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BOGOTA - COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-039097 - -00002-0000

Señor

SUPERINTENDENTE DE INDUSTRIA Y COME
DIVISION DE NUEVAS CREACIONES

Fecha: 2008-06-10 15:53:26 Dep. 2020 NUECREACION
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALI
Act. 538 PETICION Folios: 2

juicco@lloredacamacho.com

E. _____ S. _____ D. _____

Re: P1.-F. HOFFMANN-LA ROCHE AG.-Solicitud de Patente de Invención
en Fase Nacional PCT para "PIRAZOLOBENZODIAZEPINAS
DISUSTITUIDAS UTILES COMO INHIBIDROES DE CDK2 Y
ANGIOGENESIS, Y PARA EL TRATAMIENTO DE CANCER DE
SENO, COLON, PULMON Y PROSTATA".-Clase C07D 487/04.-
Expediente número 07-039.097.-

1. Me refiero a mi memorial de 25 de Septiembre de 2007.
2. De conformidad con lo dispuesto en el Artículo 5.2.12 de la Circular Externa No. 03 de 28 de Abril de 2003, y estando dentro del plazo de seis meses contados desde la fecha de la publicación de la solicitud en la Gaceta de la Propiedad Industrial número **583** de 18 de Diciembre de 2007, solicito a ese Despacho proceder a realizar el examen de patentabilidad de la presente invención.
3. Me permito recordar a usted, que tal como se informó en el Petitorio de la invención, la presente solicitud corresponde a Capítulo II.
4. Anexo me permito acompañar recibo número 08-43.650, expedido por la Superintendencia de Industria y Comercio, que acredita el pago de la tasa que dicho examen causa.

Señor Superintendente,

ALICIA LLOREDA RICAURTE

T.P. 53.215

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 900175049-2

RECIBO OFICIAL DE CAJA : 08 - 43,650
FECHA : JUNIO 3 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGOS	FECHA PGO	Vr. PAGO
LA RECTORIA S.A.	CONSIGNACION	BANCO DE BOGOTA	062794207	2758095	03/06/2008	6,621,000.00

***** D O N D E P O R T A *****

CANT. PENTITUD	CONCEPTO	TOTAL CONCEPTO
1 50005-01-01 SOLICITUDES	114 PTO CAP-II EXAMEN DE PATENTIBILIDAD	210,000.00
	TOTAL :	\$ 210,000.00

SOM: DOSCIENTOS DIEZ MIL PESOS

RECEPCIONABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

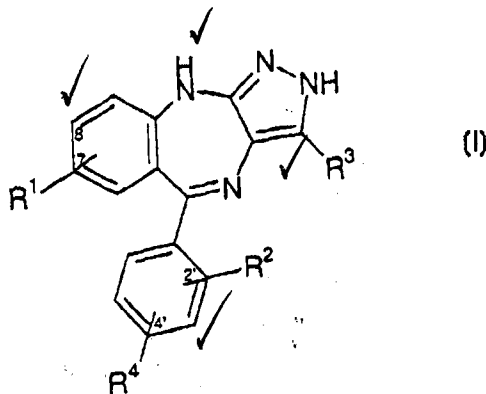
PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau208
D1

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07D 487/04, 243/22, A61K 31/41, A61P 35/00		A1	(11) International Publication Number: WO 00/64900
			(43) International Publication Date: 2 November 2000 (02.11.00)
(21) International Application Number: PCT/EP00/03394		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 14 April 2000 (14.04.00)		Published <i>With international search report.</i>	
(30) Priority Data: 60/130,370 21 April 1999 (21.04.99) <u>US</u>			
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(54) Title: PYRAZOLOBENZODIAZEPINES AS CDK2 INHIBITORS



(57) Abstract

Novel pyrazolobenzodiazepines having formula (I) and the pharmaceutically acceptable salts thereof, wherein R¹, R², R³ and R⁴ are as defined herein inhibit cyclin-dependent kinases (CDKs), in particular CDK2, are anti-proliferative agents useful in the treatment or control of cell proliferative disorders, in particular breast, colon, lung and prostate tumors.

