Abstract: The present invention provides methods for inhibiting JAK2 tyrosine kinase. Further, the present invention also provides methods of treating or preventing myeloproliferative disorders.
TITLE OF THE INVENTION
JAK2 TYROSINE KINASE INHIBITION

BACKGROUND OF THE INVENTION

Myeloproliferative disorders (MPD) include idiopathic myelofibrosis (IMF), systemic mastocytosis (SM), chronic neutrophilic leukaemia (CNL), unclassified MPD (UN), myelodysplastic syndrome (MDS), polycythemia vera (PV), essential thrombocythemia (ET), myeloid metaplasia with myelofibrosis (MMM), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), hypereosinophilic syndrome (HES), juvenile myelomonocytic leukemia (JMML) and systemic mast cell disease (SMCD). It has been suggested that abnormalities in signal transduction mechanisms, including constitutive activation of protein tyrosine kinases, initiate MPD.

JAK2 is a member of the JANUS family of protein tyrosine kinases and is a cytoplasmic protein-tyrosine kinase that catalyzes the transfer of the gamma-phosphate group of adenosine triphosphate to the hydroxyl groups of specific tyrosine residues in signal transduction molecules. JAK2 mediates signaling downstream of cytokine receptors after ligand-induced autophosphorylation of both receptor and enzyme. The main downstream effectors of JAK2 are a family of transcription factors known as signal transducers and activators of transcription (STAT) proteins.

The myeloproliferative disorders, a subgroup of myeloid malignancies, are clonal stem cell diseases characterized by an expansion of morphologically mature granulocyte, erythroid, megakaryocyte, or monocye lineage cells. Studies have disclosed an association between an activating JAK2 mutation (JAK2V617F) and MPD and between other mutations, e.g., JAK2DeltaIREED, or other aberrations of JAK2 function and other malignancies.

SUMMARY OF THE INVENTION

This invention relates to inhibition of JAK2 tyrosine kinase. This invention also relates to methods of treating or preventing myeloproliferative disorders.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods for inhibiting JAK2 tyrosine kinase. Further, the present invention also provides methods of treating or preventing myeloproliferative disorders.

Applicants have demonstrated that Compound I is a potent inhibitor of JAK2 tyrosine kinase activity.

Accordingly, one embodiment of this invention provides a method for inhibiting JAK2 tyrosine kinase, comprising contacting:

or a pharmaceutically acceptable salt thereof, and the JAK2 tyrosine kinase.

Accordingly, another embodiment of this invention provides a method for inhibiting mutant JAK2 tyrosine kinase, comprising contacting Compound I and the mutant JAK2 tyrosine kinase.

Accordingly, another embodiment of this invention provides a method for inhibiting JAK2V617F tyrosine kinase, comprising contacting Compound I and the JAK2V617F tyrosine kinase.

In certain embodiments, the JAK2 tyrosine kinase is in a patient in need of JAK2 tyrosine kinase inhibition and the method comprises administering a therapeutically effective amount of Compound I to the patient.

In certain embodiments, the JAK2 tyrosine kinase is in a patient in need of mutant JAK2 tyrosine kinase inhibition and the method comprises administering a therapeutically effective amount of Compound I to the patient.

In certain embodiments, the JAK2 tyrosine kinase is in a patient in need of JAK2V617F tyrosine kinase inhibition and the method comprises administering a therapeutically effective amount of Compound I to the patient.
This invention also provides a method of treating a patient having a myeloproliferative
disorder(s), comprising administering to the patient a therapeutically effective amount of:

![Chemical Structure]

or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having polycythemia vera
(PV), comprising administering to the patient a therapeutically effective amount of Compound I, or a
pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having essential
thrombocytethmia (ET), comprising administering to the patient a therapeutically effective amount of
Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having myeloid metaplasia
with myelofibrosis (MMM), comprising administering to the patient a therapeutically effective amount of
Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having chronic myelogenous
leukemia (CML), comprising administering to the patient a therapeutically effective amount of
Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having chronic
myelomonocytic leukemia (CMML), comprising administering to the patient a therapeutically effective
amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having hypereosinophilic
syndrome (HES), comprising administering to the patient a therapeutically effective amount of
Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having juvenile
myelomonocytic leukemia (JMML), comprising administering to the patient a therapeutically effective
amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having systemic mast cell
disease (SMCD), comprising administering to the patient a therapeutically effective amount of
Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having agnogenic myeloid
metaplasia (AMM), comprising administering to the patient a therapeutically effective amount of
Compound I, or a pharmaceutically acceptable salt thereof.
This invention also provides a method of treating a patient having post-polycythemic myeloid metaplasia (PPMM), comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having post-thrombocythemic myeloid metaplasia (PTMM), comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having idiopathic myelofibrosis (IMF), comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having systemic mastocytosis (SM), comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having chronic neutrophilic leukaemia (CNL), comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having unclassified MPD (UN), comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having myelodysplasia syndrome (MDS), comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

Compound I may also be useful for treating any JAK2-driven malignancy, including specific translocation disorders arising from TEL/JAK2 translocations (Laconique, V. et al., Science [1997], 278: 1309-1312; Ho, J. et al., Blood [2002], 100:1438-1448; Peeters, P. et al., Blood [1997], 90: 2535-2540), BCR/JAK2 translocations (Griesinger, F. et al., Genes, Chromosomes & Cancer [2005], 44:329-333), PCM1/JAK2 translocations (Murati, A. et al., Leukemia [2005], 19:1692-1696) and activating mutations in JAK2(V617F) which cause polycythemia vera (Zhao et al., 2005, JBC, James et al., 2005, Nature., Kralovics et al., 2005, NEJM, and Levine et al., 2005, Cancer Cell).

This invention also provides a method of treating a patient having any JAK2-driven malignancy, comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having a TEL/JAK2 translocation disorder, comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

This invention also provides a method of treating a patient having a BCR/JAK2 translocation disorder, comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.
This invention also provides a method of treating a patient having a PCM1/JAK2 translocation disorder, comprising administering to the patient a therapeutically effective amount of Compound I, or a pharmaceutically acceptable salt thereof.

Compound I is also useful in preparing a medicament that is useful in treating myeloproliferative disorders.

In another embodiment, Compound I, compositions and methods provided herein are particularly deemed useful for the treatment of cancer. Cancers that may be treated by Compound I, compositions and methods of the invention include, but are not limited to: Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: non small cell lung, bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma; Gastrointestinal: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma), colon, colorectal, rectal; Genitourinary tract: kidney (adenocarcinoma, Wilms's tumor [nephroblastoma], lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma); Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma; Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochondroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiform, oligodendrogloma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, menigioma, glioma, sarcoma); Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma], granulosa-theal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma [embryonal rhabdomyosarcoma], fallopian tubes (carcinoma); Hematologic: blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease,
non-Hodgkin's lymphoma [malignant lymphoma]; Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and Adrenal glands: neuroblastoma. Thus, the term "cancerous cell" as provided herein, includes a cell afflicted by any one of the above-identified conditions.

In another embodiment, cancers that may be treated by Compound I, compositions and methods of the invention include, but are not limited to: breast, prostate, colon, colorectal, lung, non-small cell lung, brain, testicular, stomach, pancreas, skin, small intestine, large intestine, throat, head and neck, oral, bone, liver, bladder, kidney, thyroid and blood.

In another embodiment, cancers that may be treated by Compound I, compositions and methods of the invention include: breast, prostate, colon, ovarian, colorectal and lung.

In another embodiment, cancers that may be treated by Compound I, compositions and methods of the invention include: colorectal and non-small cell lung.

In another embodiment, cancers that may be treated by the compounds, compositions and methods of the invention include: lymphoma and leukemia.

Compound I may be synthesized according to the General Scheme and Examples herein (see also WO 04/000833, which is incorporated herein by reference). Additionally, Compound I may be synthesized by methods known to skilled practitioners.

**General Scheme:**

![Chemical Diagram]

In another embodiment, this invention provides pharmaceutical compositions comprising Compound I and a pharmaceutically acceptable carrier, adjuvant or vehicle.

A "pharmaceutically acceptable carrier, adjuvant, or vehicle" refers to a non-toxic carrier, adjuvant, or vehicle that does not destroy the pharmacological activity of the compound with which it is formulated. Pharmaceutically acceptable carriers, adjuvants or vehicles that may be used in the compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum...
stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

Pharmacologically acceptable salts of Compound I include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, palmitate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmacologically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining Compound I and pharmaceutically acceptable acid addition salts thereof.

