20°C.

255
102
254

A working stock of 40 mM $MnCl_2$ is prepared by mixing 96 mL of dH_2O and 4 mL of $MnCl_2$ from the 1 M stock solution immediately prior to use.

One hundred mL of kinase buffer mix is enough for about 40 assay plates. Kinase Dilution Buffer (KDB) is prepared by mixing 88.56 mL of dH_2O with 10 mL of 1M Hepes pH 7.5, 1 mL of 5M NaCl, 40ml of 100 mM sodium vanadate and 0.4 mL of 5% BSA (in dH_2O).

One hundred mL of kinase buffer mix is enough for about 40 assay plates. Kinase assays are performed in NUNC 96-well V bottom polypropylene plates (Applied Scientific, Catalog # AS-72092).

10 Ethylenediamine-tetraacetic acid (EDTA) is prepared as in Example 52.

1° and 2° Antibody Dilution Buffer is prepared by mixing 89.5 mL of PBSTw with 0.5 mL of milk in PBS, 1 mL of 10 mM Sodium vanadate and 10 mL of 5% BSA in PBS.

Anti-phosphotyrosine rabbit polyclonal antisera and goat anti-rabbit HRP conjugate are commercially available (Biosource, catalog #A110404).

15 ABTS Solution is prepared as in Example 52.

Hydrogen peroxide 30% solution (Fisher, Catalog # H325). Store in the dark at 4°C until ready to use.

ABTS/ H_2O_2 is prepared as in Example 52. Take out ABTS about 60 minutes prior to use and warm to room temperature. Or warm quickly by placing tube in 37°C water bath.

20 Add 3 μL H_2O_2 prior to use.

0.2 M HCl as described above and store at room temperature.

Procedure

Coat Corning 96 well ELISA plates with 2 μg of polyEY peptide in sterile PBS.

25 Remove unbound liquid from wells by inverting plate. Wash 1x TBSTw. Pat the plate on a paper towel to remove excess liquid.

Add 100 μL of 1% BSA in PBS to each well. Incubate for 1 hour at room temperature, with shaking. Repeat removal of unbound liquid from wells by inverting plate. Wash 1x TBSTw. Pat the plate on a paper towel to remove excess liquid.

30 Soak wells with 50 mM Hepes pH 7.5 using 150 μL /well.

Dilute drugs with the extracts at 4x the desired final assay concentration in dH_2O + 4% DMSO (unless specified otherwise) in NUNC 96-well polypropylene plates. Always add the larger volume of water to the smaller volume of compound to ensure rapid mixing. Add 25 μL diluted drugs/extracts to ELISA plate. To control wells (wells which do not receive any drug) add 25 μL of dH_2O + 4% DMSO.

35 Dilute GST-FIkI 0.005 μg (5 ng)/well in KDB. For 50 mL KDB add 100 μL of 0.050 mg/mL GST-FIkI enzyme. This is enough for a 10 assay plate. Add 50 μL of diluted enzyme to each well. Add 25 μL 0.5 M EDTA to negative control wells.

EN
C2
1-2
✓
1-4
TU
C8
C8
1-4
TU
TU
1-2
1-2

256
106
260

Add 25 μ L of 40 mM $MnCl_2$ with 4x ATP (2 μ M) directly to all wells (100 μ L final volume in well with 0.5 μ M ATP final concentration in well.) Incubate for 15 minutes while shaking at room temperature. After 15 minutes stop reaction with addition of 25 μ L of 500 mM EDTA pH 8.0 for 50 mM final in well. Wash 3x with TBSTw and pat plate on paper towel to remove excess liquid.

Add 100 μ L per well of anti-phosphotyrosine antisera (1:10,000 dilution in antibody dilution buffer). Incubate 90 minutes at room temperature, with shaking. Wash 1x with TBSTw and pat plate on paper towel to remove excess liquid.

Add 100 μ L per well of goat anti-rabbit HRP conjugate (1:6,000 dilution in antibody dilution buffer). Incubate 90 minutes at room temperature with shaking. Wash 3x TBSTw. Pat the plate on a paper towel to remove excess liquid.

Add 100 μ L at room temperature. ABTS/ H_2O_2 solution to each well. Incubate 15 to 30 minutes while shaking. Remove any bubbles. If necessary stop reaction with the addition of 100 μ L of 0.2M HCl per well.

Read assay on Dynatech MR7000 ELISA reader: Test Filter: 410 nm; Reference Filter: 630 nm. A preferred template for the placement of controls in this experiment is provided above in Example 52.

5.54. EXAMPLE 54: ASSAY FOR MODULATION OF HER-2 ACTIVITY

Using the following reagents, supplies, and method, the ability of compounds of the invention to affect the activity of HER-2 in whole cells in an ELISA format can be assigned.

Materials and Reagents

Tissue Culture

Liquid culture media, DMEM, (GIBCO, Catalog #11965-092), and heat inactivated Fetal Bovine Serum (FBS), (GIBCO, Catalog #16000-044) are commercially available. Trypsin, (GIBCO, Catalog #25200-056), L-Glutamine stored in 6 mL aliquots at -20°C (GIBCO, Catalog #25030-081), and HEPES, (GIBCO, Catalog #15630-080) are also commercially available.

Growth Media is prepared by adding to a 500 mL bottle of DMEM, 10% heat inactivated FBS (55 mL), 10 mL HEPES and 5.5 mL L-Glutamine. Starvation Media is prepared by adding to a 500 mL bottle of DMEM, 0.5% heat inactivated FBS (2.5 mL), 10 mL HEPES and 5.5 mL L-Glutamine.

Flat Bottom 96-well Tissue Culture Micro Titer Plates, (Corning, Catalog #25860), 15 cm Tissue Culture Dishes, (Corning, Catalog #08757 148), Corning 96-well ELISA Plates, (Corning, Catalog # 25805-96), NUNC 96-well V bottom polypropylene plates (Applied Scientific, Catalog #AS-72092), and Costar Transfer Cartridges for the Transtar 96,

257
10X
201

Catalog #76 10 are all commercially available.

SUMO1 is a monoclonal anti-BGFR antibody. More specifically, SUMO1 is ascites-derived anti-BGFR monoclonal antibody produced by the hybridoma clone mAb108 (PNAS 86: 925-929 (1989)). The antibody is purified by conventional means using Protein A-agarose as described (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

PBS (GIBCO, Catalog # 450-1300EB), is obtained from a commercial supplier or prepared as described in Example 52.

TEST Buffer is prepared in a stock solution by adding 6.057 grams of Tris, and 8.766 grams of NaCl to 900 mL of dH₂O and pH to 7.2. 1 mL of Triton X-100 is next added to the dH₂O and the mixture is brought to 1L with dH₂O.

5% Blocking Buffer is prepared by weighing 5 grams of Non-fat Instant and pouring 100 mL of TEST (see above) into a clean beaker. Next add instant milk to TEST, stir at room temperature until the milk has dissolved. Note that when stored at 4°C, this solution is stable for 1 to 2 weeks.

The EGF Ligand: BG-201, (Shinko American, Japan) is commercially available. Store powder vials (100 µg/vial) at 4°C.

BGF Ligand at a stock concentration of 16.5 µM is prepared by resuspending all of the powder in the vial with 100 µL of 10mM HCl and adding 100 µL of 10 mM NaOH. Then add 800 µL of PBS to the vial and transfer to an eppendorf tube for storage at -20°C. Note that for best results, make up one EGF vial right before use. Resuspended EGF is typically stable at -20°C for several weeks.

A stock solution of 5x HNTG Lysis Buffer is prepared by mixing 23.83 grams of HEPES and 43.83 grams of NaCl in 350 mL of dH₂O. The mixture is brought to pH 7.2 and 500 mL of glycerol and 100 mL of Triton X-100 are added. The volume is brought to 1L with dH₂O, and stirred to the reagents.

1x HNTG* buffer is prepared by mixing 2 mL of HNTG with 100 µL of 1 mM Na₃VO₄, 240 µL of 25 mM Na₄P₂O₇, and 100 µL of 5 mM EDTA. The mix is brought to 10 mL with dH₂O.

Prepare EDTA as described in Example 52.

