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(71) Applicant *(for all designated States except US)*: **GEORGETOWN UNIVERSITY** [US/US]; 37th And O Streets, NW, Washington, DC 20057-1408 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KOZIKOWSKI, Alan, P.** [US/US]; 222 North Dayton Street, Chicago, IL 60614 (US). **GAYSINA, Irina** [RU/US]; 911 Washington Blvd., Apt. 3, Oak Park, IL 60302 (US). **PETUKHOV, Pavel** [RU/US]; 12209 Village Square Terrace, Apt. 401, Rockville, MD 20852 (US). **SRIDHAR, Jayalakshmi** [IN/US]; 991 West Blaine Street, Apt. 39, Riverside, CA 92507 (US). **TUECKMANTEL, Werner** [DE/US]; 9225 E. Tanque Verde Road, Tucson, AZ 85749 (US).

(74) Agents: **GORDON, Dana, M.** et al.; Foley Hoag LLP, Seaport World Trade Center West, 155 Seaport Boulevard, Boston, MA 02210-2600 (US).

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WO 2007/008514 A2

(54) Title: INHIBITORS OF GLYCOGEN SYNTHASE KINASE 3

(57) Abstract: One aspect of the present invention relates to heterocyclic compounds that inhibit mammalian or human glycogen synthase kinase 3. Another aspect of the invention relates to pharmaceutical compositions comprising such a heterocyclic compound. The present invention also relates to methods of treating a mammal or human suffering from a malady that is based at least in part on abnormal activity of glycogen synthase kinase 3. The present invention also relates to methods of treating a mammal or human suffering from diabetes, Alzheimer's disease, Huntington's disease, Parkinson's disease, AIDS-associated dementia, ALS, MS, or schizophrenia.

Inhibitors of Glycogen Synthase Kinase 3

Related Applications

This application claims the benefit of priority to United States Provisional Patent Application serial number 60/697,084, filed July 7, 2005; and United States Provisional Patent Application serial number 60/717,277, filed September 15, 2005.

Background of the Invention

The search for new therapeutic agents has been greatly aided in recent years by better understanding of the structure of enzymes and other biomolecules associated with target diseases. One important class of enzymes that has been the subject of extensive study is the protein kinases.

Protein kinases mediate intracellular signal transduction. They do this by effecting a phosphoryl extracellular and other stimuli cause a variety of cellular responses to occur inside the cell. Examples of such stimuli include environmental and chemical stress signals (e.g. osmotic shock, heat shock, ultraviolet radiation, bacterial endotoxin, H_2O_2), cytokines (e.g. interleukin-1 (IL-1) and tumor necrosis factorα (TNF-α)), and growth factors (e.g. granulocyte macrophage-colony-stimulating factor (GM-CSF), and fibroblast growth factor (FGF). An extracellular stimulus may effect one or more cellular responses related to cell growth, migration, differentiation, secretion of hormones, activation of transcription factors, muscle contraction, glucose metabolism, control of protein synthesis and regulation of cell cycle.

Many diseases are associated with abnormal cellular responses triggered by protein kinase-mediated events. These diseases include autoimmune diseases, inflammatory diseases, neurological and neurodegenerative diseases, cancer, cardiovascular diseases, allergies and asthma, Alzheimer's disease or hormone-related diseases. Accordingly, there has been a substantial effort in medicinal chemistry to find protein kinase inhibitors that are effective as therapeutic agents.

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase comprised of α and β isoforms that are each encoded by distinct genes. Coghlan et al., *Chemistry & Biology*, 7, 793-803 (**2000**); Kim and Kimmel, *Curr. Opinion Genetics Dev.*, 10, 508-514

(2000). GSK-3 has been implicated in various diseases including diabetes, Alzheimer's disease, CNS disorders such as manic depressive disorder and neurodegenerative diseases, and cardiomyocete hypertrophy. WO 99/65897; WO 00/38675; and Haq et al., *J. Cell Biol.* (2000) 151, 117. These diseases may be caused by, or result in, the abnormal operation of certain cell signaling pathways in which GSK-3 plays a role. GSK-3 has been found to phosphorylate and modulate the activity of a number of regulatory proteins. These proteins include glycogen synthase which is the rate limiting enzyme necessary for glycogen synthesis, the microtubule associated protein Tau, the gene transcription factor β-catenin, the translation initiation factor e1F2B, as well as ATP citrate lyase, axin, heat shock factor-1, c-Jun, c-Myc, c-Myb, CREB, and CEPBα. These diverse protein targets implicate GSK-3 in many aspects of cellular metabolism, proliferation, differentiation and development.

In a GSK-3 mediated pathway that is relevant for the treatment of type II diabetes, insulin-induced signaling leads to cellular glucose uptake and glycogen synthesis. Along this pathway, GSK-3 is a negative regulator of the insulin-induced signal. Normally, the presence of insulin causes inhibition of GSK-3 mediated phosphorylation and deactivation of glycogen synthase. The inhibition of GSK-3 leads to increased glycogen synthesis and glucose uptake. Klein et al., *PNAS*, 93, 8455-9 (**1996**); Cross et al., *Biochem. J.*, 303, 21-26 (**1994**); Cohen, *Biochem. Soc. Trans.*, 21, 555-567 (**1993**); Massillon et al., *Biochem J.* 299, 123-128 (**1994**). However, in a diabetic patient where the insulin response is impaired, glycogen synthesis and glucose uptake fail to increase despite the presence of relatively high blood levels of insulin. This leads to abnormally high blood levels of glucose with acute and long term effects that may ultimately result in cardiovascular disease, renal failure and blindness. In such patients, the normal insulin-induced inhibition of GSK-3 fails to occur. It has also been reported that in patients with type II diabetes, GSK-3 is overexpressed. WO 00/38675. Therapeutic inhibitors of GSK-3 are therefore potentially useful for treating diabetic patients suffering from an impaired response to insulin.