Salts derived from appropriate bases include alkali metal (e.g., sodium and potassium), alkaline earth metal (e.g., magnesium), ammonium and N+(Cl,4 alkyl)4 salts. This invention also envisions the quaternization of any basis nitrogen-containing groups of Compound I. Water or oil-soluble or dispersible products may be obtained by such quaternization.

For examples of specific salts of Compound I, see WO 04/000833.

The compositions of the present invention may be administered orally, parenterally by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrastemal, intrathecal, intrahepatic, intrasional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously. Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in
their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

The pharmaceutically acceptable compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the pharmaceutically acceptable compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutically acceptable compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs. Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the pharmaceutically acceptable compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of Compound I include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutically acceptable compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutically acceptable compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzyalkonium chloride.
Alternatively, for ophthalmic uses, the pharmaceutically acceptable compositions may be formulated in an ointment such as petrolatum.

The pharmaceutically acceptable compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

In another embodiment, pharmaceutically acceptable compositions of this invention are formulated for oral or intravenous administration.

The amount of Compound I that maybe combined with the carrier materials to produce a composition in a single dosage form will vary depending upon the host treated, the particular mode of administration. Preferably, the compositions should be formulated so that a dosage of between 0.01 - 100 mg/kg body weight/day of the compound can be administered to a patient receiving these compositions.

It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of a compound of the present invention in the composition will also depend upon the particular compound in the composition.

Depending upon the particular condition, or disease, to be treated or prevented, additional therapeutic agents, which are normally administered to treat or prevent that condition, may also be present in the compositions of this invention. As used herein, additional therapeutic agents that are normally administered to treat or prevent a particular disease, or condition, are known as "appropriate for the disease, or condition, being treated".

For example, chemotherapeutic agents or other antiproliferative agents may be combined with Compound I to treat proliferative diseases and cancer. Examples of known chemotherapeutic agents include, but are not limited to, Gleevec™, adriamycin, dexamethasone, vincristine, cyclophosphamide, fluorouracil, topotecan, taxol, interferons, and platinum derivatives.

Other therapies or anticancer agents that may be used in combination with Compound I include surgery, radiotherapy (in but a few examples, gamma-radiation, neutron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, and systemic radioactive isotopes, to name a few), endocrine therapy, biologic response modifiers (interferons, interleukins, and tumor necrosis factor (TNF) to name a few), hyperthermia and cryotherapy, agents to attenuate any adverse effects (e.g., antiemetics), and other approved chemotherapeutic drugs, including, but not limited to, alkylating drugs (mechlorethamine, chlorambucil, Cyclophosphamide, Melphalan, Ifosfamide), antimetabolites (Methotrexate), purine antagonists and pyrrolidine antagonists (6-Mercaptopurine, 5-Fluorouracil,
Cytarabile, Gemcitabine), spindle poisons (Vinblastine, Vincristine, Vinorelbine, Paclitaxel),
podophyllotoxins (Etoposide, Mnotecan, Topotecan), antibiotics (Doxorubicin, Bleomycin, Mitomycin),
nitrosoureas (Carmustine, Lomustine), inorganic ions (Cisplatin, Carboplatin), enzymes (Asparaginase),
and hormones (Tamoxifen, Leuprolide, Flutamide, and Megestrol), Gleevec™, adriamycin,
dexamethasone, and cyclophosphamide. For a more comprehensive discussion of updated cancer
therapies see, http://www.nci.nih.gov/, a list of the FDA approved oncology drugs at
total contents of which are hereby incorporated by reference.

The amount of additional therapeutic agent present in the compositions of this invention
will be no more than the amount that would normally be administered in a composition comprising that
therapeutic agent as the only active agent. Preferably the amount of additional therapeutic agent in the
presently disclosed compositions will range from about 50% to 100% of the amount normally present in
a composition comprising that agent as the only therapeutically active agent.

If Compound I is used in combination with an additional agent, the additional agent may
be used in the same (i.e., a single) dosage form or in separate dosage forms.

In another embodiment, Compound I may be administered to mammals, including
humans, either alone or, in combination with pharmaceutically acceptable carriers, excipients or diluents,
in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be
administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal,
subcutaneous, rectal and topical routes of administration.

In another embodiment, the pharmaceutical compositions containing the active
ingredient (Compound I) may be in a form suitable for oral use, for example, as tablets, troches,
lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules,
or syrups or elixirs. Compositions intended for oral use may be prepared according to any method
known to the art for the manufacture of pharmaceutical compositions and such compositions may contain
one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring
agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations.
Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients
which are suitable for the manufacture of tablets. These excipients may be, for example, inert diluents,
such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate;
granulating and disintegrating agents, for example, microcrystalline cellulose, sodium croscarmellose,
corn starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone or acacia,
and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be
uncoated or they may be coated by known techniques to mask the unpleasant taste of the drug or delay
disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a
longer period. For example, a water soluble taste masking material such as hydroxypropylmethyl-
cellulose or hydroxypropylecellulose, or a time delay material such as ethyl cellulose, cellulose acetate
butyrate may be employed.
In another embodiment, formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as polyethylene glycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

In another embodiment, aqueous suspensions contain the active material (Compound I) in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxyctanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

In another embodiment, oily suspensions may be formulated by suspending the active ingredient (Compound I) in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as butylated hydroxyanisol or alpha-tocopherol.

In another embodiment, dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient (Compound I) in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

In another embodiment, the pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsion. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring agents, preservatives and antioxidants.
In another embodiment, syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

In another embodiment, the pharmaceutical compositions may be in the form of sterile injectable aqueous solutions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution.

In another embodiment, the sterile injectable preparation may also be a sterile injectable oil-in-water microemulsion where the active ingredient (Compound I) is dissolved in the oily phase. For example, the active ingredient (Compound I) may be first dissolved in a mixture of soybean oil and lecithin. The oil solution then introduced into a water and glycerol mixture and processed to form a microemulsion.

In another embodiment, the injectable solutions or microemulsions may be introduced into a patient's blood-stream by local bolus injection. Alternatively, it may be advantageous to administer the solution or microemulsion in such a way as to maintain a constant circulating concentration of the instant compound. In order to maintain such a constant concentration, a continuous intravenous delivery device may be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous pump.

In another embodiment, the pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

In another embodiment, Compound I may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-imitating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

In another embodiment, for topical use, creams, ointments, jellies, solutions or suspensions, etc., containing Compound I are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

In another embodiment, Compound I can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than
intermittent throughout the dosage regimen. Compound I may also be delivered as a suppository employing bases such as cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

A 20 mg/mL lactic acid formulation of Compound I (also known as VX-680 or MK-0457) may be prepared according to the following steps: Prepare a 20 mg/mL concentration of lactic acid in water by weighing 2 mg of lactic acid (either L-lactic acid, D-lactic acid or a racemic mixture) into a 100 mL volumetric flask. Next, weigh out 200 mg of Compound I into a 10 mL volumetric flask. Next, add approximately 8 mL of the 20 mg/mL lactic acid solution to the 10 mL volumetric flask. Next, add the appropriate amount of sugar (for example, 15 mg/mL, 50 mg/mL or 100 mg/mL, depending on the desired toxicity). Stir the solution until all the drug contents are dissolved. QS’d the solution to 10 mL with the 20 mg/mL lactic acid solution and adjust the pH as needed to aid in solubilization.

A 20 mg/mL lactic acid formulation of Compound I (large scale manufacture) may be prepared according to the following steps: Add water for injection equal to 80 percent of batch weight to a suitable mixing vessel. Add the necessary amount of compendial lactic acid (either L-lactic acid, D-lactic acid or a racemic mixture) equaling to 20 mg/mL and mix to insure homogeneity. Add Compound I equal to 20 mg/mL free base to the vessel and mix to dissolve. Add the appropriate amount of sugar (for example, 15 mg/mL, 50 mg/mL or 100 mg/mL, depending on the desired toxicity) and 0.05 mg/mL EDTA (edetate disodium dihydrate) to the vessel and mix to dissolve. Adjust the pH as needed. QS’d the batch to final weight with water for injection. Sterile filter and collect the filtered formulation in an appropriate sterile receiving vessel. Fill and stopper the formulation in appropriate vials using aseptic technique in a properly classified area. Cap and terminally sterilize product as required. Store the formulation at the appropriate temperature conditions.

