Señores

DIVISION DE NUEVAS CREACIONES

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Santafé de Bogotá, D.C.

02-01-329-

Re.: Expediente No. 01-15.772

Solicitud de Patente

Debidamente traducidas, adjunto acompaño la copia oficial de la solicitud de patente Estadounidenses No. 60/186.532 la cual le solicito agregar a este expediente para los fines legales respectivos.

De Usted atentamente,

GERMAN CASTILLO GRAU

T.P. No. 2978 del C.S.J.

C.C.No.17.017.671 de Bogotá

ESTADOS UNIDOS DE AMÉRICA

A TODOS A QUIENES LLEGUE EL PRESENTE:

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS
Oficina de Marcas y Patentes

01 de marzo de 2001

LA PRESENTE CERTIFICA QUE LA ADJUNTA ES UNA COPIA TOMADA DE LOS REGISTROS DE LA OFICINA DE MARCAS Y PATENTES DE LOS ESTADOS UNIDOS, DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE ABAJO IDENTIFICADA Y LA CUAL CUMPLE CON LOS REQUISITOS PARA QUE LE SEA CONFERIDA UNA FECHA DE RADICACIÓN SEGÚN 35 USC 111.

NÚMERO DE SOLICITUD *60/186,532*
FECHA DE RADICACIÓN: *2 de marzo de 2000*

Por Autoridad del
COMISIONADO DE MARCAS Y PATENTES

(FIRMA) L. EDELEN
Oficial de Certificaciones

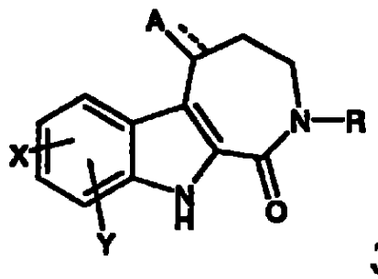
(SELLO DORADO DE OBLEA)

* * * *

INHIBIDORES DE LA QUINASA DE MITILO

REIVINDICACIONES:

1. Un compuesto según la Fórmula (I) siguiente:



(I)

en donde X y Y son, independientemente, seleccionados del grupo compuesto por H, Br, Cl, CH_3, NO_2, CN, NR_1R_2, C=ONR_1R_2MeO, HO, Me y OR;

R_1 y R_2 son seleccionados independientemente del grupo compuesto por H, alquilo más bajo, sustituido o insustituido por X, y un anillo aromático o heterocíclico, sustituido o insustituido con Y y X;

A es O, =O, =NR, =N-NHR, =NH-NH-CS-NH_2, =NH-NH-CO-NH_2; A es alquilo más bajo, sustituido o insustituido por X, arilo o anillo heterocíclico; o

A es un anillo heterocíclico.

2. Un compuesto según la reivindicación 1, seleccionado del grupo compuesto por:

5-(Piridin-2-il-hidrazono)-3,4,5,10-tetrahidro-2H-azepino[3,4-b]-indol-1-ona; y

3,4-Dihidro-2H,10*H*-azepino[3,4-*b*]indol-1,5-diona semicarbazona;

3. Un método para antagonizar un receptor de quinasa de mitilo que consiste en administrar a un sujeto que lo requiera, una cantidad efectiva de un compuesto según la reivindicación 1.

4. Un método según la reivindicación 3, en donde el compuesto es seleccionado del grupo compuesto por:

3,4 Dihidro 2*H*,10*H* azepino[3,4,*b*]indol 1,5 diona;

5-(Piridin-2-il-hidrazono)-3,4,5,10-tetrahidro-2H-azepino[3,4-b]-indol-1-ona; y

3,4-Dihidro-2H,10*H*-azepino[3,4-*b*]indol-1,5-diona semicarbazona;

5. Un método para el tratamiento de una enfermedad o trastorno seleccionado del grupo compuesto por leucemias, cánceres tumorales sólidos y metástasis, cánceres del tejido blando, cáncer del cerebro, cáncer esofágeo, cáncer estomacal, cáncer pancreático, cáncer del hígado, cáncer del pulmón, cáncer de la vejiga; cáncer en los huesos, cáncer de próstata, cáncer de ovarios, cáncer cervical, cáncer uterino, cáncer testicular, cáncer renal, cáncer en la cabeza y cáncer en el cuello, trastornos proliferativos inflamatorios crónicos, trastornos cardiovasculares proliferativos, trastornos oculares proliferativos y trastornos hiperproliferativos benignos, que consiste en administrar a un sujeto que lo requiera, una cantidad efectiva de un compuesto según la reivindicación 1.