A 0.1 M Na₃VO₄ stock solution is prepared by adding about 90 mL dH₂O to a 250 mL beaker and then adding Na₃VO₄. With pH probe in beaker, pH to 10 (turns orange) and then bring to a boil by microwaving for about 1 minute (solution turns clear). Cool to room temperature and put in water bath. Check pH and bring to pH 10 before boiling and cooling until pH remains at 10. Use HCl or NaOH to adjust pH and make 1 mL aliquots to keep at -80°C.

A 0.2 M Na₄P₂O₇ stock solution (Fisher, Catalogue # 5390) is prepared by adding 8.92 grams to 100 mL of dH₂O. Filter the solution prior to use.

258
100
262

Rabbit polyclonal antiserum specific for phosphotyrosine (anti-Ptyr antibody) is generated as described by *Methods of Enzymology*, 201:65-79 (1991).

Affinity purified antiserum, goat anti-rabbit IgG antibody, peroxidase conjugate is commercially available (Biosource antibody Cat # ALI0404).

- 5 ABTS Solution is prepared as in Example 52.

Hydrogen peroxide 30% solution (Fisher, Catalog # H325). Store in the dark at 4°C until ready to use.

ABTS/H₂O₂ is prepared as in Example 52. Take out ABTS about 60 minutes prior to use and warm to room temperature. Or warm quickly by placing tube in 37°C water bath.

- 10 Add 3 µL H₂O₂ prior to use.

0.2 M HCl as described above and store at room temperature.

Procedure

A. Pre-coating ELISA plate

- 15 Coat Corning 96 well ELISA plates with SUMOI at 1.0 µg per well in PBS, 100 µL final volume/well, overnight at 4°C. Coated plates are good for up to 10 days when stored at 4°C. If necessary, plates may be coated at room temperature for two hours while shaking.

- On day of use, remove coating buffer and wash plate 3 times with deionized water
20 and once with TEST buffer. Note that all washes should be done in this manner, unless otherwise specified. Add 100 µL of blocking buffer to each well. Incubate plate, shaking, at room temperature for 30 minutes. Just prior to use, wash plate as described above.

B. Seeding Cells

- 25 EGFR/HER-2chimera/#T3-C7 cell lines are used for this assay. Choose dishes having 80-90 % confluence for the experiment. Collect cells by trypsinization and centrifuge at 1000 rpm at room temperature for 5 minutes.

- Resuspend cells in starve medium and count with trypan blue. Only viability above 90 % is acceptable. Seed cells in starve medium at a density of 2,500 cells per well, 90 µL
30 per well, in a 96 well microtiter plate. Incubate seeded cells in 5 % CO₂ at 37°C overnight. Start the assay two days after seeding.

C. Assay Procedures

Primary Screening

- 35 Samples are diluted directly into a polypropylene plate containing starve-DMEM (non-sterile is okay). This dilution will be 1:10 or greater, depending on the samples being screened. The same amount of DMSO is put into the control wells. All cells are then transferred to the cell plate at a 1:10 dilution (10 µL of sample and media into 90 µL of

starve media). The final DMSO concentration will be 1% or lower.

Secondary Screening

- Ten samples are put into wells 2-11 of row A of the NUNC 96 well polypropylene plate. These wells contain straight starve-DMEM. For a 1:10 dilution, one would put 10 μ L of drug into 90 μ L of media. The rest of the wells (including the controls) will have a DMSO/media mixture. The percentage of DMSO in this mixture is determined by the first dilution factor, in this case 1:10. The DMSO concentration is therefore 10%. An equal amount of drug and media from row A is put into row B, containing DMSO and media.
- 10 The same amount is then taken out and put into row C. This is repeated for all rows. These are 1:2 dilutions. All wells are then transferred to the cell plate at a 1:10 dilution (10 μ L of sample and media into 90 μ L of starve-DMEM. The final DMSO concentration will be 1% or lower.

- Incubate in 5 % CO₂ at 37°C for 2 hours and in the incubation period, prepare EGF ligand by diluting stock EGF (16.5 μ M) in warm DMEM (non-sterile is okay) to 150 nM and prepare fresh HNTG* sufficient for 100 μ L per well and place on ice.

After a 2 hour incubation with the drug, add prepared EGF ligand to cells at 50 μ L per well, for a final concentration of 50 nM. Positive control wells (8) receive the same amount of EGF. Negative controls do not receive EGF. Incubate at 37°C for 10 minutes.

- 20 Remove drug, EGF, and DMEM by dumping in sink. Wash cells once with PBS.

Transfer HNTG* to cells, 100 μ L per well. Place on ice for 5 minutes. Meanwhile, remove blocking buffer from ELISA plate and wash as described above. With a pipette tip securely fitted to a micropipettor, scrape cells from plate and homogenize cell material by repeatedly aspirating and dispensing the HNTG* lysis buffer. Transfer lysate to a coated,

- 25 blocked, washed ELISA plate. Alternatively, one may use a Costar transfer cartridge to transfer lysate to the ELISA plate. Incubate, shaking, at room temperature for one hour.

Remove lysate by dumping in sink, then wash as described earlier. Transfer freshly diluted anti-phosphotyrosine (anti-Ptyr) antibody (1:3000 in TEST) to ELISA plate, 100 μ L per well. Incubate, shaking, at room temperature, for 30 minutes.

- 30 Remove anti-Ptyr antibody by dumping in sink, then wash as described earlier. Transfer freshly diluted BIOSOURCE antibody to ELISA plate, (1:8000 in TEST), 100 μ L per well. Incubate, shaking, at room temperature for 30 minutes.

- Remove BIOSOURCE antibody by dumping in sink, then wash as described earlier. Transfer freshly prepared ABTS/H₂O₂ solution to ELISA plate, 100 μ L per well. Incubate 35 5-10 minutes while shaking. Remove any bubbles and if necessary, stop reaction with the addition of 100 μ L of 0.2 M HCl per well.

Read assay on Dynatech MR7000 ELISA reader. Test Filter: 410 nm; Reference Filter: 630 nm. A preferred template for the placement of controls in this experiment is

260
110
264

provided below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	POS											POS
B	POS											POS
5 C	POS											POS
D	POS											POS
E	NEG											NEG
F	NEG											NEG
G	NEG											NEG
10 H	NEG											NEG

5.55. EXAMPLE 55: ASSAY FOR INHIBITION OF CDK2

Using the following reagents, supplies, and method, the ability of compounds of the invention to assay for modulation of the *in vitro* kinase activity of human cdk2/cyclin A in a
15 Scintillation Proximity Assay (SPA).

Reagents and Supplies

Assays are performed in Wallac 96-well polyethylene terephthalate (flexi) plates (Wallac, Catalog # 1450-401). Amersham Redivue [$\gamma^{32}\text{P}$] ATP (Amersham, Catalog #AH
20 9968).

Beads for the assay are Amersham streptavidin coated polyvinyltoluene SPA beads (Amersham, Catalog #RPNQ0007). Reconstitute beads in PBS without magnesium or calcium, at 20 mg/ mL. Store reconstituted beads at 4°C.

Activated cdk2/cyclin A enzyme complex is purified from Sf9 cells and stored at
25 -80°C in 200 μL aliquots. Human cdk2/cyclin A protein is expressed, activated, and purified from baculovirus infected sf9 cells as described previously. (*J. Mol. Biol.*, 230:1317-1319 (1993)).

The substrate is a biotinylated peptide substrate (Debtide), which is a peptide biotin-X-PKTPKKAKKL dissolved in dH_2O at a concentration of 5 mg/ mL and stored at
30 -80°C in 100 μL aliquots.

The Peptide/ATP mixture is prepared by mixing 9.979 mL dH_2O with 0.00125 mL of cold 10 mM ATP, 0.010 mL of 5mg/mL Debtide, and 0.010 mL of 10 $\mu\text{Ci/mL}$ $\gamma^{32}\text{P}$ ATP.

A 2.5x kinase buffer is prepared by mixing 8.85 mL of dH_2O with 0.625 mL of 1M Tris pH 7.4, 0.25 mL of 1M MgCl_2 , 0.25 mL of 10% NP40, and 0.025 mL of 1M DTT.