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PYRAZOLOBENZODIAZEPINES AS CDK2 INHIBITORS

✓ The present invention is directed to novel pyrazolobenzodiazepines which
5 ✓ inhibit cyclin-dependent kinases (CDKs), in particular CDK2. These compounds
✓ and their pharmaceutically acceptable salts, and prodrugs of said compounds,
✓ are anti-proliferative agents useful in the treatment or control of cell proliferative
✓ disorders, in particular cancer. The invention is also directed to pharmaceutical
✓ compositions containing such compounds, and to methods for the treatment
10 ✓ and/or prevention of cancer, particularly in the treatment or control of solid
✓ tumors. The compounds of the invention are especially useful in the treatment
✓ or control of breast, colon, lung and prostate tumors. The invention is also
directed to intermediates useful in the preparation of the above anti-proliferative
agents.

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Uncontrolled cell proliferation is the hallmark of cancer. Cancerous tumor
cells typically have some form of damage to the genes that directly or indirectly
regulate the cell-division cycle.

20

Cyclin-dependent kinases (CDKs) are enzymes which are critical to cell
cycle control. These enzymes regulate the transitions between the different
phases of the cell cycle, such as the progression from the G₁ phase to the S
phase (the period of active DNA synthesis), or the progression from the G₂ phase
to the M phase, in which active mitosis and cell-division occurs.

25

CDKs are composed of a catalytic CDK subunit and a regulatory cyclin
subunit. The cyclin subunit is the key regulator of CDK activity, with each CDK
✓ interacting with a specific subset of cyclins: e.g. cyclin A (CDK1, CDK 2). The
different kinase/cyclin pairs regulate progression through specific stages of the
30 cell cycle.

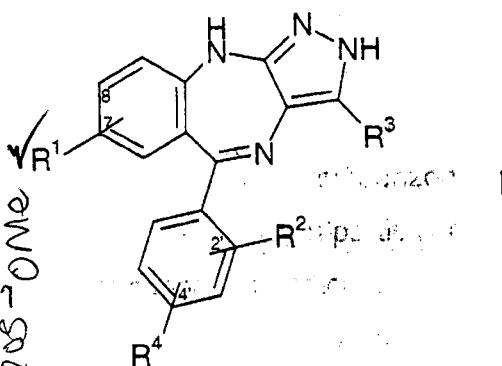
Aberrations in the cell cycle control system have been implicated in the uncontrolled growth of cancerous cells. There is an extensive body of literature validating the use of compounds inhibiting CDKs as anti-proliferative therapeutic agents.

5

The present invention relates to pyrazolobenzodiazepines capable of inhibiting the activity of one or more CDKs, in particular CDK2. Such compounds are useful for the treatment of cancer, in particular solid tumors. In particular the compounds of the present invention are especially useful in the treatment or control of breast, colon, lung and prostate tumors. The invention is also directed to intermediate compounds useful in the preparation of the above-mentioned pyrazolobenzodiazepines.

The compounds of the present invention are compounds of formula I

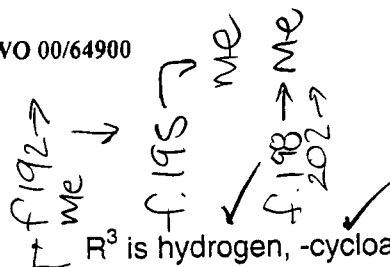
15 below



wherein

20 R^1 is hydrogen, $-NO_2$, $-CN$, $-halogen$, $-OR^5$, $-R^6OR^7$, $-COOR^7$, $-CONR^8R^9$, $-NR^{10}R^{11}$, $-NHCOR^{12}$, $-NHSO_2R^{13}$, or straight-chained lower alkyl which optionally is substituted by hydroxy and/or halogen;