PA 366661

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

March 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: *60/186,532*
FILING DATE: *March 02, 2000*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

L. Edelen

L. EDELEN
Certifying Officer

03-03-00 A/PRDY 39

jc712 U.S. PTO 03/02/00

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Date Of Deposit: March 2, 2000

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Signature

jc641 U.S. PTO 60/186532 03/02/00

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION for PATENT under 37 CFR 1.53(c).

Docket No.	P51104P		

INVENTOR(s) / APPLICANT(s)

Last Name	First Name	Middle Initial	Residence (City and Either State or Foreign Country)
LAGO	Maria	A.	Audubon, Pennsylvania

TITLE OF THE INVENTION (280 characters max)

MYT1 KINASE INHIBITORS

CORRESPONDENCE ADDRESS

SMITHKLINE BEECHAM CORPORATION
Corporate Intellectual Property - UW2220
709 Swedeland Road
King of Prussia

Telephone No. 610-270-5019
Facsimile No. 610-270-5090

State	PA	Zip Code	19406-2799	Country	United States of America

ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification	Number of Pages	19	☐ Small Entity Statement	
☐ Drawings	Number of Sheets		☐ Other (specify)	

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 19-2570	PROVISIONAL FILING FEE AMOUNT ($)	$150.00

Respectfully submitted,
Signature:
Soma G. Sinha

Date: 3/2/00
Registration No.: 37,444

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

PROVISIONAL APPLICATION FILING ONLY

SEND TO: Assistant Commissioner for Patents, Box Provisional Application, Washington, D.C. 20231.

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02020.02558US01

MYT1 KINASE INHIBITORS

FIELD OF THE INVENTION

The present invention relates to membrane-associated tyrosine and threonine kinase ("myt1 kinase") enzyme inhibitors, pharmaceutical compositions comprising these compounds and methods for identifying these compounds and methods of using these compounds to treat various forms of cancer and hyperproliferative diseases.

BACKGROUND OF THE INVENTION

Entry into mitosis is initiated by the M phase-promoting factor (MPF), a complex containing the cdc2 protein kinase and cyclin B. Proper regulation of MPF ensures that mitosis occurs only after earlier phases of the cell cycle are complete. Phosphorylation of cdc2 at Tyr-15 and Thr-14 suppresses this activity during interphase (G1, S, and G2). At G2-M transition, cdc2 is dephosphorylated at Tyr-15 and Thr-14 allowing MPF to phosphorylate its mitotic substrates. A distinct family of cdc-regulatory kinases (Wee1) is known to be responsible for phosphorylation of the cdc Tyr-15. A new member of this family, Myt1was recently described as the Thr-14 and Tyr-15-specific cdc2 kinase, and shown to be an important regulator of cdc2/cyclin B kinase activity (Science 270:86-90, 1995; Mol. Cell. Biol. vol 17:571, 1997). The inhibitory phosphorylation of cdc2 is important for the timing of entry into mitosis. Studies have shown that premature activation of cdc2 leads to mitotic catastrophe and cell death. Inhibition of Myt1 is predicted to cause premature activation of cdc2, and thus would kill rapidly proliferating cells. In addition, Myt1 inhibition is predicted to reduce resistance to conventional DNA-damaging chemotherapeutics, because the mechanisms by which cells avoid death involve arrest in the G2 phase of the cell cycle, and repair or DNA damage prior to division. That arrest should be prevented by blocking Myt1 inhibitory phosphorylation of cdc2.. Thus forcing the cell to enter mitosis prematurely.

Myt1 kinase is an important cell cycle regulator, particularly at the G2/M phase. Inhibitors would therefore be attractive for the treatment of cancer. Current cancer therapies, including surgery, radiation, and chemotherapy, are often

unsuccessful in curing the disease. The patient populations are large. For example, in colon cancer alone there are 160,000 new cases each year in the US, and 60,000 deaths. There are 600,000 new colon cancer cases each year worldwide. The number for lung cancer is twice that of colon cancer. The largest deficiency of chemotherapies for major solid tumors is that most patients fail to respond. This is due to cell cycle regulation and subsequent repair of damage to DNA or mitotic apparatus, the targets for most effective chemotherapeutic agents. Myt1 kinase offers a point of intervention downstream from these mechanisms by which tumor cells develop resistance. Inhibition of Myt1 could in and of itself have therapeutic benefit in reducing tumor proliferation, and in addition, could be used in conjunction with conventional chemotherapies to overcome drug resistance.

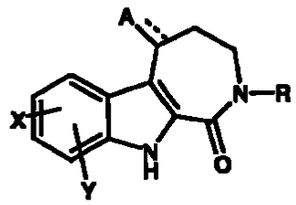
Based on the foregoing, there is a need to identify a potent myt1 kinase enzyme inhibitor for the treatment of various indications, including cancer, associated with the present receptor.