35 Ten milliliters of kinase buffer mix is sufficient for about 4.5 assay plates.

10 mM Adenosine-5'-triphosphate (from Equine muscle) ATP (Sigma, Catalog

SECRET - 110000 - 110000 - 110000

261
265

#A-5394) is prepared as in Example 53. Note that this reagent can be stored at -20°C in small aliquots to be taken out just prior to use and kept on ice. Do not freeze/thaw aliquots; discard any unused portion.

- PBS (Dulbecco's Phosphate-Buffered Saline) without magnesium or calcium
- 5 (Gibco, Catalog # 14 190-144) is purchased from a commercial supplier or prepared as in Example 53 and a stock solution of 500 mM EDTA is prepared as in Example 52.

Prior to the experiment, prepare the following stock solution:

10

Reagent	Stock solution	Amount per 10 mL	Working Concentration
PBS		9.25 mL	
ATP	100 mM	0.005 mL	50 µM
EDTA	0.5 M	0.1 mL	5 mM
TritonX-100	10%	0.1 mL	0.1%
SPA beads	20 mg/mL	1.25 mL	0.5 mg/well (200 µL)

15

Procedure

Prepare solutions of inhibitors at 5x the desired final concentration in 5% DMSO. Add 10 µL to each well. For negative controls, add 10 µL 5% DMSO.

- Dilute 5 µL of cdk2/cyclin A solution into 2.1 mL 2x kinase buffer (per plate) and
- 20 add 20 µL enzyme per well. This can be added using a hand pipette or by using the Titertek Multidrop. Note that the enzyme can be freeze/thawed, but this results in loss of some activity. For each freeze/thaw cycle, double the amount of enzyme used in the assay. Also, the enzymes should be thawed quickly (e.g., in the palm of one's hand) and be kept on ice prior to use in the assay. Add 10 µL of 0.5 M EDTA to the negative control wells.

- 25 To start kinase reaction, add 20 µL of peptide/ATP mixture using either a hand pipette or the Titertek Multidrop. Let sit on benchtop behind shield for one hour. No shaking is necessary and is not recommended when using Flexiplates (plates are not easy to add/remove from shaker).

- Add 200 µL stop solution per well using either the Titertek Multidrop or hand
- 30 pipette. Let stand at least 10 minutes. Spin plate approximately 2300 rpm for 3-5 minutes. Count plate on TriLux reader.

A preferred template for the placement of controls in this experiment is shown above in Example 52.

35 **5.56. EXAMPLE 56: ASSAY FOR MODULATION OF EGFR ACTIVITY**

Using the following reagents, supplies, and method, the ability of compounds of the invention to modulate the *in vitro* activity of EGFR can be readily determined in an ELISA:

50171288-122173

262
112
266

Reagents and Supplies

Reactions are performed in Corning 96-well ELISA plates (Corning, Catalog # 25805-96).

SUMO1 is a monoclonal anti-EGFR antibody stored at -20°C, in 1 mL aliquots and is described in Example 54.

PBS (Gibco, Catalog # 450-1300EB) is purchased from a commercial source or made as described in Example 52.

TBS Buffer is prepared as in Example 52, and Triton X-100 is added to a 0.1% Blocking Buffer is prepared as in Example 53.

A431 mouse cell lysate is stored at -80°C (ATCC, Catalog # HB-9629).

TBS +10% DMSO is prepared as in Example 52.

A 1.0 mM ATP stock solution and a 1M MnCl₂ stock solution are prepared as described above.

ATP/MnCl₂ phosphorylation mix is prepared by adding 300 µL of 1.0 mM ATP and 500 µL of 1M MnCl₂ to 9.2 mL of dH₂O and mixing. Ten mL of phosphorylation mix is enough for about 6 assay plates. Note that the mixture should be made fresh and kept on ice immediately before use, and that although the preferred ATP is fresh from powder stock, any remaining stock solution of ATP may be frozen at -20°C in small aliquots to be used at a later time.

NUNC 96-well V bottom polypropylene plates are used for the reactions (Applied Scientific, Catalog # AS-72092).

EDTA is prepared as described in Example 52.

Rabbit polyclonal anti-phosphotyrosine serum is prepared as described above and stored at -80°C, 1 mL aliquots. Thaw 1 mL vial and aliquot in smaller volumes to store at -80°C. Antiserum is stable for several weeks when thawed and stored at 4°C. Goat anti-rabbit IgG peroxidase conjugate (Biosource, Catalog # ALI0404) is commercially available.

ABTS Solution is prepared as described in Example 52.

Hydrogen peroxide 30% solution is commercially available (Fisher, Catalog # H325). Store in the dark at 4°C until ready to use.

ABTS/H₂O₂ is prepared as in Example 52.

Take out ABTS about 60 minutes prior to use and warm to room temperature. Or warm quickly by placing tube in 37°C water bath. Add 3 µL H₂O₂ prior to use.

0.2 M HCl as described above and store at room temperature.

Procedure

Coat Corning 96 well ELISA plates with 0.5 µg per well with SUMO1 in a volume of 100 µL PBS overnight, at 4°C. Remove unbound SUMO1 from wells by inverting plate

263
X
267

to remove liquid. Wash 1x with distilled H₂O by filling wells. Pat the plate on a paper towel to remove excess liquid.

Add 150 µL of Blocking Buffer to each well. Incubate for 30 minutes at room temperature, with shaking. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

Dilute lysate in PBS (7 µg lysate/100 µL PBS) and add 100 µL of diluted lysate to each well. Shake at room temperature for 60 minutes. Wash 1x with distilled H₂O by filling wells. Pat the plate on a paper towel to remove excess liquid.

Add 120 µL TBST to ELISA plate containing captured EGFR.

10 Dilute drugs/extracts 1:10 (unless specified otherwise) in TBST in 96-well polypropylene plates. (i.e., 10 µL compound + 90 µL TBST). Add 13.5 µL diluted drugs/extracts to ELISA plate. To control wells (wells which do not receive any drug) add 13.5 µL of TBST + 10% DMSO. Incubate for 30 minutes while shaking at room temperature.

15 Add 15 µL phosphorylation mix directly to all wells except negative control well which does not receive ATP/MnCl₂. Approximately 150 µL final volume in well with 3 µM ATP/5 mM MnCl₂ final concentration in well. Incubate 5 minutes while shaking. Note that this is a timed event. It is critical that ATP/MnCl₂ phosphorylates the receptor for only 5 minutes. It is best to add the ATP/MnCl₂ with an automated 96 place pipettor workstation,
20 then 5 minutes later stop the reaction with EDTA using the same workstation.

Alternatively, addition can be performed using a 12 channel pipettor 1 row at a time, leaving 20 seconds between each row so that the reaction may be stopped with EDTA exactly 5 minutes later (this depends on the number of plates being phosphorylated in one batch). Shake between each addition.

25 After 5 minutes, to stop the reaction, add 16.5 µL of 200 mM EDTA pH 8.0 for 20 mM final in well, shaking continuously between each addition. This is done using either the same work station as above, or the same timing method employed with the 12 channel pipettor. After the EDTA has been added, shake for 1 minute. Wash 4x with deionized water, twice with TBST.

30 Add 100 µL per well of anti-phosphotyrosine (1:3000 dilution in TBST). Incubate 30-45 minutes at room temperature, with shaking. Wash 1x with distilled H₂O by filling wells. Pat the plate on a paper towel to remove excess liquid.

Add 100 µL per well of biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST). Incubate 30 minutes at room temperature, with shaking. Wash
35 1x with distilled H₂O by filling wells. Pat the plate on a paper towel to remove excess liquid.

Add 100 µL of ABTS/H₂O₂ solution to each well. Incubate 5 to 10 minutes while shaking. Remove any bubbles. If necessary stop reaction with the addition of 100 µL of

264
114
258

0.2M HCl per well

Read assay on Dynatech MR7000 ELISA reader. Test Filter: 410 nM; Reference Filter: 630 nM. A preferred template for the placement of controls in this experiment is shown above in Example 52.