R^2 and R^4 are each independently hydrogen, -halogen, -NO₂, -CF₃, or straight chained lower alkyl;



R^3 is hydrogen, -cycloalkyl, -aryl, -heterocycle, -heteroaryl, -COOR⁷-CN, -alkenyl, -CONR⁸R⁹, -alkynyl, or lower alkyl which optionally is substituted by hydroxy, -OR⁹, F, and/or aryl;

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R^5 is lower alkyl which optionally is substituted by halogen;

R^6 is lower alkyl;

10

R^7 is hydrogen or lower alkyl;

R^8 and R^9 are each independently hydrogen or lower alkyl which itself optionally are substituted by hydroxy and/or -NH₂; alternatively, R^8 and R^9 may form a 5- or 6-membered heterocycle which optionally is substituted by hydroxy, -NH₂, and/or lower alkyl;

15

R^{10} , R^{11} and R^{12} are each independently hydrogen or lower alkyl;

R^{13} is lower alkyl which optionally is substituted by halogen and/or -NR¹⁴R¹⁵; and

20

R^{14} and R^{15} are each independently hydrogen or lower alkyl which optionally are substituted halogen, or alternatively, -NR¹⁴R¹⁵ is a heterocycle.

25

Further, the invention relates to prodrugs and pharmaceutically active metabolites of compounds of formula I, and the pharmaceutically acceptable salts of the foregoing compounds.

The present invention is further directed to the use of the compounds of formula I, or prodrugs and pharmaceutically active metabolites of compounds of formula I, and the pharmaceutically acceptable salts of the foregoing compounds

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as medicaments; and to pharmaceutical compositions comprising a pharmaceutically effective amount of any one or more of the above-described compounds, or a pharmaceutically acceptable salt or prodrug thereof, and a pharmaceutically acceptable carrier or excipient.

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The present invention is also directed to the use of the compounds of formula I, prodrugs or pharmaceutically active metabolites of compounds of formula I, or the pharmaceutically acceptable salts of the foregoing compounds for the manufacture of a medicament for treating solid tumors, in particular breast, colon, lung and prostate tumors, more specifically breast and colon tumors.

As used herein, the following terms shall have the following definitions.

15 "Aryl" means an aromatic group having 5 to 10 atoms and consisting of 1 or 2 rings. Examples of aryl groups include phenyl and 1- or 2-naphthyl.

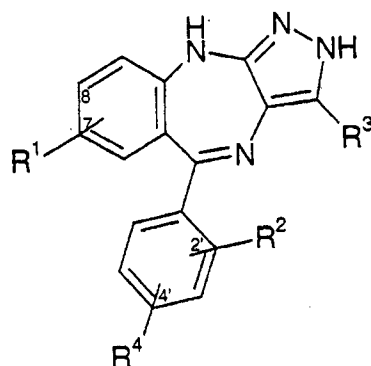
"Alkenyl" means a straight-chain or branched, substituted or unsubstituted, aliphatic unsaturated hydrocarbon having 2 to 6, preferably 2 to 4, carbon atoms and containing double bonds. Typical alkenyl groups include ethylene, propylene, isopropylene, butylene and the like. Preferred alkenyl groups are straight-chained.

25 "Alkynyl" means a straight-chain or branched, substituted or unsubstituted, aliphatic unsaturated hydrocarbon having 2 to 6, preferably 2 to 4, carbon atoms and containing triple bonds. Typical alkynyl groups include acetylene and the like. Preferred alkynyl groups are straight-chained.