SUMMARY OF THE INVENTION

The present invention involves compounds represented by Formula (I) hereinbelow, pharmaceutical compositions comprising such compounds and methods of antagonizing the myt1 kinase receptor using these compounds.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compounds of Formula (I), hereinbelow:



(I)

wherein X and Y are, independently, selected from the group consisting of H, Br, Cl, CH_3, NO_2, CN, NR_1R_2, $C{=}ONR_1R_2$, MeO, HO, Me, and OR;

R_1 and R_2 are independently selected from the group consisting of H, lower alkyl, unsubstituted or substituted by X, and an aromatic or heterocyclic ring, unsubstituted or substituted with Y and X;

A is O, =O, =NR, =N-NHR, =NH-NH-CS-NH_2, =NH-NH-CO-NH_2; A is lower alkyl substituted or unsubstituted by X, aryl or heteroaryl ring; or

A is a heterocyclic ring.

Preferred compounds of the present invention are selected from the group consisting of:

3,4-Dihydro-2*H*,10*H*-azepino[3,4-*b*]indole-1,5-dione;

5-(Pyridin-2-yl-hydrazono)-3,4,5,10-tetrahydro-2H-azepino[3,4-b]-indol-1-one; and

3,4-Dihydro-2*H*,10*H*-azepino[3,4-*b*]indole-1,5-dione semicarbazone;

As used herein, "alkyl" refers to an optionally substituted hydrocarbon group joined together by single carbon-carbon bonds. The alkyl hydrocarbon group may be linear, branched or cyclic, saturated or unsaturated. Preferably, the group is saturated linear or cyclic.

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds and diastereomers are contemplated to be within the scope of the present invention.

The present compounds can also be formulated as pharmaceutically acceptable salts and complexes thereof. Pharmaceutically acceptable salts are non-toxic salts in the amounts and concentrations at which they are administered.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, fumarate, maleate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, maleic acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, fumaric acid, and quinic acid.

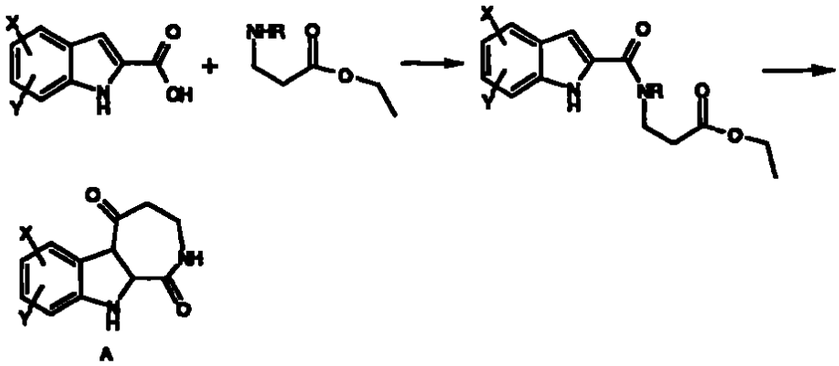
Pharmaceutically acceptable salts also include basic addition salts such as those containing benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium,

potassium, sodium, ammonium, alkylamine, and zinc, when acidic functional groups, such as carboxylic acid or phenol are present.

Preferred salts include hydrobromide, dihydrobromide and bistrifluoroacetate.

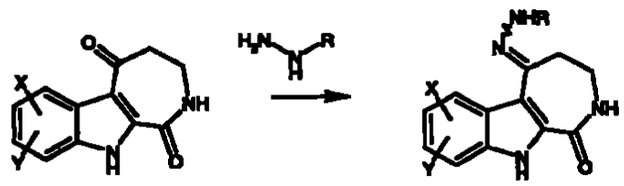
The present compounds are readily prepared by the schemes represented below:

Scheme 1



A

Scheme 2



A

X, Y are independently H, Br, Cl, CH_3, NO_2, CN, NR_1R_2, $C{=}ONR_1R_2$, MeO, HO, Me, and OR.

General Preparation

The general procedures for the synthesis of these compounds are illustrated in Schemes 1 and 2. Preparation of the 3,4-Dihydro-2*H*,10*H*-azepino[3,4-*b*]indole-1,5-dione ring was accomplished following the procedure described in Scheme 1, e.g. the coupling of the β-aminoalanine ethyl ester with indole-2-carboxylic acid was carried out under standard amide coupling conditions. Cyclization of the thus

obtained ester was carried out by treatment under strong acid conditions, such as methanesulfonic acid or polyphosphoric acid leading to the formation of the azepinone ring. Reaction of 3,4-Dihydro-2*H*,10*H*-azepino[3,4-*b*]indole-1,5-dione with several amine nucleophiles such as hydrazines, semicarbazides or amines was carried out under standard literature conditions leading to the formation of the corresponding hydrazones, semicarbazones and imines respectively.