5

5.57. **EXAMPLE 57: KINASE INHIBITION OF SELECTED COMPOUNDS**

Using methods such as those described above, the ability of selected compounds of the invention to inhibit the activity of the kinases FlkGST, EGFR, PDGFR, cdk2SPA, and HER-2 are provided below:

10

Compound Formula	bio FlkGST IC ₅₀ (Mm)	bio EGFR IC ₅₀ (μM)	bio PDGFR IC ₅₀ (μM)	cdk2SPA IC ₅₀ (μM)	HER-2 kinase IC ₅₀ (μM)
3	0.06	0.51	>100	>10	0.272
4	0.33	0.006	>100	>10	0.16
5	>20	>20	>100		61.16
6	3.31	0.001	>100		0.13
7	3.24	0.16	85.96		0.04
8	15.93	0.06	16.39	2.62	0.1
9	16.49	0.04	>100		0.01
10	>20	>20	>100		>100
11	>20	>20	28.14	0.01	>100
12	0.78	0.004	>100		0.28
13	>20	19.07	>100		>100
14	>20	>20	>100	0.04	>100
15	>20	0.02	>100		0.13
16	9.28	0.1	>20		1.02
17	16.22	0.02	>20		0.1
18	4.18	0.18	15.29		0.03
19	1.05	0.17	12.75		
24	2.73	0.89	>20		
25	5.12	0.058			11.92
26	15.16	0.16	>20		
27	5.3	0.15	>20		0.64

50171228-122127

265
H
269

Compound Formula	bio FlkGST IC ₅₀ (Mm)	bio EGFR IC ₅₀ (μM)	bio PDGFR IC ₅₀ (μM)	cdk2SPA IC ₅₀ (μM)	HER-2 kinase IC ₅₀ (μM)
28	8.07	0.21	>20		0.16
29	14.88	0.45	>20		
30	13.87	0.77	>20		
31	19.42	0.21	>20		0.37
32	13.5	7.14	>20		18.74
33	18.68	0.9	>20		
34	14.73	>20	>20		>20
35	>20	0.4	>20		0.59
36	>20	17.39	>20		>20
37	11.99	0.24	>20		1.07
38	5.98	5.6			
39	>20	0.004	>20		
40	>20	4.2			
41	>20	0.54	>20		
42	13.17	10.43			
43	13.09	0.86	>20		
44	13.76	>20			
45	6.61	0.05	>20		
46	7.41	0.0012			
47	10.16	0.036	>20		
48	>20	>20			
49	17.25	0.005	>20		1.8
50	3.46	0.377			
51	7.15	0.038			0.86
52	1.62	0.0011			
53	4.68	<0.156			
54	>20	<0.156			

From these data, it is readily apparent that preferred compounds of the invention are
 30 capable of inhibiting the activities of a variety of different kinases. It is further apparent

50171288-122177

266
116
270

that particular compounds are capable of exhibiting highly selective inhibition. For example, the hydrochloride salt of compound 6 (i.e., 5-[4-(1-benzyl-1H-indol-5-ylamino)-6-oxo-6,7-dihydro-pyrrolo[2,3-d]pyrimidin-5-ylidenemethyl]-4-methyl-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)-amide hydrochloride) inhibits EGFR at very low concentrations, yet exhibits relatively little effect on the activity of PDGFR.

5.58. EXAMPLE 58: ORAL FORMULATION

Hard gelatin capsule dosage forms containing kinase inhibitors of the invention can be prepared using the following ingredients:

10

Component	5 mg capsule	10 mg capsule	20 mg capsule
Kinase Inhibitor	5.0	10.0	20.0
Microcrystalline Cellulose	90.0	90.0	90.0
Pre-gelatinized Starch	100.3	97.8	82.8
Croscarmellose	7.0	7.0	7.0
Magnesium Stearate	0.2	0.2	0.2

15

20

The kinase inhibitor is sieved and blended with the excipients listed. The mixture is filled into suitably sized two-piece hard gelatin capsules using suitable machinery and methods well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16th or 18th Editions, each incorporated herein in its entirety by reference. Other doses can be prepared by altering the fill weight and, if necessary, changing the capsule size to suit. Any of the stable, non-lactose hard gelatin capsule formulations above can be formed.

Compressed tablet dosage forms of kinase inhibitors can be prepared using the following ingredients:

SECRET - 122173

267
117
271

5

Component	5 mg capsule	10 mg capsule	20 mg capsule
Kinase Inhibitor	5.0	10.0	20.0
Microcrystalline Cellulose	90.0	90.0	90.0
Pre-gelatinized Starch	100.3	97.8	82.8
Croscarmellose	7.0	7.0	7.0
Magnesium Stearate	0.2	0.2	0.2

10

The kinase inhibitor is sieved through a suitable sieve and blended with the non-lactose excipients until a uniform blend is formed. The dry blend is screened and blended with the magnesium stearate. The resulting powder blend is then compressed into tablets of desired shape and size. Tablets of other strengths can be prepared by altering the ratio of the active ingredient to the excipient(s) or modifying the table weight.

15

The embodiments of the invention described above are intended to be merely exemplary, and those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, numerous equivalents of the specific materials and procedures. All such equivalents are considered to be within the scope of the invention and are encompassed by the appended claims.

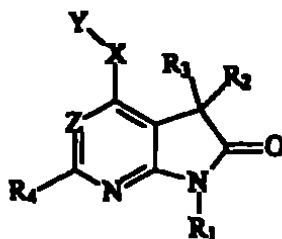
20

50171288.122170

CLAIMS

What is claimed is:

1. A compound of Formula 1



1

or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof, wherein:

R₁ is H or methyl;

each of R₂ and R₃ is independently H, halogen, (C₁-C₃)alkyl, or (C₁-C₃)alkoxy; or R₂

- 15 and R₃ taken together form an optionally substituted methylinane or a 3- to 7-membered ring optionally comprising 0-3 heteroatoms;

R₄ is H, methyl, trifluoromethyl, (C₁-C₃)alkyl, alkoxy, amido, amino, or optionally substituted aryl;

X is a chemical bond, ethynyl, -O-, -S-, -S(O)-, -S(O)₂-, -NR₂C(O)-, or -NR₂-;

- 20 wherein R₂ is H, methyl, or substituted methylene;

Y is a 5- to 10-membered mono or bicyclic, saturated, unsaturated, or aromatic ring comprising 0-3 heteroatoms and optionally substituted; and

Z is N or CR₆, wherein R₆ is H, halogen, nitro, cyano, alkoxy, sulfonamide, amino, or amide.

25

2. The compound of claim 1 wherein X is a chemical bond, -O-, -S-, or -NR₂-.

3. The compound of claim 1 wherein Y is selected from the group consisting of phenyl, indolyl, indolinyl, 1H-indazolyl, 2,3-dihydro-1H-indazolyl, 1H-benzimidazolyl, 2,3-dihydro-1H-benzimidazolyl, benzotriazolyl, pyridyl, pyrimidyl, 4-substituted piperazin-1-yl, morpholino, piperidinyl, pyrrolidin-1-yl, furanyl, thiophenyl, pyrrolyl, pyrazolyl, imidazolyl, pyridopyrrolyl, pyridazopyrrolyl, pyrimidopyrrolyl, pyrazopyrrolyl, pyridofuranyl, and derivatives thereof.