30 "Cycloalkyl" means a non-aromatic, partially or completely saturated cyclic aliphatic hydrocarbon group containing 3 to 8 atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

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In one embodiment, the current invention is directed to compounds having the formula:



I } ✓

- 5 and prodrugs and pharmaceutically active metabolites of compounds of formula I, and the pharmaceutically acceptable salts of the foregoing compounds, wherein R^1 through R^{15} are as defined above. ✓

- 10 In a preferred embodiment of the compounds of formula I, R^1 is hydrogen, NO_2 , CN, $CONH_2$, halogen or unsubstituted lower alkyl. Preferred lower alkyls are methyl and ethyl. More preferably, R^1 is NO_2 , CN, or $CONH_2$. R^1 is preferably on the 7- or 8- position. ✓

- 15 In another preferred embodiment of the compounds of formula I, R^2 is on the 2' position and is hydrogen or halogen.

- In another preferred embodiment of the compounds of formula I, R^3 is unsubstituted lower alkyl, hydroxy lower alkyl, cycloalkyl, heterocycle, or heteroaryl. Preferred lower alkyl groups are methyl, ethyl and hydroxymethyl.
20 Preferred cycloalkyl groups are unsubstituted C_3-C_5 . ✓

In another preferred embodiment of the compounds of formula I, R^4 is at the 4' position and is hydrogen or halogen, most preferably R^4 is hydrogen.

wherein, in each of the immediately foregoing formulas, each of R^1 , R^2 , R^3 and R^4 are as previously defined herein. These intermediates are useful in the synthesis of compounds of formula I.

5

The compounds disclosed herein and covered by the above formulae may exhibit tautomerism or structural isomerism. It is intended that the invention encompasses any tautomeric or structural isomeric form of these compounds, or mixtures of such forms, and is not limited to any one tautomeric or structural isomeric form utilized within the formulae drawn above.

10

Synthesis of Compounds of Formula I

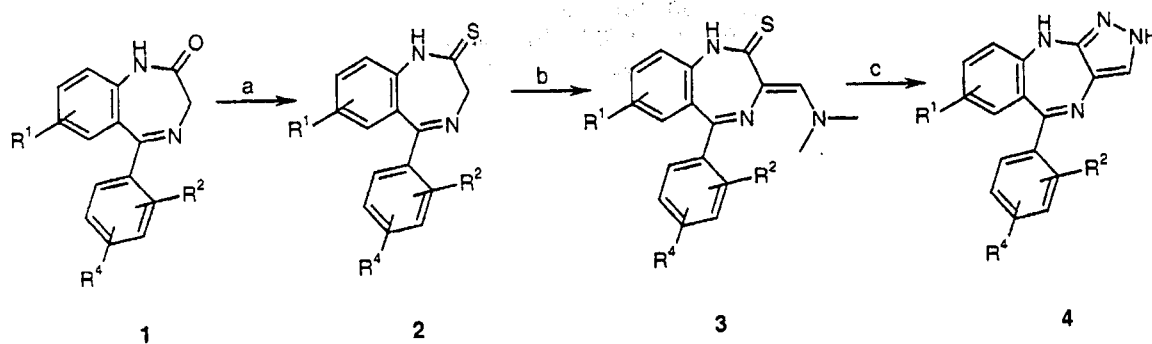
The compounds of the invention may be prepared by processes known in the art. Suitable processes for synthesizing these compounds are provided in the examples. Generally, these compounds may be prepared according to the synthesis schemes provided below.

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} ✓

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Scheme 1

 $R^3 = H$ 

a) Lawesson's reagent (a known reaction for most substitutions)