Nuclear magnetic resonance spectra were recorded at either 300 or 400 MHz using, respectively, a Bruker AM 300 or Bruker AC 400 spectrometer. $CDCl_3$ is deuteriochloroform, DMSO-d_6 is hexadeuteriodimethylsulfoxide, and CD_3OD is tetradeuteriomethanol. Chemical shifts are reported in parts per million (δ) downfield from the internal standard tetramethylsilane. Abbreviations for NMR data are as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=doublet of doublets, dt=doublet of triplets, app=apparent, br=broad. J indicates the NMR coupling constant measured in Hertz. Continuous wave infrared (IR) spectra were recorded on a Perkin-Elmer 683 infrared spectrometer, and Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 400 D infrared spectrometer. IR and FTIR spectra were recorded in transmission mode, and band positions are reported in inverse wavenumbers (cm^{-1}). Mass spectra were taken on either VG 70 FE, PE Syx API III, or VG ZAB HF instruments, using fast atom bombardment (FAB) or electrospray (ES) ionization techniques. Elemental analyses were obtained using a Perkin-Elmer 240C elemental analyzer. All temperatures are reported in degrees Celsius.

Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates were used for thin layer chromatography. Both flash and gravity chromatographies were carried out on E. Merck Kieselgel 60 (230-400 mesh) silica gel. Analytical and preparative HPLC were carried out on Rainin or Beckman chromatographs. ODS refers to an octadecylsilyl derivatized silica gel chromatographic support. 5 µ Apex-ODS indicates an octadecylsilyl derivatized silica gel chromatographic support having a nominal particle size of 5 µ, made by Jones Chromatography, Littleton, Colorado. YMC ODS-AQ® is an ODS chromatographic support and is a registered trademark of YMC Co. Ltd., Kyoto, Japan. PRP-1® is a polymeric (styrene-

-divinylbenzene) chromatographic support, and is a registered trademark of Hamilton Co., Reno, Nevada) Celite® is a filter aid composed of acid-washed diatomaceous silica, and is a registered trademark of Manville Corp., Denver, Colorado.

With appropriate manipulation and protection of any chemical functionality, synthesis of the remaining compounds of Formula (I) is accomplished by methods analogous to those above.

In order to use a compound of Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

The present ligands can be administered by different routes including intravenous, intraperitoneal, subcutaneous, intramuscular, oral, topical, transdermal, or transmucosal administration. For systemic administration, oral administration is preferred. For oral administration, for example, the compounds can be formulated into conventional oral dosage forms such as capsules, tablets and liquid preparations such as syrups, elixirs and concentrated drops.

Alternatively, injection (parenteral administration) may be used, e.g., intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds of the invention are formulated in liquid solutions, preferably, in physiologically compatible buffers or solutions, such as saline solution, Hank's solution, or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms can also be produced.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration, for example, may be through nasal sprays, rectal suppositories, or vaginal suppositories.

For topical administration, the compounds of the invention can be formulated into ointments, salves, gels, or creams, as is generally known in the art.

The amounts of various compounds to be administered can be determined by standard procedures taking into account factors such as the compound IC_{50}, EC_{50}, the biological half-life of the compound, the age, size and weight of the patient, and the disease or disorder associated with the patient. The importance of these and other factors to be considered are known to those of ordinary skill in the art.

Amounts administered also depend on the routes of administration and the degree of oral bioavailability. For example, for compounds with low oral bioavailability, relatively higher doses will have to be administered.

Preferably the composition is in unit dosage form. For oral application, for example, a tablet, or capsule may be administered, for nasal application, a metered aerosol dose may be administered, for transdermal application, a topical formulation or patch may be administered and for transmucosal delivery, a buccal patch may be administered. In each case, dosing is such that the patient may administer a single dose.

Each dosage unit for oral administration contains suitably from 0.01 to 500 mg/Kg, and preferably from 0.1 to 50 mg/Kg, of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base. The daily dosage for parenteral, nasal, oral inhalation, transmucosal or transdermal routes contains suitably from 0.01 mg to 100 mg/Kg, of a compound of Formula(I). A topical formulation contains suitably 0.01 to 5.0% of a compound of Formula (I). The active ingredient may be administered from 1 to 6 times per day, preferably once, sufficient to exhibit the desired activity, as is readily apparent to one skilled in the art.