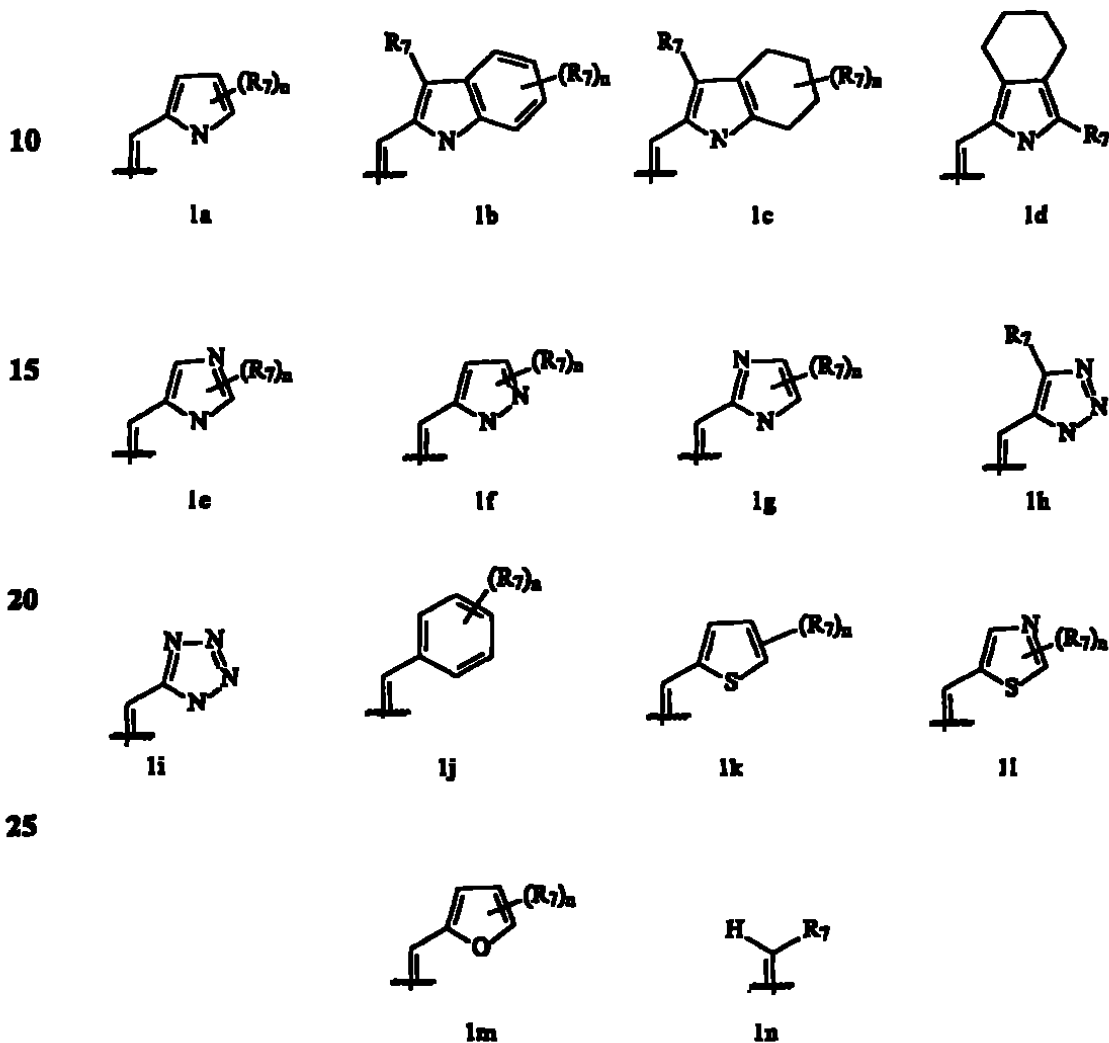
- 35 4. The compound of claim 1 wherein Z is N or CH.

5. The compound of claim 1 wherein R₂ and R₃ are both H, halogen, or methyl.

269
X
273

6. The compound of claim 1 wherein R_1 and R_2 are taken together to form a ring selected from the group consisting of 1,3-dioxolane, 1,3-dioxane, cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

5 7. The compound of claim 1 wherein R_2 and R_3 are taken together to form an optionally substituted methylinene selected from those of Formulas 1a-1n:



wherein:

n is an integer of 0-3;

each R_7 is independently H, alkyl, carboxylic acid, amine, halogen, nitro, cyano, X_1 , X_2 -(C-C)_nalkyl- R_8 , X_2 -(C-C)_nalkenyl- R_8 , or X_2 -(C-C)_nalkynyl- R_8 ;

35 X_1 is -C(O)NR₉, -NR₉C(O)-, -C(O)O-, C(O)R₁₁, -OC(O)-, -O-, -NR₉, -S-, -S(O₂), or -S(O₂)NR₉;

X_2 is a chemical bond, -C(O)NR₉, -NR₉C(O)-, -C(O)O-, C(O)R₁₁, -OC(O)-, -O-, -NR₉, -S-, -S(O₂), or -S(O₂)NR₉;

50171288.122177

270
 120
 374

R_8 is selected from the group consisting of hydrogen, dialkylamino, carboxyl, hydroxyl, alkoxy, sulfonamide, urea, carbamate, diol, alkylsulphonyl, and R_{10} ;

R_9 is H or (C_1-C_3) alkyl;

R_{10} is an optionally substituted 5- or 6-membered saturated, unsaturated, or aromatic heterocycle comprising from 1 to 4 heteroatoms; and

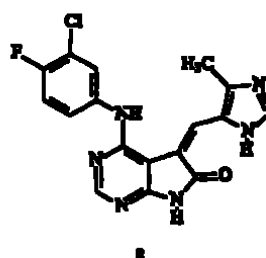
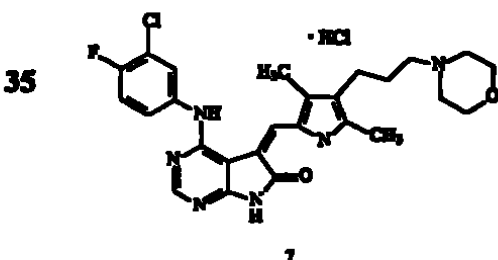
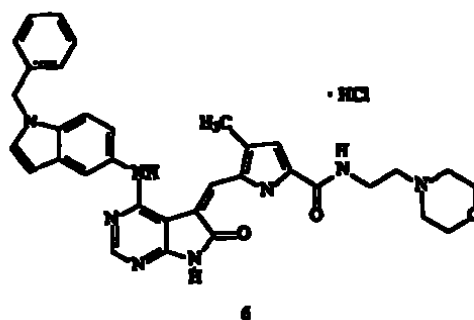
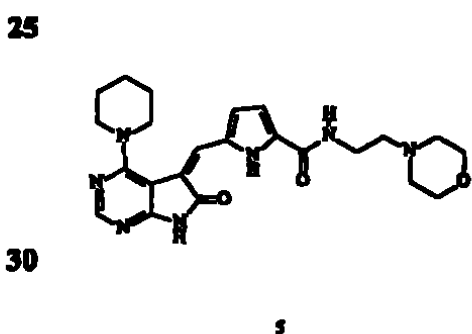
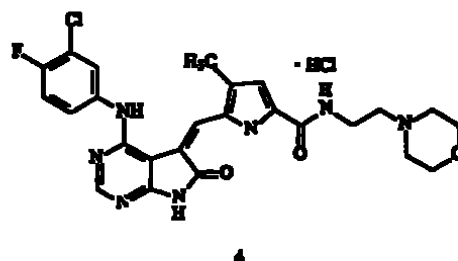
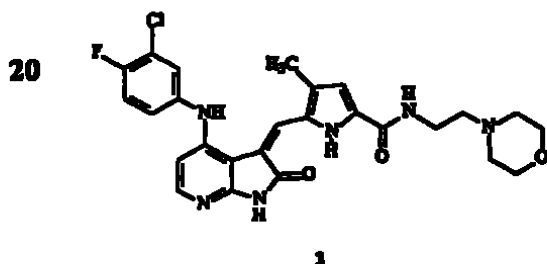
R_{11} is an optionally substituted 5- to 6-membered saturated heterocyclic ring.

8. The compound of claim 7 wherein R_7 is $X_2-(C_1-C_4)$ alkyl- R_8 , $X_2-(C_1-C_4)$ alkenyl- R_8 , or $X_2-(C_1-C_4)$ alkynyl- R_8 , and R_8 is selected from the group consisting of alkylsulfonyl, alkoxy, carboxyl, morpholino, 1-alkyl-piperazin-4-yl, pyrrolidinyl, piperidinyl, pyridyl, imidazolo, triazolo, tetrazolo, and thiazolo.

9. The compound of claim 1 wherein R_4 is H, methyl, or trifluoromethyl.

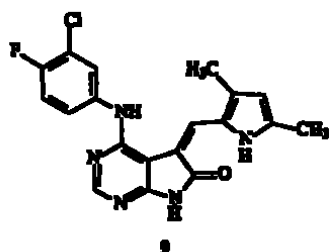
10. The compound of claim 1 wherein if Z is CH, R_1 is CH₃ or R_2 and R_2 do not form an optionally substituted methyldiene.