b) reaction with $\text{Me}_2\text{N}-\text{CH}(\text{OEt})_2$

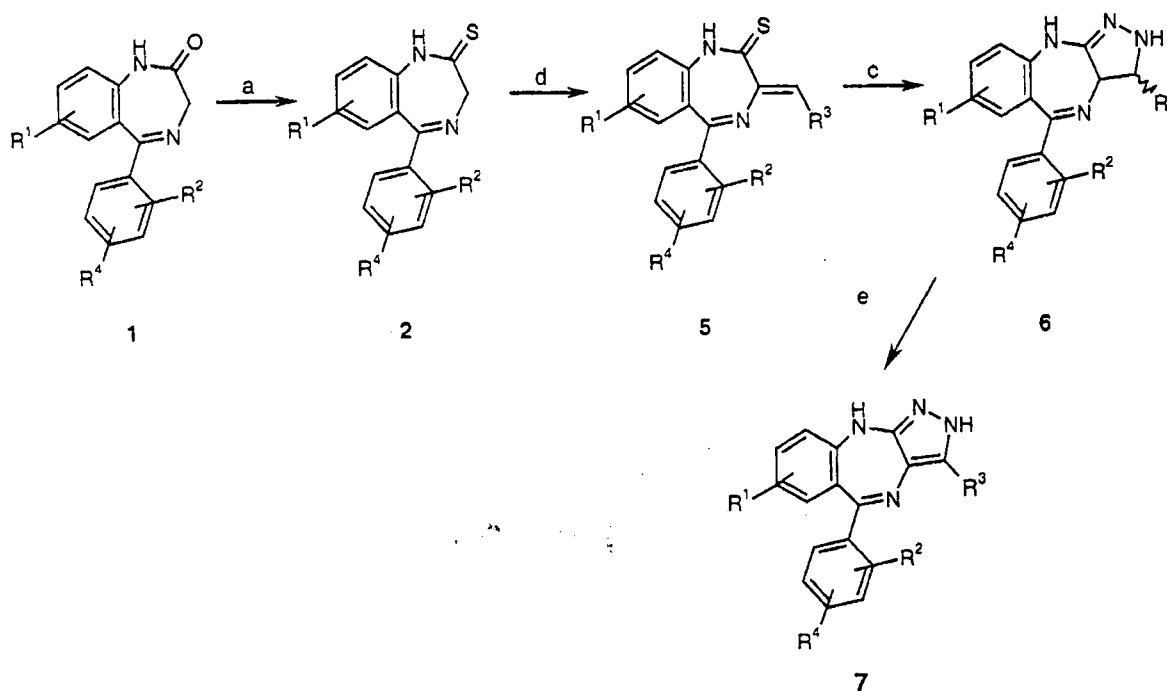
c) reaction with hydrazine.

Compound 1 is either available from commercial sources or is synthesized by methods known in the art.

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Scheme 2 R^3 is other than H

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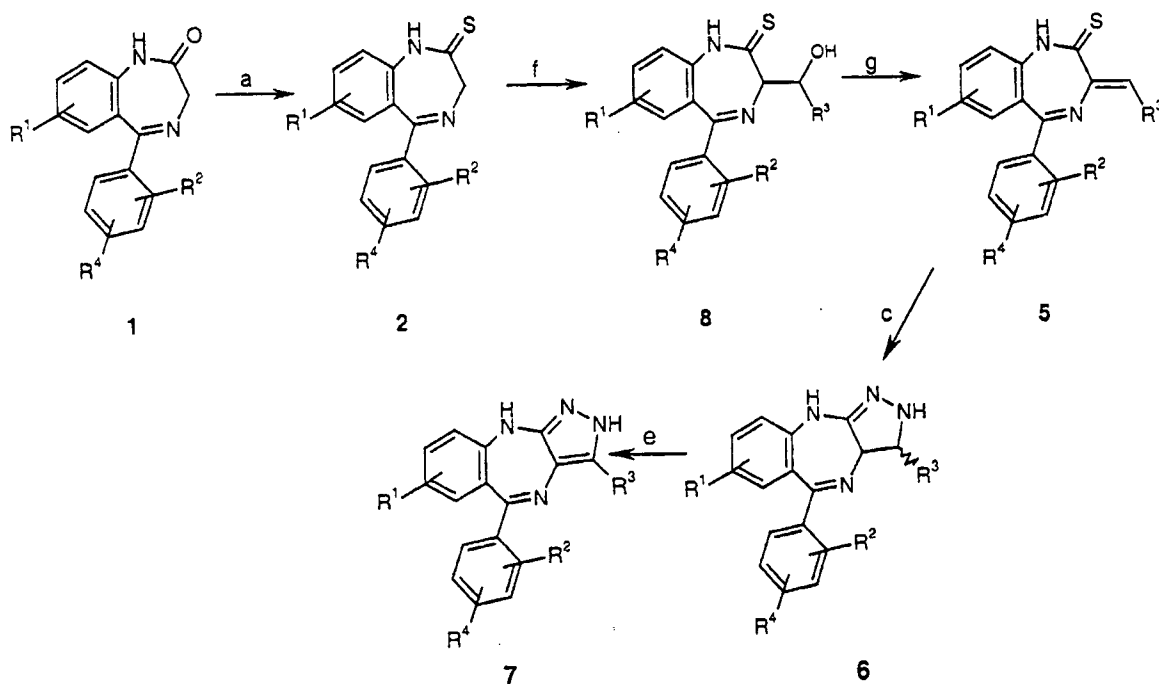
a) Lawesson's reagent (a known reaction for most substitutions)