As used herein, "treatment" of a disease includes, but is not limited to prevention, retardation and prophylaxis of the disease. As used herein, "diseases" treatable using the present compounds include, but are not limited to leukemias, solid tumor cancers, metastases, soft tissue cancers, brain cancer, esophageal cancer, stomach cancer, pancreatic cancer, liver cancer, lung cancer, bladder cancer, bone cancer, prostate cancer, ovarian cancer, cervical cancer, uterine cancer, testicular

cancer, kidney cancer, head cancer and neck cancer, chronic inflammatory proliferative diseases such as psoriasis and rheumatoid arthritis; proliferative cardiovascular diseases such as restenosis; proliferative ocular disorders such as diabetic retinopathy; and benign hyperproliferative diseases such as hemangiomas.

Composition of Formula (I) and their pharmaceutically acceptable salts which are active when given orally can be formulated as syrups, tablets, capsules and lozenges. A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, peanut oil. olive oil, glycerine or water with a flavoring or coloring agent. Where the composition is in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, terra alba, talc, gelatin, acacia, stearic acid, starch, lactose and sucrose. Where the composition is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums, celluloses, silicates or oils, and are incorporated in a soft gelatin capsule shell.

Typical parenteral compositions consist of a solution or suspension of a compound or salt in a sterile aqueous or non-aqueous carrier optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil or sesame oil.

Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

A typical suppository formulation comprises a compound of Formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent, for example polymeric glycols, gelatins, cocoa-butter or other low melting vegetable waxes or fats or their synthetic analogs.

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Typical dermal and transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

Preferably the composition is in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patient may administer a single dose.

No unacceptable toxological effects are expected when compounds of the present invention are administered in accordance with the present invention.

The biological activity of the compounds of Formula (I) is demonstrated by the tests indicated hereinbelow.

<u>In vitro assays</u>:

Compounds capable of inhibiting myt1 kinase can be identified with in vitro assays and cellular assays as described below. Variations of these assays would be obvious to those skilled in the art.

<u>Expression of GST-Myt1</u>:

A GST-Myt1 expression construct was constructed which has the glutathione-S-transferase gene fused to the amino terminus of Myt1 kinase via a linker containing a thrombin cleavage site. This clone has been truncated at amino acid 362 of Myt1, just prior to the to the transmembrane domain. This construct was cloned into the Baculovirus expression vector, pFASTBAC, and this was used to make the viral stock for the subsequent infection. Spodoptera frugiperda cells (Sf21) were infected with the virus expressing the GST-Myt1 and the cells were grown for 3 days, then harvested and frozen down.

<u>Purification of GST-Myt1</u>:

The GST-Myt1 protein was purified as follows: An Sf21 cell pellet expressing GST-Myt1 was resuspended on ice in 10mls of lysis buffer (50mM Tris-Cl, pH 7.5, 250mM $NaCl_2$, 1mM dithiothreitol (DTT), 0.1%NP-40, 5% (v/v) protease inhibitor cocktail, 1mM sodium orthovanadate), cells were lysed by sonication and centrifuged at 100,000xg for 30min. The supernatant was added to 5mls (packed volume) of Glutathione Sepharose 4B, equilibrated in wash buffer (20mM Tris-Cl, pH 7.0, 10mM $MgCl_2$, 100mM $NaCl_2$, 1mM DTT, 0.5%(v/v) protease inhibitor cocktail, 1mM sodium orthovanadate). The mixture was rocked

for 30min. The resin with the bound GST-Myt1 was spun down at 500xg for 5min and washed with 14mls of wash buffer. The beads were spun as above and resuspended in another 14mls of wash buffer. The suspension was transferred into a column and allowed to pack, then the wash buffer was allowed to flow through by gravity. The GST-Myt1 was eluted from the column with 10mls of 10mM Glutathione in 50mM Tris-Cl, pH 8.0 in 500ul fractions. Protein concentrations were determined on the fractions using Bio-Rad's Protein assay kit as per instructions. Fractions containing the GST-Myt1 were pooled and diluted to a concentration of ~0.5mg/ml and dialyzed for 4 hours at 4^0C in dialysis buffer (20mM HEPES, pH 7.0, 1mM Manganese Acetate, 100mM $NaCl_2$, 0.05% Brij-35, 10% glycerol, 1mM DTT, 0.2% (v/v) protease inhibitor cocktail, 1mM sodium orthovanadate). The protein was aliquoted and stored at -80^0.