11. A compound selected from the group consisting of:

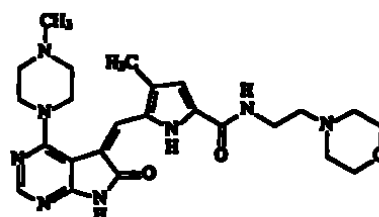


221
~~221~~
 275

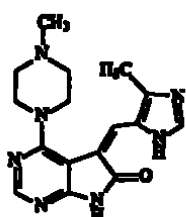
5



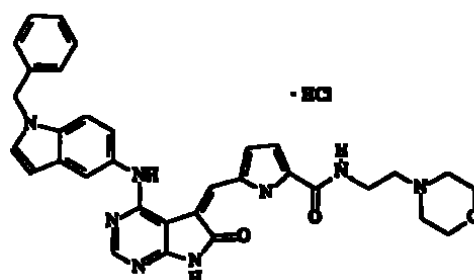
10



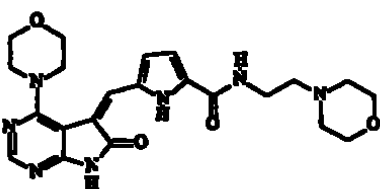
10



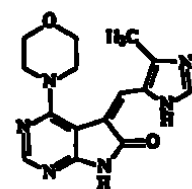
- HCl



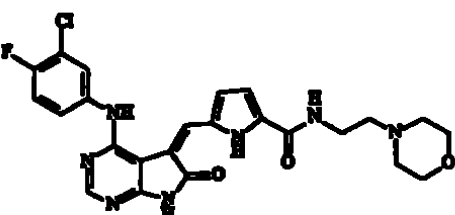
15



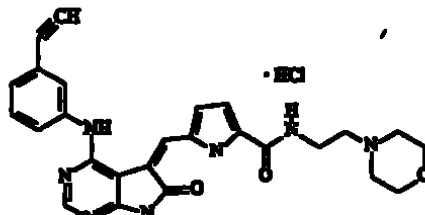
14



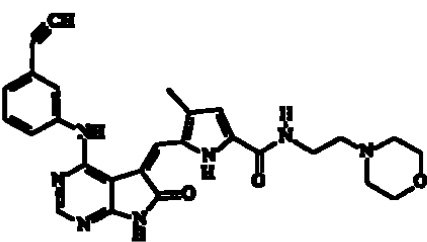
20



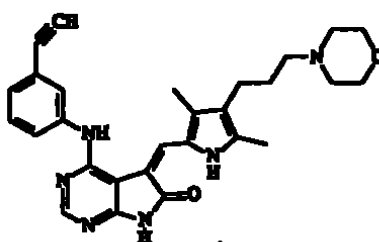
16



30



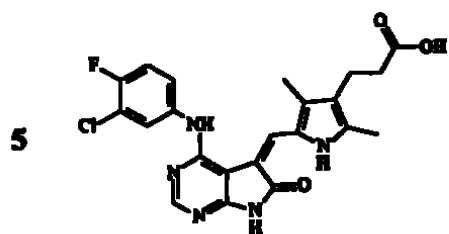
18



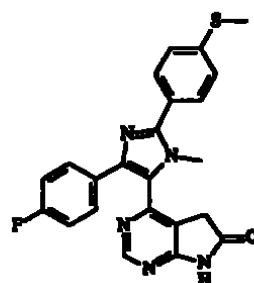
35

50171288-122175

272
 276

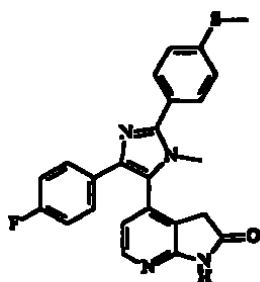


19

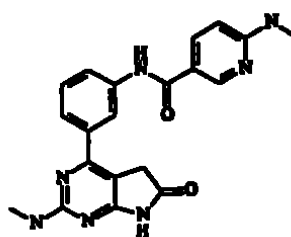


20

10

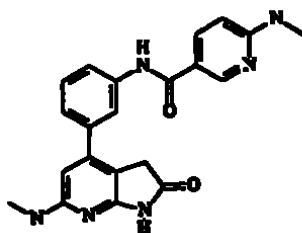


21

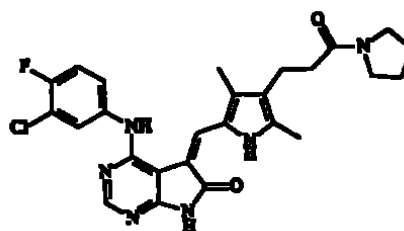


22

15

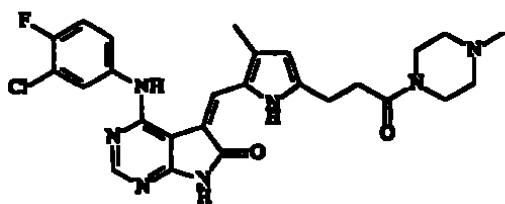


23

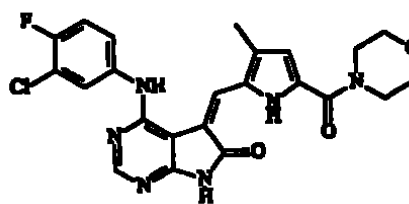


24

20



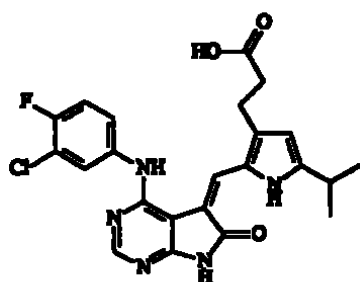
25



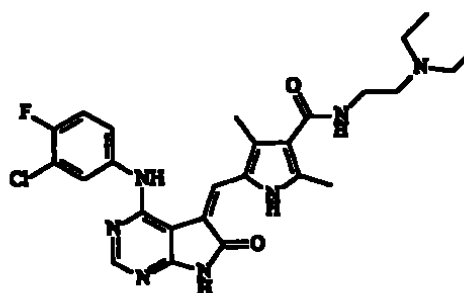
26

25

30



27

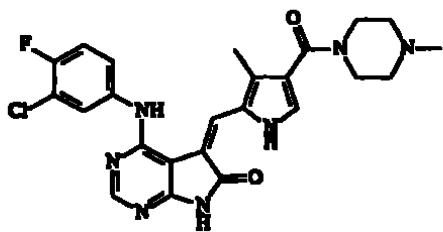


28

35

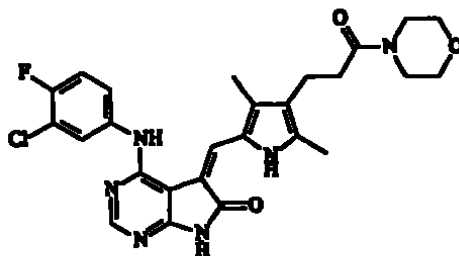
273
277

5

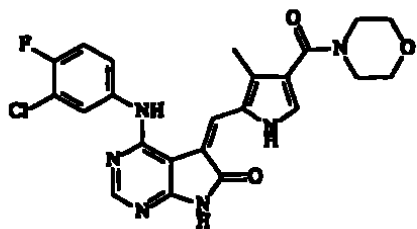


29

30

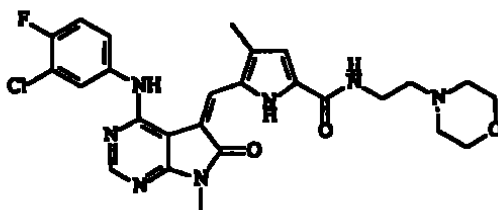


10