In another embodiment, a 20 mg/mL lactic acid formulation of Compound I (large scale manufacture) may be prepared according to the following steps: Add water for injection equal to 80 percent of batch weight to a suitable mixing vessel. Add the necessary amount of compendial lactic acid (either L-lactic acid, D-lactic acid or a racemic mixture) equaling to 20 mg/mL and mix to insure homogeneity. Add Compound I equal to 20 mg/mL free base to the vessel and mix to dissolve. Add the appropriate amount of sugar (for example, 15 mg/mL, 50 mg/mL or 100 mg/mL, depending on the desired toxicity) and 0.05 mg/mL EDTA (edetate disodium dihydrate) to the vessel and mix to dissolve. Adjust the pH as needed. QS’d the batch to final weight with water for injection. Sterile filter and collect the filtered formulation in an appropriate sterile receiving vessel. Fill and stopper the formulation in appropriate vials using aseptic technique in a properly classified area. Cap and terminally sterilize product as required. Store the formulation at the appropriate temperature conditions.

A lyophilized powder formulation for reconstitution with sterile water for injection may be prepared according to the following steps: Place approximately 90% of the final batch weight of water for injection, USP into a tared, clean agitated vessel. Add the specified amount of mannitol, USP, agitate for at least 15 minutes to dissolve. Add the specified amount of the sulfate salt of Compound I; agitate for at least 30 minutes to dissolve. Add water for injection, USP to the final batch weight. For purposes of this exemplary formulation, the final batch contains the following proportions:
<table>
<thead>
<tr>
<th>Component</th>
<th>mg/mL</th>
<th>mg/vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound I-sulfate</td>
<td>12.1</td>
<td>91.0</td>
</tr>
<tr>
<td>(as equivalent free base)</td>
<td>(10.0)</td>
<td>(75.0)</td>
</tr>
<tr>
<td>Mannitol</td>
<td>50</td>
<td>375</td>
</tr>
<tr>
<td>Water for Injection</td>
<td>q.s. to</td>
<td>q.s. to</td>
</tr>
<tr>
<td></td>
<td>1.0 mL</td>
<td>7.5 mL</td>
</tr>
</tbody>
</table>

Cool the solution thus prepared to 22° C. and filter through a .22 µm sterilizing filter into appropriate sterile containers. Lyophilize to form a white powder.

The sulfate salt of Compound I (dry powder) may be prepared according to the following steps: To Compound I in solution in ethanol at 70°C. (7mg of free base/ml), add one equivalent of concentrated sulfuric acid. Stir the reaction mixture at this temperature 10 minutes. After cooling, collect the precipitate by filtration and dry in a vacuum oven at 50°C. overnight.

In another embodiment, when a composition according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, and response of the individual patient, as well as the severity of the patient's symptoms.

In another embodiment, the dosage regimen utilizing Compound I can be selected in accordance with a variety of factors including type, species, age, weight, sex and the type of cancer being treated; the severity (i.e., stage) of the cancer to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to treat, for example, to prevent, inhibit (fully or partially) or arrest the progress of the disease. For example, Compound I can be administered in a total daily dose of up to 10,000 mg. Compound I can be administered once daily (QD), or divided into multiple daily doses such as twice daily (BED), and three times daily (TID). Compound I can be administered at a total daily dosage of up to 10,000 mg, e.g., 2,000 mg, 3,000 mg, 4,000 mg, 6,000 mg, 8,000 mg or 10,000 mg, which can be administered in one daily dose or can be divided into multiple daily doses as described above.

In another embodiment, for example, Compound I can be administered in a total daily dose of up to 1,000 mg. Compound I can be administered once daily (QD), or divided into multiple daily doses such as twice daily (BED), and three times daily (TBD). Compound I can be administered at a total daily dosage of up to 1,000 mg, e.g., 200 mg, 300 mg, 400 mg, 600 mg, 800 mg or 1,000 mg, which can be administered in one daily dose or can be divided into multiple daily doses as described above.

In another embodiment, the administration can be continuous, i.e., every day, or intermittently. The terms "intermittent" or "intermittently" as used herein means stopping and starting at either regular or irregular intervals. For example, intermittent administration of Compound I may be administration one to six days per week or it may mean administration in cycles (e.g. daily administration for two to eight consecutive weeks, then a rest period with no administration for up to one week) or it may mean administration on alternate days.
In another embodiment, Compound I may be administered according to any of the schedules described above, consecutively for a few weeks, followed by a rest period. For example, Compound I may be administered according to any one of the schedules described above from two to eight weeks, followed by a rest period of one week, or twice daily at a dose of 100 - 500 mg for three to five days a week. In another particular embodiment, Compound I may be administered three times daily for two consecutive weeks, followed by one week of rest.

In another embodiment, Compound I can be administered intravenously for a 5-day continuous infusion at 24-64 mg/m²/hr with a cycle duration every 14-28 days. In another embodiment, Compound I can be administered intravenously for a 5-day continuous infusion at 6-12 mg/m²/hr with a cycle duration every 14-28 days. In another embodiment, Compound I can be administered intravenously for a 5-day continuous infusion at 8-10 mg/m²/hr with a cycle duration every 14-28 days. In another embodiment, Compound I can be administered intravenously for a 24 hr infusion every 14-21 days at 32-200 mg/hrVhr. In another embodiment, Compound I can be administered intravenously for a 24 hr infusion every 14-21 days at 32-64 mg/m²/hr. In another embodiment, Compound I can be administered intravenously for a 24 hr infusion every 14-21 days at 32-64 mg/m²/hr. In another embodiment, Compound I can be administered intravenously for a 48 hr infusion every 21-28 days at 8-12 mg/m²/hr. In another embodiment, Compound I can be administered intravenously for a 6 hr infusion every 14-21 days at 32-200 mg/m²/hr. In another embodiment, Compound I can be administered intravenously for a 6 hr infusion every 14-21 days at 32-64 mg/m²/hr. In another embodiment, Compound I can be administered intravenously for a 3 hr infusion every 14-21 days at 32-200 mg/m²/hr. In another embodiment, Compound I can be administered intravenously for a 3 hr infusion every 14-21 days at 32-64 mg/m²/hr.

In another embodiment, Compound I can be administered intravenously for a 5-day continuous infusion at 24-64 mg/m²/hr with a cycle duration every 14-28 days. In another embodiment, Compound I can be administered intravenously for a 5-day continuous infusion at 8-10 mg/m²/hr with a cycle duration every 21 days. In another embodiment, Compound I can be administered intravenously for a 24 hr infusion every 21 days at 64-96 mg/m²/hr. In another embodiment, Compound I can be administered intravenously for a 24 hr infusion every 21 days at 32-64 mg/m²/hr. In another embodiment, Compound I can be administered intravenously for a 6 hr infusion every 14-21 days at 32-200 mg/m²/hr. In another embodiment, Compound I can be administered intravenously for a 3 hr infusion every 14-21 days at 32-200 mg/m²/hr.

In another embodiment, any one or more of the specific dosages and dosage schedules of Compound I, may also be applicable to any one or more of the therapeutic agents to be used in the combination treatment (hereinafter referred to as the "second therapeutic agent").

In another embodiment, the specific dosage and dosage schedule of this second therapeutic agent can further vary, and the optimal dose, dosing schedule and route of administration will be determined based upon the specific second therapeutic agent that is being used.

In another embodiment, Compound I is also useful in combination with therapeutic, chemotherapeutic and anti-cancer agents. Combinations of Compound I with therapeutic, chemotherapeutic and anti-cancer agents are within the scope of the invention. Examples of such agents can be found in Cancer Principles and Practice of Oncology by V.T. Devita and S. Hellman (editors), 6th
edition (February 15, 2001), Lippincott Williams & Wilkins Publishers. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the cancer involved. Such agents include the following: estrogen receptor modulators, androgen receptor modulators, retinoid receptor modulators, cytotoxic/cytostatic agents, antiproliferative agents, prenyl-protein transferase inhibitors, HMG-CoA reductase inhibitors and other angiogenesis inhibitors, HIV protease inhibitors, reverse transcriptase inhibitors, inhibitors of cell proliferation and survival signaling, bisphosphonates, aromatase inhibitors, siRNA therapeutics, γ-secretase inhibitors, agents that interfere with receptor tyrosine kinases (RTKs) and agents that interfere with cell cycle checkpoints. Compound I is particularly useful when co-administered with radiation therapy.

"Estrogen receptor modulators" refers to compounds that interfere with or inhibit the binding of estrogen to the receptor, regardless of mechanism. Examples of estrogen receptor modulators include, but are not limited to, tamoxifen, raloxifene, idoxifene, LY353381, LYL1 17081, toremifene, fulvestrant, 4-[7-(2,2-dimethyl-1-oxopropoxy-4-methyl-2-[4-[2-(1-piperidinyl)ethoxy]phenyl]-2H-l-benzopyran-3-yl]-phenyl-2,2-dimethylpropanoate, 4,4'-dihydroxybenzo phenone-2,4-dinitrophenyl-hydrazone, and SH646.