10 d) reaction with R^3 -CHO, in the presence of a base preferably piperidine

c) reaction with hydrazine

e) oxidation of dihydropyrazole to pyrazole (use of air in DMSO RT-150° C, or in the presence of air and base ($\text{Cs}_2\text{CO}_3/\text{DMF}$)).

15

Scheme 3Alternative Scheme when R³ is other than H

a) Lawesson's reagent (a known reaction for most substitutions)

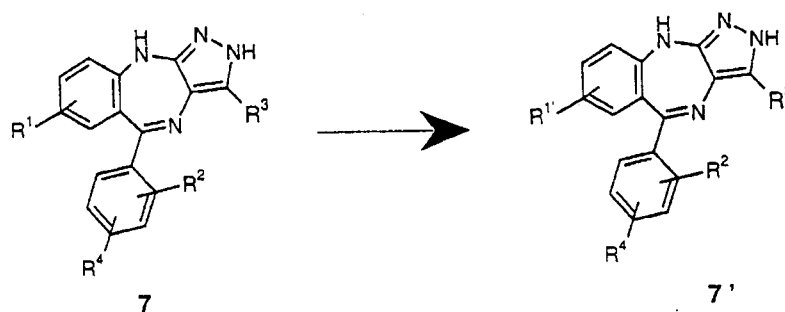
f) reaction with R³-CHO, in the presence of a base preferably diazabicycloundecane or 2,2,6,6-tetramethylpiperidine.

10 g) dehydration, by treatment with weak acid (pyridinium p-toluenesulfonate, pyridinium acetate etc.) or with chlorotrimethylsilane in pyridine at reflux

c) reaction with hydrazine

e) oxidation of dihydropyrazole to pyrazole (use of air in DMSO RT-150° C, or in the presence of air and base (Cs₂CO₃/DMF).

Scheme 4

Transformation of R¹ or R³ Functional Groups

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wherein R^{1'} can be any of the options for R¹ as defined above and, similarly, R^{3'} can be any of the options for R³ as defined above.

10 Several substitutions may be obtained by chemical modification of existing functional groups using known methods as is exemplified in scheme 4 above. For example, when the desired R¹ = NH₂, this substitution may be obtained by reduction of the corresponding nitro group. Similarly, when the desired R¹ = NHR' (where R' = -COR¹², -SO₂R¹³, or -R¹⁰R¹¹), this substitution may be

15 obtained by reaction of the corresponding R¹ = NH₂ compound with an acid halide or anhydride. When the desired R¹ = CONRR" (where R = hydrogen or lower alkyl, and R" = lower alkyl), this substitution may be obtained by reaction of the corresponding compound where R¹ = I, with carbon monoxide and a primary or secondary amine in the presence of a palladium catalyst.