<u>Enzyme Assays</u>:

GST-Myt1 autophosphorylation-DELFIA assay

Delayed fluorescent immunoassays (DELFIA) were performed in 96well NUNC maxisorp plates, at 50ul/well with 0.25ug GST-Myt1, in BufferA (50mM HEPES, pH 7.4, 2mM $Mn(OAc)_2$, 5uM ATP, 1mM DTT). For determination of pH optimum, divalent cation usage and K_m of ATP, the appropriate component was varied as indicated in the figures. Autophosphorylation reactions were initiated by the addition of GST-Myt1 in buffer and were allowed to proceed at room temperature with shaking for 20min. The reactions were stopped with the addition of EDTA to a 20mM final concentration, and the protein was allowed to continue to bind to the wells for an additional 40min. Wells were washed three times with 300ul TBS/Tween (50mM Tris, pH 7.4, 150mM $NaCl_2$, 0.2% Tween-20). After washing, the plate was blocked using Pierce's Superblock in TBS at 100ul/well. This was immediately decanted and the blocking was repeated two more times. The plate was then washed again with three washes of 300ul/well of TBS-Tween. Then 100ul of Eu-labeled anti-phosphotyrosine antibody diluted to 0.125ug/ml in TBS/Tween containing 0.15mg/ml BSA was added to the wells and allowed to incubate for 30min. with shaking at room temperature. Wells were then washed three times with 300ul of TBS/Tween, 200ul of Enhancement solution was added per well and

incubated with shaking for 10min. The plate was then read on the 1420 VICTOR plate counter from Wallac, Inc. The identical conditions are used for inhibitor studies except that ATP is at 1uM and inhibitors are added, in dimethyl sulfoxide (DMSO) to a final concentration of 1%. Typical concentration ranges in which test compounds are expected to inhibit myt1 autophosphorylation are 0.001 to 10 uM.

Biological Studies:

Cell Cycle Studies

Drug studies considering cellular effects were performed in the Hela S3 adherent cell line. Cells were plated at a concentration sufficiently low such that 24 hours later they were at 10-20% confluence (typically $2x10^5$ cells/15cm e3). Cells were then synchronized in S phase by a repeated thymidine block. Briefly, cells were treated with 2mM thymidine for 18hours, released for 8 hours by 3 washes, and then treated again with thymidine. Following the second release from thymidine, 95% of cells were in S phase. Synchronized cells were then returned to complete media containing a DNA-damaging drug such as 50nM topotecan (a dosage we have found to be sufficient to arrest cells in early G2 phase without inducing apoptosis) alone and in combination with test compounds for up to 18 hours. Cell Cycle profiles were then performed cytometrically using a procedure for propidium iodide staining of nuclei. (Vindelov et al, Cytometry Vol.3, No.5, 1983, 323-327) Myt1 inhibitors would be expected to reverse the G2 arrest caused by the DNA damaging agent. Typical concentration ranges for such activity would be 0.001 to 10 uM.

Proliferation/Apoptosis Studies:

Proliferation studies were performed in a variety of adherent and non-adherent cell lines including Hela S3, HT29, and Jurkat. The proliferation assay utilized a colorimetric change resulting from reduction of the tetrazolium reagent XTT into a formazan product by metabolically active cells (Scudiero et al. Cancer Research, 48, 1981, 4827-4833) Cells were seeded in 100uls in 96 well plates to roughly 10% confluence (cell concentration varied with cell lines) and grown for 24 hours. Compounds were then added with or without sufficient vehicle- containing media to raise the cells to a 200ul final volume containing chemical reagents in 0.2% DMSO. Cells received multiple concentrations of DNA-damaging anti-proliferative

drugs such as topotecan, test compounds, and combination treatment at 37°C 5% CO_2. 72 hours later, 50 uls of an XTT/ phenazine methosulfate mixture were added to each well and cells were left to incubate for 90mins. Plate was read at 450nm, and anti-proliferative effects were compared relative to vehicle treated cells. Myt1 inhibitors are expected to inhibit the proliferation of such cancer cell lines and/or enhance the cytotoxicity of DNA-damaging chemotherapeutic drugs. Typical concentration ranges for such activity would be 0.001 to 10 uM. Other assays for cellular proliferation or cytotoxicity could also be used with test compounds, and these assays are known to those skilled in the art.

The present invention includes but is not limited to the examples below.

Nuclear magnetic resonance spectra were recorded at 300 MHz using a Bruker AM 300 spectrometer. $CDCl_3$ is deuteriochloroform, DMSO-d_6 is hexadeuteriodimethylsulfoxide, and CD_3OD is tetradeuteriomethanol. Chemical shifts are reported in parts per million (?) downfield from the internal standard tetramethylsilane. Abbreviations for NMR data are as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=doublet of doublets, dt=doublet of triplets, app=apparent, br=broad. J indicates the NMR coupling constant measured in Hertz. Continuous wave infrared (IR) spectra were recorded on a Perkin-Elmer 683 infrared spectrometer, and Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 400 D infrared spectrometer. IR and FTIR spectra were recorded in transmission mode, and band positions are reported in inverse wavenumbers (cm^{-1}). Mass spectra were taken on either VG 70 FE, PE Syx API III, or VG ZAB HF instruments, using fast atom bombardment (FAB) or electrospray (ES) ionization techniques. Elemental analyses were obtained using a Perkin-Elmer 240C elemental analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. All temperatures are reported in degrees Celsius.

Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates were used for thin layer chromatography. Both flash and gravity chromatography was carried out on E. Merck Kieselgel 60 (230-400 mesh) silica gel. Analytical and preparative HPLC were carried out on Rainin or Beckman chromatographs. ODS refers to an octadecylsilyl derivatized silica gel chromatographic support. 5 µ Apex-

ODS indicates an octadecylsilyl derivatized silica gel chromatographic support having a nominal particle size of 5 μ, made by Jones Chromatography, Littleton, Colorado. YMC ODS-AQ® is an ODS chromatographic support and is a registered trademark of YMC Co. Ltd., Kyoto, Japan. PRP-1® is a polymeric (styrene-divinylbenzene) chromatographic support, and is a registered trademark of Hamilton Co., Reno, Nevada) Celite® is a filter aid composed of acid-washed diatomaceous silica, and is a registered trademark of Manville Corp., Denver, Colorado.

Following the general procedure described above the following compounds have been synthesized:

Example 1

Preparation of 3,4-Dihydro-2*H*,10*H*-azepino[3,4-*b*]indole-1,5-dione

a) 3-[(1-1*H*-Indol-2-yl-methanoyl)-amino]-propionic acid ethyl : Indole 2-carboxylic acid (4g, 25mmol), ethyl β-alanine (50mmol) and BOP reagent was dissolved in 150 mL of acetonitrile. Then triethyl amine was added to give clear solution. The resultant mixture was stirred at room temperature overnight. The product precipitated out as white solid, it was then filtered to afford 3.5 g of the desired product. ^{1}H-NMR (DMSO-d6): δ 8.56 (1H, t, J=5.6Hz), 7.60 (1H, d, J=8Hz), 7.42 (1H, d, J=8Hz), 7.17 (1H, m), 7.08 (1H, s), 7.00 (1H, m), 4.08 (2H, q), 3.5(2H, q), 2.5 (2H, q), 1.17 (3H, t, J=7.1Hz)

b) 3, 4-dihydro-2*H*, 10*H*azepino[3, 4-b]indole-1, 5-dione trifluoroacetate : The ethyl ester obtained in experiment 1 (a) (1g) was dissolved in 5mL $MeSO_3H$ and refluxed for 15 h, cooled down to RT, then poured into ice water, neutralized to PH=7 using Sat. $NaHCO_3$, followed by extraction of EtOAc, dried over anhydrous sodium sulfate and removed the solvent to give yellow solid as crude product, the crude was subjected to chromatography column (20:80=EtOAc:Hexane) to afford 300mg white solid as the final product. ^{1}HNMR (DMSO-d6): δ8.77 (1H, t, J=5Hz), 8.30 (1H, d, J=8Hz), 7.53 (1H, d, J=8Hz), 7.33 (1H, t, J=7Hz), 7.25 (1H, t, J=7Hz), 3.46 (2H, dd, J=5.2Hz, 10.5Hz), 2.83 (2H, d, J=5.2Hz, 10.5Hz).

M+1($C_{12}H_{10}N_2O_2$): 215.1

Example 2

Preparation of 5-(Pyridin-2-yl-hydrazono)-3, 4, 5, 10-tetrahydro-2*H*-azepino[3, 4-b]-indol-1-one 3, 4-dihydro-2*H*, 10*H*-azepino[3, 4-b]indole-1, 5-dione (100mg, 0.47mmol) and 2-hydrazinepyridine (61mg, 0.56mmol) was dissolved in 10 mL EtOH, followed by addition of catalytic amount of glacial acetic acid, then after refluxing for 3 h, light yellow solid was precipitated out, filtered and washed with a little bit EtOH to afford 100 mg product. ^{1}HNMR (DMSO-d6): δ9.57 (1H, s), 8.44 (1H, d, J=8Hz), 8.37 (1H, t, J=5.4Hz), 8.16 (1H, dd, J=1.oHz, 4.8Hz), 7.73 (1H, m), 7.46 (1H, m), 7.29 (1H, m), 7.27 (1H, m), 6.80 (1H, m), 3.38 (2H, m), 3.00 (2H, m). M+1(C17H15N5O): 306

Example 3

Preparation of 3,4-Dihydro-2*H*,10*H*-azepino[3,4-*b*]indole-1,5-dione semicarbazone : The procedure is analogous to the preparation of Example number 2 but using semicarbazide instead of the hydrazine to obtain the title compound as a crystalline yellow solid. ^{1}HNMR (DMSO-d6): δ9.23 (1H, s), 8.36 (1H, t, J=5.32), 8.18 (1H, d, J=8,10Hz), 7.45 (1H, d, J=8.20Hz), 7.28 (1H, m), 7.16 (1H, m), 6.3 (2H, br), 3.33 (2H, m), 2.86 (3H, m). M+1(C13H13N5O2): 272

Formulations for pharmaceutical use incorporating compounds of the present invention can be prepared in various forms and with numerous excipients. Examples of such formulations are given below:

Example 4

Inhalant Formulation:

A compound of Formula (I), (1 mg to 100 mg) is aerosolized from a metered dose inhaler to deliver the desired amount of drug per use.