"Androgen receptor modulators" refers to compounds which interfere or inhibit the binding of androgens to the receptor, regardless of mechanism. Examples of androgen receptor modulators include finasteride and other 5α-reductase inhibitors, nilutamide, flutamide, bicalutamide, liarozole, and abiraterone acetate.

"Retinoid receptor modulators" refers to compounds which interfere or inhibit the binding of retinoids to the receptor, regardless of mechanism. Examples of such retinoid receptor modulators include bexarotene, tretinoin, 13-cis-retinoic acid, 9-cis-retinoic acid, α-difluoromethylomithine, ILX23-7553, trans-N-(4'-hydroxyphenyl) retinamide, and N-4-carboxyphenyl retinamide.

"Cytotoxic/cytostatic agents" refer to compounds which cause cell death or inhibit cell proliferation primarily by interfering directly with the cell's functioning or inhibit or interfere with cell myosis, including alkylating agents, tumor necrosis factors, intercalators, hypoxia activatable compounds, microtubule inhibitors/microtubule-stabilizing agents, inhibitors of mitotic kinesins, histone deacetylase inhibitors, inhibitors of kinases involved in mitotic progression, inhibitors of kinases involved in growth factor and cytokine signal transduction pathways, antimitabolites, biological response modifiers, hormonal/anti-hormonal therapeutic agents, haematopoietic growth factors, monoclonal antibody targeted therapeutic agents, topoisomerase inhibitors, proteosome inhibitors, ubiquitin ligase inhibitors, and aurora kinase inhibitors.

Examples of cytotoxic/cytostatic agents include, but are not limited to, sertenef, cachectin, ifosfamide, tasonermin, loxidamine, carboplatin, altretamine, prednimustine, dibromodulcitol, ranimustine, fotemustine, nedaplatin, oxaliplatin, temozolomide, heptaplatin, estramustine, improsulfan tosilate, trofosfamide, nimustine, dibromodiposphium chloride, pumitepa, lobaplatin, satraplatin, proflromycin, cisplatin, irofulven, dexifosfamide, cis-aminedichloro(2-methyl-pyridine)platinum, benzylguanine,

- 16 -
glufosfamide, GPX100, (trans, trans, trans)-bis-mu-(hexane-1,6-diamme)-mu-[diamine-platinum(II)]bis[diainine(chloro) platinum (II)]tetrachloride, diarizidinylspermine, arsenic trioxide, 1-(11-dodecylamino-10-hydroxyundecyl)-3,7-dimethylxanthine, zorubicin, idarubicin, daunorubicin, bisantrene, mitoxantrone, pirarubicin, pinafide, valrubicin, amrubicin, antineoplaston, 3’-deamino-3’-morpholino-13-deoxy-IO-hydroxycarminomycin, annamycin, galarubicin, elinafide, MEN10755, 4-demethoxy-3-deamino-3-aziridinyl-4-methylsulphonyl-daunorubicin (see WO 00/50032), Raf kinase inhibitors (such as Bay43-9006) and mTOR inhibitors (such as Wyeth’s CCI-779).

An example of a hypoxia activatable compound is tirapazamine.

Examples of proteosome inhibitors include but are not limited to lactacystin and MLN-341 (Velcade).

Examples of microtubule inhibitors/microtubule-stabilising agents include paclitaxel, vindesine sulfate, S'-nitrovincaleukoblastine, docetaxel, rhizoxin, dolastatin, mivobulin isethionate, auristatin, cemadotin, RPR109881, BMS184476, vinflunine, cryptophycin, 2,3,4,5,6-pentafuoro-N-(3-fluoro-4-methoxyphenyl) benzene sulfonamide, anhydrovinblastine, N,N-dimethyl-L-valyl-L-valyl-N-methyl-L-valyl-L-prolyl-L-proline-t-butylamide, TDX258, the epothilones (see for example U.S. Pat. Nos. 6,284,781 and 6,288,237) and BMS188797. In an embodiment the epothilones are not included in the microtubule inhibitors/microtubule-stabilising agents.


Examples of inhibitors of mitotic kinesins, and in particular the human mitotic kinesin KSP, are described in Publications WO03/039460, WO03/050064, WO03/050122, WO03/049527, WO03/049679, WO03/049678, WO04/038977, WO04/039973, WO03/099211, WO03/105855, WO03/106417, WO04/037171, WO04/058148, WO04/126699, WO05/018638, WO05/019206, WO05/019205, WO05/018547, WO05/017190, US2005/0176776. In an embodiment inhibitors of mitotic kinesins include, but are not limited to inhibitors of KSP, inhibitors of MKLP1, inhibitors of CEÑP-E, inhibitors of MCAK and inhibitors of Rab6-KIFL.
Examples of "histone deacetylase inhibitors" include, but are not limited to, SAHA, TSA, oxamflatin, PXD101, MG98 and scriptaid. Further reference to other histone deacetylase inhibitors may be found in the following manuscript; Miller, T.A. et al. J. Med. Chem. 46(24):5097-5116 (2003).

"Inhibitors of kinases involved in mitotic progression" include, but are not limited to, inhibitors of aurora kinase, inhibitors of Polo-like kinases (PLK; in particular inhibitors of PLK-1), inhibitors of bub-1 and inhibitors of bub-R1. An example of an "aurora kinase inhibitor" is VX-680.

"Antiproliferative agents" includes antisense RNA and DNA oligonucleotides such as G3139, ODN698, RVASKRAS, GEM231, and ESX3001, and antimetabolites such as enocitabine, carmofur, tegafur, pentostatin, doxifluridine, trimetrexate, fludarabine, capcitabine, galocitabine, cytarabine ocfosfate, fosteabine sodium hydrate, raltitrexed, palitrexid, emitefur, tiazofurin, decitabine, nalotrexed, pemetrexed, nelzarabine, 2'-deoxy-2'-methylidinecytidine, 2'-fluoromethylene-2'-deoxyctydine, N-[5-(2,3-dihydro-benzofuryl)sulfonyl]-N'-(3,4-dichlorophenyl)urea, N6-[4-deoxy-4-[N2(2E),4(E)-tetradecadienoyl][glycylamino]-L-glycero-B-L-manno-heptopyranosyl]adenine, aplidine, ecteinascidin, traxocitabine, 4-[2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimidino[5,4-b][1,4]thiazin-6-yl-(S)-ethyl]-2,5-thienoyl-L-glutamic acid, aminopterin, 5-florouracil, alanosine, 11-acetyl-8-(carbamoyloxymethyl)-4-formyl-6-methoxy-14-oxa-1,11-diazatetracyclo(7.4.0.0^7.10)-tetradeca-2,4,6-trien-9-yl acetic acid ester, swainsonine, lometrexol, dextraoxane, methioninase, 2'-cyano-2'-deoxy-N4-palmitoyl-1-B-D-arabinofuranosyl cytosine, 3-aminopyridine-2-carboxaldehydehydrosenicularbazone and trastuzumab.

Examples of monoclonal antibody targeted therapeutic agents include those therapeutic agents which have cytotoxic agents or radioisotopes attached to a cancer cell specific or target cell specific monoclonal antibody. Examples include Bexxar.

"HMG-CoA reductase inhibitors" refers to inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase. Examples of HMG-CoA reductase inhibitors that may be used include but are not limited to lovastatin (MEVACOR®; see U.S. Patent Nos. 4,231,938, 4,294,926 and 4,319,039), simvastatin (ZOCOR®; see U.S. Patent Nos. 4,444,784, 4,820,850 and 4,916,239), pravastatin (PRAVACHOL®; see U.S. Patent Nos. 4,346,227, 4,537,859, 4,410,629, 5,030,447 and 5,180,589), fluvastatin (LESCOL®; see U.S. Patent Nos. 5,354,772, 4,911,165, 4,929,437, 5,189,164, 5,118,853, 5,290,946 and 5,356,896), atorvastatin (LIPITOR®; see U.S. Patent Nos. 5,273,995, 4,681,893, 5,489,691 and 5,342,952) and cerivastatin (also known as rivastatin and BAYCHOL®; see US Patent No. 5,177,080). The structural formulas of these and additional HMG-CoA reductase inhibitors that may be used in the instant methods are described at page 87 of M. Yalpani, "Cholesterol Lowering Drugs", Chemistry & Industry, pp. 85-89 (5 February 1996) and US Patent Nos. 4,782,084 and 4,885,314. The term HMG-CoA reductase inhibitor as used herein includes all pharmaceutically acceptable lactone and open-acid forms (i.e., where the lactone ring is opened to form the free acid) as well as salt and ester forms of compounds which have HMG-CoA reductase inhibitory activity, and therefore the use of such salts, esters, open-acid and lactone forms is included within the scope of this invention.
"Prenyl-protein transferase inhibitor" refers to a compound which inhibits any one or any combination of the prenyl-protein transferase enzymes, including farnesyl-protein transferase (FPTase), geranylgeranyl-protein transferase type I (GGPTase-I), and geranylgeranyl-protein transferase type-H (GGPTase-π, also called Rab GGPTase).