20

In addition, if R³ in the starting material is CO₂Et, standard chemical modification may be used to produce compounds having the following corresponding R³ groups:

25 CH₂OH (reduction); CHO (partial reduction); CH₂NMe₂ (reductive amination of the aldehyde); CH₂OMe (alkylation of the alcohol); CH=CH₂ (olefination of the aldehyde); CONRR" (where R = H or lower alkyl and R"

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= H or lower alkyl, aminolysis with the corresponding amine HNRR" where R = H or lower alkyl and R" = H or lower alkyl); CONHNHR (where R=H, lower alkyl or aryl) (hydrazinolysis - reaction with hydrazine); CN (dehydration of the amide CONH₂).

5

In the foregoing schemes, compound 1 is either commercially available, for example from Sigma, or can be readily synthesized by methods known in the art. Thus, compound 2 is prepared from the corresponding lactam (compound 1) by the procedure of Sternbach et al., J. Org. Chem. 29:231 (1964) or by reaction with Lawesson's reagent.

10

Compositions/Formulations

In an alternative embodiment, the present invention is directed to pharmaceutical compositions comprising at least one compound of formula I or a prodrug thereof, or a pharmaceutically acceptable salt of a compound of formula I or a prodrug of such compound.

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These pharmaceutical compositions can be administered orally, for example, in the form of tablets, coated tablets, dragees, hard or soft gelatin capsules, solutions, emulsions or suspensions. They can also be administered rectally, for example, in the form of suppositories, or parenterally, for example, in the form of injection solutions.

20

The pharmaceutical compositions of the present invention comprising compounds of formula I, prodrugs of such compounds, or the salts thereof, may be manufactured in a manner that is known in the art, e.g. by means of conventional mixing, encapsulating, dissolving, granulating, emulsifying, entrapping, dragee-making, or lyophilizing processes. These pharmaceutical preparations can be formulated with therapeutically inert, inorganic or organic carriers. Lactose, corn starch or derivatives thereof, talc, steric acid or its salts can be used as such carriers for tablets, coated tablets, dragees and hard

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gelatin capsules. Suitable carriers for soft gelatin capsules include vegetable oils, waxes and fats. Depending on the nature of the active substance, no carriers are generally required in the case of soft gelatin capsules. Suitable carriers for the manufacture of solutions and syrups are water, polyols, saccharose, invert sugar and glucose. Suitable carriers for injection are water, alcohols, polyols, glycerine, vegetable oils, phospholipids and surfactants. Suitable carriers for suppositories are natural or hardened oils, waxes, fats and semi-liquid polyols.

The pharmaceutical preparations can also contain preserving agents, solubilizing agents, stabilizing agents, wetting agents, emulsifying agents, sweetening agents, coloring agents, flavoring agents, salts for varying the osmotic pressure, buffers, coating agents or antioxidants. They can also contain other therapeutically valuable substances, including additional active ingredients other than those of formula I.

Dosages

As mentioned above, the compounds of formula I, prodrugs thereof, and their salts, and compositions containing these compounds are useful in the treatment or control of cell proliferative disorders, in particular oncological disorders. These compounds and formulations containing said compounds are particularly useful in the treatment or control of solid tumors, such as, for example, breast and colon tumors.

A therapeutically effective amount of a compound in accordance with this invention means an amount of compound that is effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated. Determination of a therapeutically effective amount is within the skill in the art.

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extracts washed successively with water, brine and then dried over anhydrous sodium sulfate. The mixture was filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (elution with ethyl acetate/methanol (95:5)) gave 0.5 g of Compound C19 (pyrazole 7 wherein $R^1 =$ 5 $CONH_2$, $R^2 = F$, $R^3 = CH_3$, $R^4 = H$) as a yellow solid. mp 323-324 °C. ^1H nmr: (DMSO- d_6 , 300 MHz) 11.79 (s, 1H), 8.08 (s, 1H), 7.64 (s, 1H), 7.58 (dd, $J = 2, 9$ Hz, 1H), 7.38-7.52 (m, 2H), 7.02-7.30 (m, 4H), 6.73 (d, $J = 9$ Hz, 1H), 2.05 (s, 3H).