Example 5

Tablet Formulation:

Tablets/Ingredients		Per Tablet
1.	Active ingredient (Cpd of Form. (I)	40 mg
2.	Corn Starch	20 mg
3.	Alginic acid	20 mg
4.	Sodium Alginate	20 mg
5.	Mg stearate	1.3 mg

Procedure for Tablet Formulation:

Ingredients 1, 2, 3 and 4 are blended in a suitable mixer/blender. Sufficient water is added portion-wise to the blend with careful mixing after each addition until the mass is of a consistency to permit its conversion to wet granules. The wet mass is converted to granules by passing it through an oscillating granulator using a No. 8 mesh (2.38 mm) screen. The wet granules are then dried in an oven at 140°F (60 °C) until dry. The dry granules are lubricated with ingredient No. 5, and the lubricated granules are compressed on a suitable tablet press.

Example 6

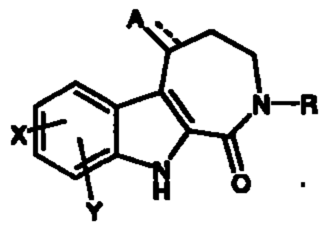
Parenteral Formulation

A pharmaceutical composition for parenteral administration is prepared by dissolving an appropriate amount of a compound of Formula I in polyethylene glycol with heating. This solution is then diluted with water for injections (to 100 mL). The solution is then rendered sterile by filtration through a 0.22 micron membrane filter and sealed in sterile containers.

All publications, including but not limited to patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference as though fully set forth.

What is claimed is:

1. A compound according to Formula (I), hereinbelow:



(I)

wherein X and Y are, independently, selected from the group consisting of H, Br, Cl, CH_3, NO_2, CN, NR_1R_2, $C{=}ONR_1R_2$MeO, HO, Me, and OR;

R_1 and R_2 are independently selected from the group consisting of H, lower alkyl, unsubstituted or substituted by X, and an aromatic or heterocyclic ring, unsubstituted or substituted with Y and X;

A is O, =O, =NR, =N-NHR, =NH-NH-CS-NH_2, =NH-NH-CO-NH_2; A is lower alkyl, substituted or unsubstituted by X, aryl or heteroaryl ring; or

or A is a heterocyclic ring .

2. A compound according to claim 1 selected from the group consisting of:

5-(Pyridin-2-yl-hydrazono)-3,4,5,10-tetrahydro-2H-azepino[3,4-b]-indol-1-one; and

3,4-Dihydro-2*H*,10*H*-azepino[3,4-*b*]indole-1,5-dione semicarbazone;

3. A method of antagonizing a myt1 kinase receptor which comprises administering to a subject in need thereof, an effective amount of a compound according to claim 1.

4. A method according to claim 3 wherein the compound is selected from the group consisting of:

3,4 Dihydro 2*H*,10*H* azepino[3,4 *b*]indole 1,5 dione;

5-(Pyridin-2-yl-hydrazono)-3,4,5,10-tetrahydro-2H-azepino[3,4-b]-indol-1-one; and

3,4-Dihydro-2*H*,10*H*-azepino[3,4-*b*]indole-1,5-dione semicarbazone;

5. A method of treating a disease or disorder selected from the group consisting of leukemias, solid tumor cancers and metastases, soft tissue cancers, brain cancer, esophageal cancer, stomach cancer, pancreatic cancer, liver cancer, lung cancer, bladder cancer, bone cancer, prostate cancer, ovarian cancer, cervical cancer, uterine

cancer, testicular cancer, kidney cancer, head cancer and neck cancer, chronic inflammatory proliferative diseases, proliferative cardiovascular diseases, prolifertive ocular disorders and benign hyperproliferative diseases which comprises administering to a subject in need thereof an effective amount of a compound according to claim 1.

6. A method according to claim 5wherein the disease or disorder treated is selected from the group consisting of psoriasis, rheumatoid arthritis, diabetic retinopathy and hemangiomas.

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ABSTRACT OF THE DISCLOSURE

Novel myt1 kinase receptor antagonists and methods of using them are provided.