In another embodiment, other therapeutic agents that modulate or inhibit angiogenesis and may also be used in combination with Compound I include agents that modulate or inhibit the coagulation and fibrinolysis systems (see review in Clin. Chem. La. Med. 38:679-692 (2000)). Examples
of such agents that modulate or inhibit the coagulation and fibrinolysis pathways include, but are not limited to, heparin (see Thromb. Haemost. 80:10-23 (1998)), low molecular weight heparins and carboxypeptidase U inhibitors (also known as inhibitors of active thrombin activatable fibrinolysis inhibitor [TAFIa]) (see Thrombosis Res. 101:329-354 (2001)). TAFIa inhibitors have been described in U.S. Ser. Nos. 60/310,927 (filed August 8, 2001) and 60/349,925 (filed January 18, 2002).

"Agents that interfere with cell cycle checkpoints" refer to compounds that inhibit protein kinases that transduce cell cycle checkpoint signals, thereby sensitizing the cancer cell to DNA damaging agents. Such agents include inhibitors of ATR, ATM, the CHK1 and CHK 12 kinases and cdk and cdc kinase inhibitors and are specifically exemplified by 7-hydroxyxystaurosporin, flavopiridol, CYC202 (Cyclacel) and BMS-387032.

"Agents that interfere with receptor tyrosine kinases (RTKs)" refer to compounds that inhibit RTKs and therefore mechanisms involved in oncogenesis and tumor progression. Such agents include inhibitors of c-Kit, Eph, PDGF, Flt3 and c-Met. Further agents include inhibitors of RTKs as described by Bume-Jensen and Hunter, Nature, 411:355-365, 2001.


As described above, the combinations with NSAID's are directed to the use of NSATD's which are potent COX-2 inhibiting agents. For purposes of this specification an NSAID is potent if it possesses an IC50 for the inhibition of COX-2 of 1 uM or less as measured by cell or microsomal assays.


Inhibitors of COX-2 that are particularly useful in the instant method of treatment are: 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(JH)-furanone; and
5-chloro-3-(4-methylsulfonyl)phenyl-2-(2-methyl-5-pyridinyl)pyr dine; or a pharmaceutically acceptable salt thereof.

Compounds that have been described as specific inhibitors of COX-2 and are therefore useful in the present invention include, but are not limited to, the following: parecoxib, BEXTRA® and CELEBREX® or a pharmaceutically acceptable salt thereof.

Other examples of angiogenesis inhibitors include, but are not limited to, endostatin, ukram, ranpimase, IM862, 5-methoxy-4-[2-methyl-3-(3-methyl-2-butenyl)oxiranyl]-l-oxaspiro[2,5]oct-6-yl(chloroacetyl)carbamate, acetyldidanaline, 5-amino-l-[3,5-dichloro-4-(4-chlorobenzoyl)phenyl]-methyl]-1H-1,2,3-triazole-4-carboxamide,CM101, squalamine, combretastatin, RPI4610, NX31838, sulfated mannohexaose phosphate, 7,7-(carbonyl-bis[immo-N-methyl-4,2-pyrolcarbonyliminopSI-methyl-4,2-pyrrole]-carbonylimmo]-bis-(1,3-naphthalene disulfonate), and 3-[2,4-dimethylpyrrol-5-yl)methylene]-2-indolinone (SU5416).

As used above, "integrin blockers" refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the α5β3 integrin, to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the ααβ5 integrin, to compounds which antagonize, inhibit or counteract binding of a physiological ligand to both the ααβ3 integrin and the ααβ5 integrin, and to compounds which antagonize, inhibit or counteract the activity of the particular integrin(s) expressed on capillary endothelial cells. The term also refers to antagonists of the α5β, ααβ6, ααβ1, α2β1, α3β1, α4β1 and ααβ4 integrins. The term also refers to antagonists of any combination of ααβ3, ααβ5, ααβ6, ααβ8, ααβ1, ααβ1, ααβ1, ααβ8, ααβ6, integrins.

Some specific examples of tyrosine kinase inhibitors include N-(4-fluoromethylphenyl)-5-methylisoxazol-4-carboxamide, 3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]indoloh-2-one, 17-(allylamino)-17-deoxygeldanamycin, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-[3-(4-morpholinyl)propoxy]quinoloxine, N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolmamine, BIBX1382, 2,3,9,10,11,12-hexahydro-10-(hydroxymethyl)-10-hydroxy-9-methyl-9,12-epoxy-1H-diindololo[1,2-fg:3,2-g]pyrrolo[3,4-i]l6-benzodiazocin-l-one, SH268, genistem, ST571, CEP2563, 4-(3-chlorophenylamino)-5,6-dimethyl-7H-pyrrolo[2,3-d]pyrimidemethane sulfonate, 4-(3-bromo-4-hydroxyphenyl)amino-6,7-dimethoxyquinazolne, 4-(4'-hydroxyphenyl)nino-6,7-dimethoxyquinazolne, SU6668, ST571A, N-4-chlorophenyl-4-(4-pyridylmethyl)-1-phthalazina nino, and EMD121974.

In another embodiment, combinations with compounds other than anti-cancer compounds are also encompassed in the instant methods. For example, combinations of Compound I with PPAR-γ (i.e., PPAR-gamma) agonists and PPAR-δ (i.e., PPAR-delta) agonists are useful in the treatment of certain mhanngncs. PPAR-γ and PPAR-δ are the nuclear peroxisome proliferator-activated receptors γ and δ. The expression of PPAR-γ on endothelial cells and its involvement in angiogenesis has been reported in the literature (see J. Cardiovasc. Pharmacol. 1998; 31:909-913; J. Biol. Chem. 1999;274:91 16-9121; Invest. Ophthamol Vis. Sci. 2000; 41:2309-2317). More recently, PPAR-γ agonists have been shown to inhibit the angiogenic response to VEGF in vitro; both troglitazone and rosightazone maleate inhibit the development of retinal neovascularization in mice. (Arch. Ophthamol.
Examples of PPAR-γ agonists and PPAR-γ/α agonists include, but are not limited to, thiazolidinediones (such as DRP2725, CS-01, troglitazone, rosiglitazone, and pioglitazone), fenofibrate, gemfibrozil, clofibrate, GW2570, SB219994, AR-H039242, JTT-501, MCC-555, GW2331, GW409544, NN2344, KRP297, NPOI 10, DRF4158, NN622, GI26570, PNU182716, DRF552926, 2-[(5,7-dipropyl-3-trifluoromethyl-1,2-benzisoxazol-6-yl)oxy]-2-methylpropionic acid (disclosed in USSN 09/782,856), and 2(R)-7-(3-(2-chloro-4-(4-fluorophenoxy) phenoxy)propoxy)-2-ethylchromane-2-carboxylic acid (disclosed in USSN 60/235,708 and 60/244,697).

In another embodiment of the instant invention is the use of Compound I in combination with gene therapy for the treatment of cancer. For an overview of genetic strategies to treating cancer see Hall et al (Am. J. Hum. Genet. 61:785-789, 1997) and Kufe et al (Cancer Medicine, 5th Ed, pp 876-889, BC Decker, Hamilton 2000). Gene therapy can be used to deliver any tumor suppressing gene. Examples of such genes include, but are not limited to, p53, which can be delivered via recombinant virus-mediated gene transfer (see U.S. Patent No. 6,069,134, for example), a uPA/uPAR antagonist ("Adenovirus-Mediated Delivery of a uPA/uPAR Antagonist Suppresses Angiogenesis-Dependent Tumor Growth and Dissemination in Mice," Gene Therapy, August 1998;5(8):1 105-13), and interferon gamma (J. Immunol. 2000;164:217-222).

In another embodiment, Compound I may also be administered in combination with an inhibitor of inherent multidrug resistance (MDR), in particular MDR associated with high levels of expression of transporter proteins. Such MDR inhibitors include inhibitors of p-glycoprotein (P-gp), such as LY335979, XR9576, OC144-093, R101922, VX853 and PSC833 (valspodar).