10 Additional compounds not specifically listed in Examples 1-3 above were prepared using the methods described above. These compounds, designated "D," are included in Tables I-IV below.

Example 4: Antiproliferative Activity

15 The antiproliferative activity of the compounds of the invention is demonstrated below. These effects indicate that the compounds of the present invention are useful in treating cancer, in particular solid tumors such as breast and colon tumors.

20

CDK2 FlashPlate Assay

To determine inhibition of CDK2 activity, purified recombinant retinoblastoma (Rb) protein was coated on 96 well FlashPlates (New England Nuclear, Boston, MA). Rb is a natural substrate for phosphorylation by CDK2 25 (Herwig and Strauss *Eurr. J. Biochem.*, Vol. 246 (1997) pp. 581-601 and references therein). Recombinant active human Cyclin E/CDK2 complexes were partially purified from extracts of insect cells. The active Cyclin E/CDK2 was added to the Rb-coated FlashPlates along with ^{33}P -ATP and dilutions of test compounds. Plates were incubated for 25 minutes at room temperature with 30 shaking, then washed and counted in the Topcount scintillation counter (Packard Instrument Co., Downers Grove, IL). Dilutions of test compounds were tested in

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duplicate in each assay. The percent inhibition of Rb phosphorylation, which is a measure of the inhibition of CDK2 activity, was determined according to the following formula:

$$100 \times \left[1 - \frac{\text{test compound} - \text{nonspecific}}{\text{total} - \text{nonspecific}} \right]$$

where "test compound" refers to the average counts per minute of the test duplicates, "nonspecific" refers to the average counts per minute when no Cyclin E/CDK2 was added, and "total" refers to the average counts per minute when no compound was added.

The results of the foregoing *in vitro* experiments are set forth in Tables IA to 1C below.

TABLE IA

Inhibition of Cdk2 - IC₅₀ Range 0.01 - 0.99 μ M

Compound Number	R ¹ (position 7)	R ² (position 2') *	R ³	R ⁴ (position 4')
A5	NO ₂	Cl	H	H
A13	NO ₂	F	H	H
A15	CN	F	H	H
A16	NO ₂	H	H	H
A17	NO ₂	CF ₃	H	H
A20	CO ₂ Et	F	H	H
A21	H	Cl	H	H
B1	NO ₂	Cl	2-pyrrolyl	H
B3	NO ₂	Cl	CH ₃	H
B4	NO ₂	Cl	CH ₂ CH ₃	H
B6	NO ₂	Cl	i-Pr	H
B7	CN	F	CH ₃	H
B9	CO ₂ Et	F	CH ₃	H
B10	NO ₂	Cl	5-(4-Me)-pyrazolyl	H
B12	NO ₂	Cl	CF ₃	H
B13	NO ₂	Cl	1-thiazolyl	H
B14	NO ₂	Cl	4-imidazolyl	H
B15	NO ₂	Cl	2-pyrazolyl	H
B16	NO ₂	Cl	3-pyrazolyl	H
B17	NO ₂	Cl	CH(Me)CH ₂ Me	H
B18	MeO	Cl	CH ₃	H
B19	Cl	H	CH ₃	H
B20	Cl	Cl	CH ₃	H
B21	H	F	CH ₃	H
B22	F	H	CH ₃	H
B23	NO ₂	Cl	Ph	H
B24	NO ₂	Cl	propyl	H
B25	NO ₂	Cl	cyclopropyl	H
B26	F	F	CH ₃	H
B27	NO ₂	H	CH ₃	H
B28	H	H	CH ₃	H
B29	I	F	CH ₃	H
B30	H	Cl	CH ₃	H
B31	NO ₂	F	CH ₃	H
B32	Cl	F	CH ₃	H