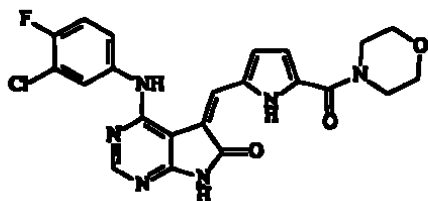


31

32

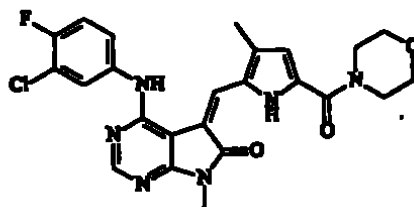


15

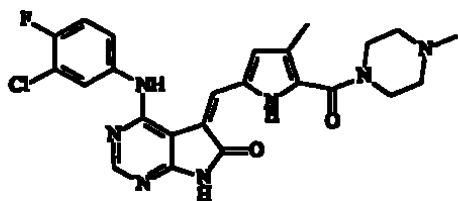


33

34



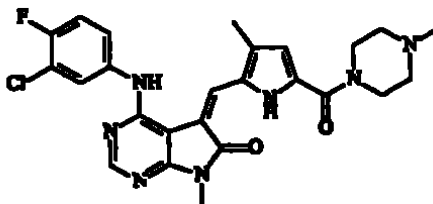
20



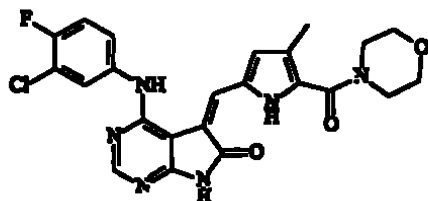
35

36

25



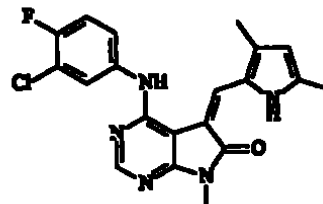
30



37

38

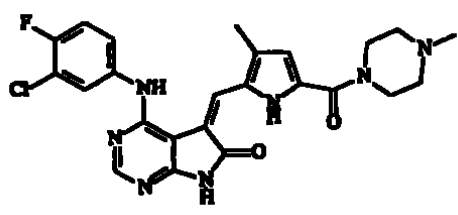
35



50171288-122177

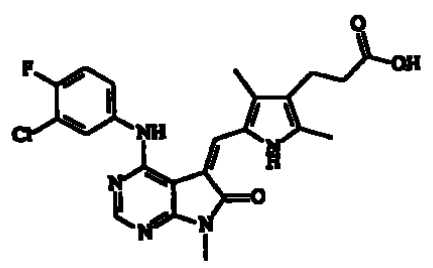
274
2A
278

5

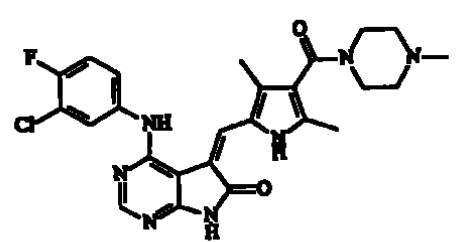


39

40

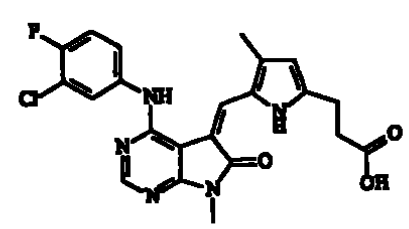


10

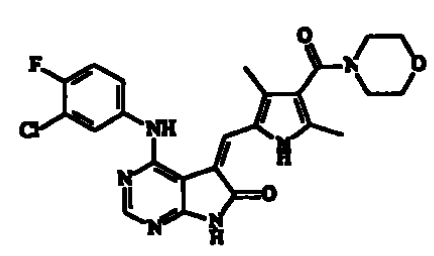


41

42

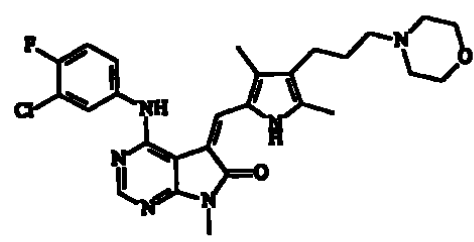


15

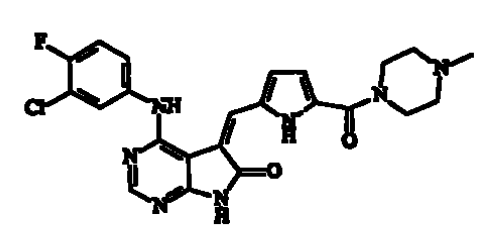


43

44

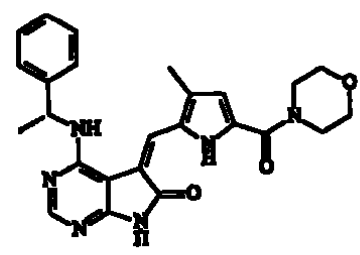


20

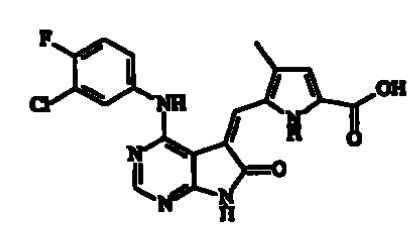


45

46

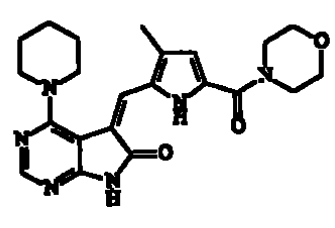


25



47

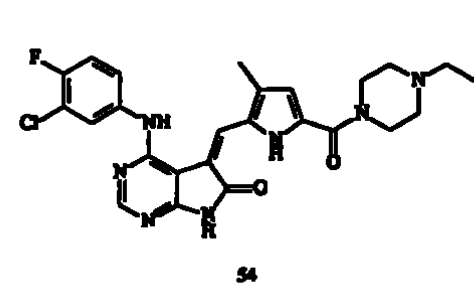
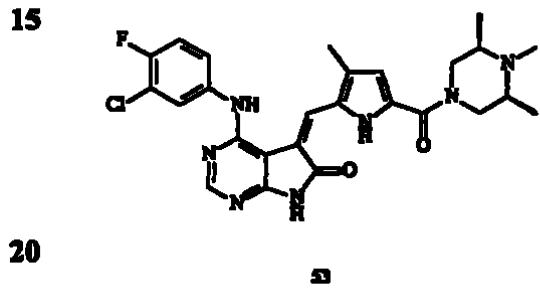
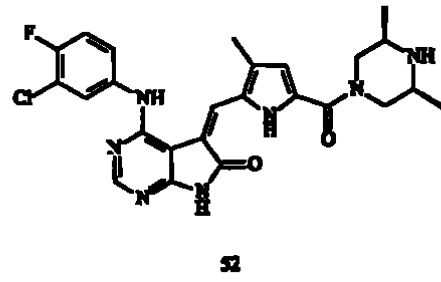
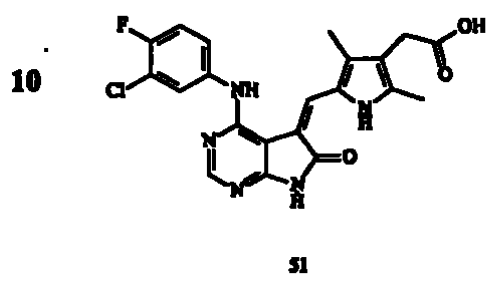
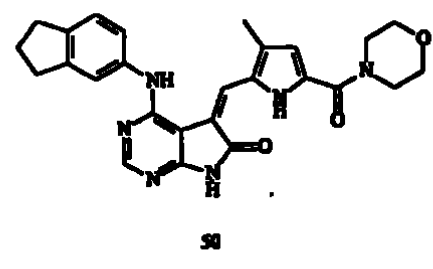
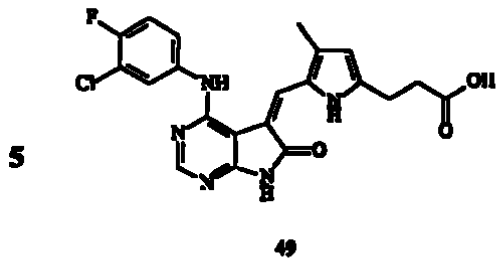
48



35

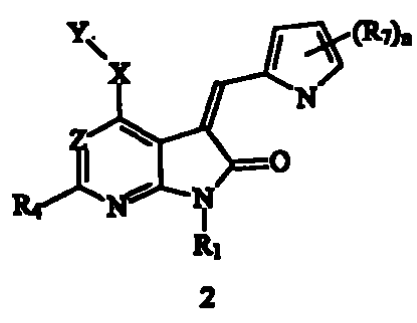
50171288-122177

275
119
879



and pharmaceutically acceptable salts, solvates, clathrates, and prodrugs thereof.