In another embodiment, Compound I may be employed in conjunction with anti-emetic agents to treat nausea or emesis, including acute, delayed, late-phase, and anticipatory emesis, which may result from the use of Compound I, alone or with radiation therapy. For the prevention or treatment of emesis, Compound I may be used in conjunction with other anti-emetic agents, especially neurokinin-1 receptor antagonists, SHT3 receptor antagonists, such as ondansetron, granisetron, tropisetron, and zatsitron, GABAB receptor agonists, such as baclofen, a corticosteroid such as Decadron (dexamethasone), Kenalog, Aristocort, Nasalide, Preferid, Benecorten or others such as disclosed in U.S. Patent Nos. 2,789,118, 2,990,401, 3,048,581, 3,126,375, 3,929,768, 3,996,359, 3,928,326 and 3,749,712, an antiadrenergic, such as the phenothiazines (for example prochlorperazine, fluphenazine, thioridazine and mesoridazine), metoclopramide or dronabinol. In another embodiment, conjunctive therapy with an anti-emesis agent selected from a neurokinin-1 receptor antagonist, a SHT3 receptor antagonist and a corticosteroid is disclosed for the treatment or prevention of emesis that may result upon administration of the instant compounds.

Neurokinin-1 receptor antagonists of use in conjunction with Compound I are fully described, for example, in U.S. Patent Nos. 5,162,339, 5,232,929, 5,242,930, 5,373,003, 5,387,595, 5,459,270, 5,494,926, 5,496,833, 5,637,699, 5,719,147; European Patent Publication Nos. EP 0 360 390, 0 394 989, 0 428 434, 0 429 366, 0 430 771, 0 436 334, 0 443 132, 0 482 539, 0 498 069, 0 499 313, 0 512 901, 0 512 902, 0 514 273, 0 514 274, 0 514 275, 0 514 276, 0 515 681, 0 517 589, 0 520 555, 0 522 808, 0 528 495, 0 532 456, 0 533 280, 0 536 817, 0 545 478, 0 558 156, 0 577 394, 0 585 913, 0 590 152,
The compounds of the present invention are selected from: 2-(R)-(1-(R)-(3,5-bis(trifluoromethyl)phenyl)ethoxy)-3-(S)-(4-fluorophenyl)-4-(3-(5-oxo-1H,4H-1,2,4triazolo)methyl)morpholine, or a pharmaceutically acceptable salt thereof, which is described in U.S. Patent No. 5,719,147.

In another embodiment, Compound I may also be administered with an agent useful in the treatment of anemia. Such an anemia treatment agent is, for example, a continuous erythropoiesis receptor activator (such as epoetin alfa).

In another embodiment, Compound I may also be administered with an agent useful in the treatment of neutropenia. Such a neutropenia treatment agent is, for example, a hematopoietic growth factor which regulates the production and function of neutrophils such as a human granulocyte colony stimulating factor (G-CSF). Examples of a G-CSF include filgrastim.

In another embodiment, Compound I may also be administered with an immunologic-enhancing drug, such as levamisole, isoprinosine and Zadaxin.

In another embodiment, Compound I may also be useful for treating or preventing cancer in combination with P450 inhibitors including: xenobiotics, quinidine, tyramine, ketoconazole, testosterone, quinine, methyrapone, caffeine, phenelzine, doxorubicin, troleandomycin, cyclobenzaprine, erythromycin, cocaine, furafyline, cimetidine, dextromethorphan, ritonavir, indinavir, amprenavir, diltiazem, terfenadine, verapamil, Cortisol, itraconazole, mibefradil, nefazodone and neflaminavir.

In another embodiment, Compound I may also be useful for treating or preventing cancer in combination with Pgp and/or BCRP inhibitors including: cyclosporin A, PSC833, GF120918, cremophorEL, fumitremorgin C, Kol32, Kol34, Iressa, Imatinib mesylate, EKI-785, C11033, novobiocin,
diethylstilbestrol, tamoxifen, reserpme, VX-710, tryprostatm A, flavonoids, ritonavir, saquinavir, nelfmavir, omeprazole, quindine, verapamil, terfenadine, ketoconazole, nifidipine, FK506, amiodarone, XR9576, indinavir, amprenavir, Cortisol, testosterone, LY335979, OC144-093, erythromycin, vincristine, digoxm and tahnol.

In another embodiment, Compound I may also be useful for treating or preventing cancer, including bone cancer, in combination with bisphosphonates (understood to include bisphosphonates, diphosphonates, bisphosphonic acids and diphosphonic acids). Examples of bisphosphonates include but are not limited to: etidronate (Didronel), pamidronate (Aredia), alendronate (Fosamax), πsedronate (Actonel), zoledronate (Zometa), ibandronate (Boniva), macadronate or cimadronate, clodronate, EB-1053, minodronate, nett.dronate, piridronate and tiludronate including any and all pharmaceutically acceptable salts, derivatives, hydrates and mixtures thereof.

In another embodiment, Compound I may also be useful for treating or preventing breast cancer in combination with aromatase inhibitors. Examples of aromatase inhibitors include but are not limited to: anastrozole, letrozole and exemestane.

In another embodiment, Compound I may also be useful for treating or preventing cancer in combination with siRNA therapeutics.


In another embodiment, Compound I may also be useful for treating or preventing cancer in combination with PARP inhibitors.

In another embodiment, Compound I may also be useful for treating cancer in combination with the following therapeutic agents: abarelix (Plenaxis depot®); aldesleukin (Prokine®); Aldesleukin (Proleukin®); Algentuzumab (Campath®); ahtretinoin (Panretin®); allopurmol (Zyloprim®); altretamine (Hexalen®); amifostine (Ethylol®); anastrozole (Arimex®); arsenic trioxide (Trisenox®); asparaginase (Elspar®); azacitidine (Vidaza®); bevacuzimab (Avastm®); bexarotene capsules (Targetin®); bexarotene gel (Targetin®); bleeomycin (Bleòxane®); bortezomib (Velcade®); busulfan intravenous (Busulfex®); busulfan oral (Myleran®); calusterone (Methosarb®); capecitabine (Xeloda®); carboptam (Paraplatin®); Carmustine (BCNU®, BiCNU®); carmustine (Gliadel®); carmustine with Pohlehprosan 20 Implant (Gliadel Wafer®); celecoxib (Celebrex®); cetuximab (Erbitux®); chlorambucil (Leukeran®); cisplatm (Platinol®); cladribine (Leustatin®, 2-CdA®); clorarabine (Clolar®); cyclophosphamide (Cytoxan®, Neosar®); cyclophosphamide (Cytoxan Injection®); cyclophosphamide (Cytoxan Tablet®); cytarabine (Cytosar-U®); cytarabine liposomal (DepoCyt®); dacarbazine (DTIC-Dome®); dactomycin, actomycin D (Cosmegen®); Darbepoetm alfa (Aranesp®); daunorubicin liposomal (Danuoxome®); daunorubicm, daunomycin (Daunorubicin®);
daunorubicin, daunomycin (Cefiibidine®); Denileukin diftitox (Ontak®); dexrazoxane (Zinecard®); docetaxel (Taxotere®); doxorubicin (Adriamycin PFS®); doxorubicin (Adriamycin®, Rubex®); doxorubicin (Adriamycin PFS Injection®); doxorubicin liposomal (Doxil®); dromostanolone propionate (dromostanolone®); dromostanolone propionate (masterone injection®); Elliott’s B Solution (Elliott’s B Solution®); epirubicin (Ellence®); Epoetin alfa (epogen®); erlotinib (Tarceva®); estramustine (Emcyt®); etoposide phosphate (Etopophos®); etoposide, VP-16 (Vepesid®); exemestane (Aromasin®); Filgrastim (Neupogen®); fluouracil (Fludara®); fluouracil, 5-FU (Adrucil®); fulvestrant (Faslodex®); gefitinib (Lressa®); gemcitabine (Gemzar®); gemtuzumab ozogamicin (Mylotarg®); goserelin acetate (Dromostanolone®); goserelin acetate (Zoladex®); histrelin acetate (Histrelin implant®); hydroxyurea (Hydrea®); Ibritumomab Tiuxetan (Zevalin®); idarubicin (Idamycin®, ifosfamide (IFEX®); imatinib mesylate (Gleevec®); interferon alfa 2a (Roferon A®); Interferon alfa-2b (Intron A®); irinotecan (Camptosar®); lenalidomide (Revlimid®); letrozole (Femara®); leucovorin (Wellcovorin®, Leucovorin®); Leuprolide Acetate (Eliqard®); levamisole (Ergamisol®); lomustine, CCNU (CeeBU®); mecloretamine, nitrogen mustard (Mustargen®); megestrol acetate (Megace®); melphanal, L-PAM (Alkeran®); mercaptopurine, 6-MP (Purinethol®); mesna (Mesnex®); mesna (Mesnex tabs®); methotrexate (Methotrexate®); methoxsalen (Uvadex®); mitomycin C (Mutamycin®); mitotane (Lysodren®); mitoxantrone (Novantrone®); nandrolone phenpropionate (Durabolin-50®); nelarabine (Arranon®); Noferumomab (Verluma®); Oprelvekin (Neumega®); oxaliplatin (Eloxatin®); paclitaxel (Paxene®); paclitaxel (Taxol®); paclitaxel protein-bound particles (Abraxane®); palifermin (Kepivance®); pamidronate (Aredia®); pegademase (Adagen (Pegademase Bovine®)); pegasparagase (Oncaspar®); Pegfilgrastim (Neulasta®); pemetrexed disodium (Alimta®); pentostatin (Nipent®); pipobroman (Vercyte®); plicamycin, mithramycin (Mithracin®); porfimer sodium (Photofrin®); procarbazine (Matulane®); quinacrine (Atabrine®); Rasburicase (Elitek®); Rituximab (Rituxan®); sargramostim (Leukine®); Sargramostim (Prokine®); sorafenib ( Nexavar®); streptozocin (Zanosar®); sunitinib maleate (Sutent®); talc (Sclerosol®); tamoxifen (Nolvadex®); temozolomide (Temodar®); teniposide, VM-26 (Vumon®); testolactone (Teslac®); thioguanine, 6-TG (Thioguanine®); thiotapec (Thioplex®); topotecan (Hycamtin®); toremifene (Fareston®); Tositumomab (Bexxar®); Tositumomab/I-131 tositumomab (Bexxar®); Trastuzumab (Herceptin®); tretonoin, ATRA (Vesanoid®); Uracil Mustard (Uracil Mustard Capsules®); valrubicin (Valstar®); vinblastine (Velban®); vincristine (Oncovin®); vinorelbine (Navelbine®); zoledronate (Zometa®); and vorinostat (Zolinza®).

In another embodiment, Compound I may also be useful for treating cancer in combination with Gleevec® or dasatinib or nilotinib.

In another embodiment, the scope of the instant invention encompasses the use of Compound I in combination with a second compound selected from: an estrogen receptor modulator, an androgen receptor modulator, a retinoid receptor modulator, a cytotoxic/cytostatic agent, an antiproliferative agent, a prenyl-protein transferase inhibitor, an HMG-CoA reductase inhibitor, an HIV protease inhibitor, a reverse transcriptase inhibitor, an angiogenesis inhibitor, PPAR-γ agonists, PPAR-δ agonists, an inhibitor of inherent multidrug resistance, an anti-emetic agent, an agent useful in the
treatment of anemia, an agent useful in the treatment of neutropenia, an immunologic-enhancing drug, an inhibitor of cell proliferation and survival signaling, a bisphosphonate, an aromatase inhibitor, an siRNA therapeutic, γ-secretase inhibitors, agents that interfere with receptor tyrosine kinases (RTKs), an agent that interferes with a cell cycle checkpoint and any of the therapeutic agents listed above.

In another embodiment, the term "administration" and variants thereof (e.g., "administering" a compound) in reference to Compound I means introducing the compound or a prodrug of the compound into the system of the animal in need of treatment. When Compound I or prodrug thereof is provided in combination with one or more other active agents (e.g., a cytotoxic agent, etc.), "administration" and its variants are each understood to include concurrent and sequential introduction of the compound or prodrug thereof and other agents.

In another embodiment, as used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

In another embodiment, the term "therapeutically effective amount" as used herein means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician.

In another embodiment, the term "treating cancer" or "treatment of cancer" refers to administration to a mammal afflicted with a cancerous condition and refers to an effect that alleviates the cancerous condition by killing the cancerous cells, but also to an effect that results in the inhibition of growth and/or metastasis of the cancer.

In another embodiment, the angiogenesis inhibitor to be used as the second compound is selected from a tyrosine kinase inhibitor, an inhibitor of epidermal-derived growth factor, an inhibitor of fibroblast-derived growth factor, an inhibitor of platelet derived growth factor, an MMP (matrix metalloprotease) inhibitor, an integrin blocker, interferon-α, interleukin-12, pentosan polysulfate, a cyclooxygenase inhibitor, carboxamidotriazole, combretastatin A-4, squalamine, 6-O-chloroacetyl-carbonyl)-fumagillol, thalidomide, angiostatin, troponin-1, or an antibody to VEGF. In an embodiment, the estrogen receptor modulator is tamoxifen or raloxifene.

In another embodiment, also included in the scope of the claims is a method of treating cancer that comprises administering a therapeutically effective amount of Compound I in combination with radiation therapy and/or in combination with a second compound selected from: an estrogen receptor modulator, an androgen receptor modulator, a retinoid receptor modulator, a cytotoxiccytostatic agent, an antiproliferative agent, a prenyl-protein transferase inhibitor, an HMG-CoA reductase inhibitor, an HTV protease inhibitor, a reverse transcriptase inhibitor, an angiogenesis inhibitor, PPAR-γ agonists, PPAR-δ agonists, an inhibitor of inherent multidrug resistance, an anti-emetic agent, an agent useful in the treatment of anemia, an agent useful in the treatment of neutropenia, an immunologic-enhancing drug, an inhibitor of cell proliferation and survival signaling, a bisphosphonate, an aromatase inhibitor, an siRNA therapeutic, γ-secretase inhibitors, agents that interfere with receptor tyrosine kinases (RTKs), an agent that interferes with a cell cycle checkpoint and any of the therapeutic agents listed above.
And yet another embodiment of the invention is a method of treating cancer that comprises administering a therapeutically effective amount of Compound I in combination with paclitaxel or trastuzumab.

In another embodiment, the invention further encompasses a method of treating or preventing cancer that comprises administering a therapeutically effective amount of Compound I in combination with a COX-2 inhibitor.

In another embodiment, the instant invention also includes a pharmaceutical composition useful for treating or preventing cancer that comprises a therapeutically effective amount of Compound I and a second compound selected from: an estrogen receptor modulator, an androgen receptor modulator, a retinoid receptor modulator, a cytotoxic/cytostatic agent, an antiproliferative agent, a prenyl-protein transferase inhibitor, an HMG-CoA reductase inhibitor, an HIV protease inhibitor, a reverse transcriptase inhibitor, an angiogenesis inhibitor, a PPAR-γ agonist, a PPAR-δ agonist, an inhibitor of cell proliferation and survival signaling, a bisphosphonate, an aromatase inhibitor, an siRNA therapeutic, γ-secretase inhibitors, agents that interfere with receptor tyrosine kinases (RTKs), an agent that interferes with a cell cycle checkpoint and any of the therapeutic agents listed above.

In another embodiment, the route of administration of Compound I is independent of the route of administration of the second therapeutic agent. In another embodiment, the administration of Compound I is oral administration. In another embodiment, the administration of Compound I is intravenous administration. Thus, in accordance with these embodiments, Compound I is administered orally or intravenously, and the second therapeutic agent can be administered orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intracellularly, via local delivery by catheter or stent, subcutaneously, intraadiposally, intraarticularly, intrathecally, or in a slow release dosage form.

In another embodiment, Compound I and second therapeutic agent may be administered by the same mode of administration, i.e. both agents administered e.g. orally, by IV. However, it is also within the scope of the present invention to administer Compound I by one mode of administration, e.g. IV, and to administer the second therapeutic agent by another mode of administration, e.g. oral or any other ones of the administration modes described hereinabove.

In another embodiment, the first treatment procedure, administration of Compound I, can take place prior to the second treatment procedure, i.e., the second therapeutic agent, after the treatment with the second therapeutic agent, at the same time as the treatment with the second therapeutic agent, or a combination thereof. For example, a total treatment period can be decided for Compound I. The second therapeutic agent can be administered prior to onset of treatment with Compound I or following treatment with Compound I. In addition, anti-cancer treatment can be administered during the period of administration of Compound I but does not need to occur over the entire treatment period of Compound I.

All patents, publications and pending patent applications identified are hereby incorporated by reference.
In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

**EXAMPLES**

Examples 1-4 refer to the General Scheme above.

**Example 1**

4,6-Dichloropyrimidine-2-methylsulfone (A): Prepared by methods substantially similar to those set forth in Koppell et al. *JOC*, 26, 1961, 792, in the following manner. To a stirred solution of 4,6-dichloro-2-(methylthio)pyrimidine (50 g, 0.26 mol) in dichloromethane (1 L) at 0°C was added meta-chloroperoxybenzoic acid (143.6 g, 0.64 mol) over a period of 20 minutes. The solution was allowed to warm to room temperature and was stirred for 4 hours. The mixture was diluted with dichloromethane (1.5 L) and then treated sequentially with 50% Na₂S₂O₅/NaHCO₃ solution (2 x 200 ml), sat NaHCO₃ solution (4 x 300 ml), and brine (200 ml) then dried (MgSO₄). The solvent was removed *in vacuo* to afford an off-white solid, which was redissolved in EtOAc (IL) and treated sequentially with sat. NaHCO₃ solution (3 x 300 ml), and bπne (100 ml) then dπed (MgSO₄). The solvent was removed *in vacuo* to afford the title compound (A) as a white solid (55.6 g, 96% yield). ¹H NMR CDCl₃ δ 3.40 (3H, s, CH₃), 7.75 (IH, s, ArH).