12. A method of preparing a compound of Formula 2:



or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof, wherein:

R₁ is H or methyl;

35 R₄ is H, methyl, trifluoromethyl, (C₁-C₄)alkyl, alkoxy, amido, amino, or optionally substituted aryl;

X is a chemical bond, ethynyl, -O-, -S-, -S(O)-, -S(O)₂-, -NR₂C(O)-, or -NR₂-,

wherein R₂ is H, methyl, or substituted methylene;

Y is a 5- to 10-membered mono or bicyclic, saturated, unsaturated, or aromatic ring.

50471288 - 122177

comprising 0-3 heteroatoms and optionally substituted; and

Z is N or CR₆, wherein R₆ is H, halogen, nitro, cyano, alkoxy, sulfonamide, amino, or amide;

n is an integer of 0-3;

5 each R₇ is independently H, alkyl, carboxylic acid, amina, halogen, nitro, cyano, X₁, X₂-(C-C₄)alkyl-R₈, X₂-(C₁-C₄)alkenyl-R₈, or X₂-(C₁-C₄)alkynyl-R₈;

X₁ is -C(O)NR₉-, -NR₉C(O)-, -C(O)O-, C(O)R₁₁-, -OC(O)-, -O-, -NR₉-, -S-, -S(O₂), or -S(O₂)NR₉;

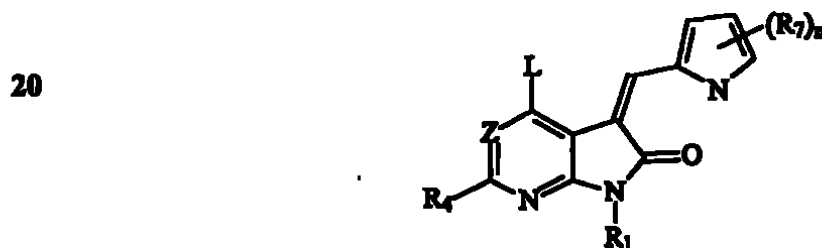
10 X₂ is a chemical bond, -C(O)NR₉-, -NR₉C(O)-, -C(O)O-, C(O)R₁₁-, -OC(O)-, -O-, -NR₉-, -S-, -S(O₂), or -S(O₂)NR₉;

R₈ is selected from the group consisting of hydrogen, dialkylamino, carboxyl, hydroxyl, alkoxy, sulfonamide, urea, carbamate, diol, alkylsulphonyl, and R₁₀;

R₉ is H or (C₁-C₃)alkyl;

15 R₁₀ is an optionally substituted 5- or 6-membered saturated, unsaturated, or aromatic heterocycle comprising from 1 to 4 heteroatoms; and

R₁₁ is an optionally substituted 5- or 6-membered saturated heterocyclic ring; which comprises reacting a compound of the formula:



25 wherein L is a leaving group with a compound of formula YXH under conditions sufficient to form a compound of Formula 2.

13. The method of claim 12 wherein L is selected from the group consisting of Br, Cl, SCH₃, and S(O)CH₃.

30

14. The method of claim 12 wherein the reaction is performed in a polar solvent.

15. The method of claim 14 wherein the polar solvent is selected from the group consisting of alcohols, DMF, and DMSO.

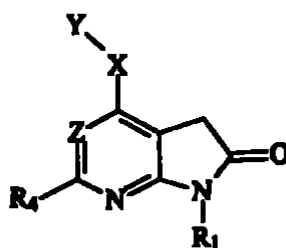
35

16. The method of claim 12 wherein the reaction is catalyzed by a catalyst selected from the group consisting of AgOTf, Pd(Ph₃)₄, and p-TsOH.

277
122
281

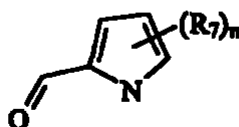
17. A method of preparing a compound of Formula 2 which comprises reacting a compound of the formula:

5



with a compound of the formula:

10



under conditions sufficient to form a compound of Formula 2.

15

18. The method of claim 17 wherein the reaction is performed in a polar solvent.

19. The method of claim 18 wherein the polar solvent is selected from the group consisting of alcohols, DMF, and DMSO.

20

20. The method of claim 17 which is catalyzed by a base.

21. The method of claim 20 wherein the base is selected from the group consisting of pyridine and piperidine.

25

22. A pharmaceutical composition comprising a compound of Formula 1, or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof, and a pharmaceutically acceptable carrier.

30

23. The pharmaceutical composition comprising a compound of claim 11 and a pharmaceutical acceptable carrier or excipient.

24. The pharmaceutical composition of claim 22 which is suitable for oral, transdermal, topical, parenteral, or mucosal administration.

35

25. A method of regulating, modulating, or inhibiting protein kinase activity which comprises contacting a compound of Formula 1, or a pharmaceutically acceptable salt or solvate thereof, with a protein kinase.

27B
120
282

26. The method of claim 25 wherein the protein kinase is a protein tyrosine kinase.

27. The method of claim 25 wherein the protein kinase is selected from the group consisting of ab1, ATK, bcr-ab1, Blk, Brk, Btk, c-fms, c-kit, c-met, c-src, CDK1, CDK2, CDK4, CDK6, cRaf1, CSF1R, CSK, EGFR, ErbB2, ErbB3, ErbB4, ERK, Fak, fcs, FGFR1, FGFR2, FGFR3, FGFR4, FGFR5, Fgr, FLK-4, flt-1, Fps, Frk, Fyn, GSK, Gst-Flk1, Hck, Her-2, Her-4, IGF-1R, INS-R, Jak, JNK, KDR, Lck, Lyn, MEK, p38, PANHER, PDGFR, PLK, PKC, PYK2, Raf, Rho, ros, SRC, tie₁, tie₂, TRK, UL97, VEGFR, Yes, and Zap70.

28. The method of claim 27 wherein the protein kinase is selected from the group consisting of PANHER, EGFR, Her-2, Her-4, PDGFR, SRC, Lck, cdk2, p38, Raf, and Rho.

29. The method of claim 28 wherein the protein kinase is selected from the group consisting of PANHER, CDK2, PDGFR, p38, and Raf.

30. The method of claim 25 wherein the protein kinase is in a cell culture.

31. The method of claim 25 wherein the protein kinase is in a mammal.

32. A method of treating or preventing a mammalian disease characterized by unregulated protein kinase activity which comprises administering to a mammal in need of such treatment or prevention a therapeutically or prophylactically effective amount of a compound of Formula 1, or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof.

33. The method of claim 30 wherein the disease characterized by unregulated protein kinase activity is selected from the group consisting of: blood vessel proliferative disorders; fibrotic disorders; mesangial cell proliferative disorders; metabolic disorders; allergies; asthma; thrombosis; nervous system diseases; and cancer.

34. The method of claim 31 wherein the disease characterized by unregulated protein kinase activity is cancer.

5
10
15
20
25
30
35

279
129
283

35. The method of claim 32 wherein the cancer is selected from the group consisting of breast, stomach, ovary, colon, lung, brain, larynx, lymphatic system, genitourinary tract (including bladder and prostate), ovarian, gastric, bone, and pancreatic cancer.

5

50171288.122177

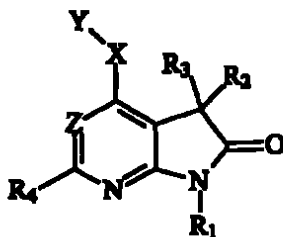
280
+38
284

**4-SUBSTITUTED 7-AZA-INDOLIN-2-ONES AND THEIR
USE AS PROTEIN KINASE INHIBITORS**

ABSTRACT

5 This invention relates to 4-substituted 7-aza-indolin-2-ones and their use as protein kinase inhibitors. Particular 4-substituted 7-aza-indolin-2-ones disclosed herein are of Formula 1

10



15 and pharmaceutically acceptable salts, solvates, clathrates, and prodrugs thereof, wherein R₁, R₂, R₃, R₄, X, Y, and Z are defined herein. The invention further relates to pharmaceutical compositions and dosage forms comprising compounds of Formula 1 and to methods of their use for the treatment and/or prevention of diseases such as, but not limited to, cancer.

20

5017122T-122177