**Example 2**

Cyclopropane carboxylic acid [4-(4,6-dichloro-pyrimidin-2-ylsulphanyi)-phenyl]-amide (C): A suspension of compound A (10g, 44.04 mmol) and cyclopropane carboxylic acid (4-mercapto-phenyl)-amide (B, 8.51 g, 44.04 mmol) in t-butanol (300 ml) was degassed by evaporation, then flushing with nitrogen. The mixture was stirred at 90°C under nitrogen atmosphere for 1 hour then the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate (600 ml) and washed with an aqueous solution of potassium carbonate and sodium chloride. The organic extract was dried over magnesium sulphate, concentrated to a low volume and allowed to crystallize. The product C was collected as colourless crystals, (11.15 g, 74%). ¹H-NMR DMSO-d⁶ δ 0.82-0.89 (4H₃, m), 1.80-1.88 (IH, m), 7.55 (2H, d), 7.70-7.76 (3H, m), 10.49 (IH, s); M+H, 340.

**Example 3**

Cyclopropane carboxylic acid[4-4-chloro-6-(5-methyl-2H-pyrazol-3-ylamino)-pyrimidin-2-ylsulphonyl-phenyl] amide (D). A mixture of compound C (10 g, 2.94 mmol)and 3-ammo-5-methylypyrazole (314 mg, 3.23 mmol) in dimethylformamide (6 ml) was treated with dπopropylethylamine (0.614 ml, 3.53 mmol) and sodium iodide (530 mg, 3.53 mmol) The mixture was stirred under nitrogen at 85 ° for 4 hours, cooled to room temperature and diluted with ethyl acetate. The solution was washed with water (x 4), dπed over magnesium sulphate and concentrated to 5 ml to afford, upon crystallization and harvesting of colourless crystals, the title compound D (920 mg, 78%). ¹H-
NIVIR DMSO-d$_6$, $\delta$ 0.80-0.87 (4H, m), 1.77-1.85 (IH, m), 1.92 (IH, s), 5.24 (IH, br s), 6.47 (IH, br s), 7.55 (2H, d), 7.70-7.80 (2H, m), 10.24 (IH, s), 10.47 (IH, s), 11.92 (IH, s).

**Example 4**

Cyclopropane carboxylic acid \{4-[4-(4-methyl-piperazin-1-yl)-6-(5-methyl-2H-pyrazol-3-ylamino)pyrimidin]-2-ylsulpha\}yl-[phenyl]-amide (I): Compound D (2.373 g, 5.92 mmol) was treated with N-methylpiperazine (10 ml) and the mixture stirred at 110°C for 2 hours. The excess N-methylpiperazine was removed in vacuo then the residue was dissolved in ethyl acetate, washed with aqueous sodium bicarbonate solution, dried over magnesium sulphate, and concentrated. The residue was crystallised from methanol to give colourless crystals of desired product 1 (1.82 g, 66%). $^1$H-NMR DMSO-d$_5$, $\delta$ 0.81 (4H, d), 1.79 (IH, m), 2.01 (3H, s), 2.18 (3H, s), 2.30 (4H, m), 3.35 (masked signal), 5.42 (IH, s), 6.02 (IH, br s), 7.47 (2H, d), 7.69 (2H, d), 9.22 (IH, s), 10.39 (IH, s), 11.69 (IH, s).

**Example 5**

**JAK2 Kinase Activity Inhibition Assay and Determination of IC$_{50}$**

The kinase activity was measured using a modified version of the homogeneous time-resolved tyrosine kinase assay described in Parket al. Anal. Biochem. 269, 94-104 (1999).

The procedure for determining the potency of a compound to inhibit JAK2 kinase comprises the following steps:

1. prepare 3-fold serial diluted compound/inhibitor solutions in 100% (DMSO) at 2OX of the final desired concentrations in a 96 well plate;
2. prepare a master reaction mix containing 6.67mM MgCl$_2$, 133.3mM NaCl, 66.7mM Tris-HCl (pH 7.4), 0.13mg/ml BSA, 2.67mM dithiothreitol, 0.27 recombinant JAK2 and 666.7nM biotinylated synthetic peptide substrate (biotin-ahx-EQEDPEGDFEWLE-CONH$_2$) (SEQ. ID.: 1);
3. in a black assay plate, add 2.5μl compound/inhibitor (or DMSO) and 37.5μl master reaction mix per well; initiate the kinase reaction by adding 10μl of 75 μM MgATP per well, allow the reactions to proceed for 80 minutes at room temperate; (the final conditions for the reactions are: 50mM JAK2 JHI domain (Upstate), 2.0μM substrate, 15μM MgATP, 5mM MgCl$_2$, 100mM NaCl, 2mM DTT, 0.1mg/ml BSA, 50mM Tris (pH 7.4) and 5% DMSO);
4. stop the kinase reaction with 50μl of Stop/Detection buffer containing 10mM EDTA, 25mM HEPES, 0.1% TRITON X-100, 0.126μg/ml Eu-chelate labeled anti-phosphotyrosine antibody PY20 (cat. # AD0067, PerkinElmer) and 45μg/ml Streptavidin-allophycocyanin conjugate (cat. # PJ25S, Prozyme); and
5. read HTRF signals on a Victor reader (PerkinElmer) in HTRF mode after 60 minutes.

IC$_{50}$ was obtained by fitting the observed relationship between compound/inhibitor concentration and HTRF signal with a 4-parameter logistic equation.
Compound I is a potent inhibitor of recombinant purified JAK2 kinase activity with an IC<sub>50</sub> of approximately 375nM.

**Example 6**

**JAK2 In-Vivo Assay**

Assays that can be utilized to assess the in-vivo efficacy of Compound I, include those well known in the art. An example of such an assay is described by Lacout et al. (Blood, 1 September 2006, Vol. 108, No. 5, pg 1652-1660).

**Example 7**

**Clinical Trial**

Currently, clinical trials are ongoing to test the activity of Compound I in patients with JAK2 positive refractory MPD. In one trial, six out of eight patients with JAK2 positive refractory MPD achieved an objective response.

While a number of embodiments of this invention have been described, it is apparent that the basic examples may be altered to provide other embodiments, which utilize Compound I and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments, which have been represented by way of example.
WHAT IS CLAIMED IS:

1. A method for treating myeloproliferative disorders in a patient comprising administering to the patient a therapeutically effective amount of:

   ![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof.

2. The method according to Claim 1 wherein the myeloproliferative disorder is polycythemia vera.

3. The method according to Claim 1 wherein the myeloproliferative disorder is essential thrombocythemia.

4. The method according to Claim 1 wherein the myeloproliferative disorder is myeloid metaplasia with myelofibrosis.

5. The method according to Claim 1 wherein the myeloproliferative disorder is chronic myelogenous leukemia.

6. The method according to Claim 1 wherein the myeloproliferative disorder is chronic myelomonocytic leukemia.

7. The method according to Claim 1 wherein the myeloproliferative disorder is hypereosinophilic syndrome.

8. The method according to Claim 1 wherein the myeloproliferative disorder is juvenile myelomonocytic leukemia.

9. The method according to Claim 1 wherein the myeloproliferative disorder is systemic mast cell disease.
10. The method according to Claim 1 wherein the myeloproliferative disorder is idiopathic myelofibrosis.

11. The method according to Claim 1 wherein the myeloproliferative disorder is systemic mastocytosis.

12. The method according to Claim 1 wherein the myeloproliferative disorder is chronic neutrophilic leukaemia.

13. The method according to Claim 1 wherein the myeloproliferative disorder is unclassified MPD.

14. The method according to Claim 1 wherein the myeloproliferative disorder is myelodysplastic syndrome.

15. A method for inhibiting JAK2 tyrosine kinase, comprising contacting:

![Chemical structure](image)

or a pharmaceutically acceptable salt thereof, and the JAK2 tyrosine kinase.
SEQUENCE LISTING

<110> Merck & Co., Inc.
Buser-Doepner, Carolyn A.
Freedman, Steven J.
Gibbs, Jackson B.
Giles, Frank J.
Marshall, Christopher G.
Pollard, John R.

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