GSK-3 activity has also been associated with Alzheimer's disease. Alzheimer's disease is among the most important health care problems in the world. The past decade has seen the adoption of the first class of medications, the cholinesterase inhibitors, effective in improving cognitive symptoms in Alzheimer's disease. These drugs provide symptomatic relief; effective disease-modifying therapy remains a major, elusive goal. Substantial efforts have been made to apply findings from laboratory research, as well as

genetic and epidemiologic studies, to the identification of potential strategies for influencing Alzheimer's disease pathology. Alzheimer's disease is a progressive dementia which develops in late middle ages (45 to 65 years old) and its etiological changes are shrinkage of cerebral cortex due to a neuronal cell loss and degeneration of the neurons while, from the pathological view, many senile plaques and neurofibrillary tangles are noted in the brain. There is no pathologically substantial difference between the disease and senile dementia caused by the so-called natural aging which develops in the senile period of 65 years and older ages and, therefore, this disease is called senile dementia of Alzheimer type.

This disease is characterized by the well-known β-amyloid peptide and the formation of intracellular neurofibrillary tangles. The neurofibrillary tangles contain hyperphosphorylated Tau protein where Tau is phosphorylated on abnormal sites. GSK-3 has been shown to phosphorylate these abnormal sites in cell and animal models. Furthermore, inhibition of GSK-3 has been shown to prevent hyperphosphorylation of Tau in cells. Lovestone et al., *Current Biology 4*, 1077-86 (**1994**); Brownlees et al., *Neuroreport*, 8, 3251-55 (**1997**). Therefore, it is believed that GSK-3 activity may promote generation of the neurofibrillary tangles and the progression of Alzheimer's disease.

Another substrate of GSK-3 is β-catenin which is degradated after phosphorylation by GSK-3. Reduced levels of β-catenin have been reported in schizophrenic patients and have also been associated with other diseases related to increase in neuronal cell death. Zhong et al., *Nature*, 395, 698-702 (**1998**); Takashima et al., *PNAS*, 90, 7789-93 (**1993**); Pei et al., *J. Neuropathol. Exp*, 56, 70-78 (**1997**).

As a result of the biological importance of GSK-3, there is current interest in therapeutically effective GSK-3 inhbitors. Small molecules that inhibit GSK-3 have recently been reported. WO 99/65897 (Chiron) and WO 00/38675 (SmithKline Beecham).

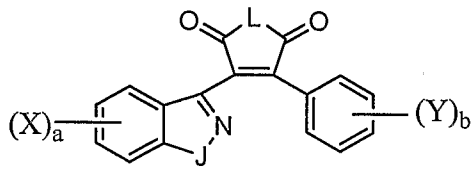
For many of the aforementioned diseases associated with abnormal GSK-3 activity, other protein kinases have also been targeted for treating the same diseases. However, the various protein kinases often act through different biological pathways. For example, certain quinazoline derivatives have been reported recently as inhibitors of p38 kinase. WO 00/12497 to Scios. The compounds are reported to be useful for treating conditions characterized by enhanced p38α activity and/or enhanced TGF-β activity. While p38

activity has been implicated in a wide variety of diseases, including diabetes, p38 kinase is not reported to be a constituent of an insulin signaling pathway that regulates glycogen synthesis or glucose uptake. Therefore, unlike GSK-3, p38 inhibition would not be expected to enhance glycogen synthesis and/or glucose uptake.

There is a continued need to find new therapeutic agents to treat human diseases. The protein kinase GSK-3 is especially attractive targets for the discovery of new therapeutics due to their important role in cancer, diabetes, Alzheimer's disease and other diseases.

Summary of the Invention

In part, the present invention relates to a compound of formula **I**:



I

wherein, independently for each occurrence

L is O, S, or NR;

J is O, S, or NR;

R is H, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or $-(CH_2)_n-R_2$

wherein,

n is an integer from 1 to 6 inclusive;

R_2 is -OH, $-NH_2$, -NH(alkyl), $-N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), $-N(aryl)_2$, or one or more saccharide units;

X and Y are -OH, halide, $-NO_2$, carboxylic, ketone, aldehyde, $-NH_2$, -NH(alkyl), $-N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), or $-N(aryl)_2$;

a is an integer from 1 to 4 inclusive; and

b is an integer from 1 to 5 inclusive.

In a further embodiment, the present invention relates to a compound of formula **I**

and the attendant definitions, wherein L is NH.

In a further embodiment, the present invention relates to a compound of formula **I** and the attendant definitions, wherein J is NH.

In a further embodiment, the present invention relates to a compound of formula **I** and the attendant definitions, wherein a is 0.

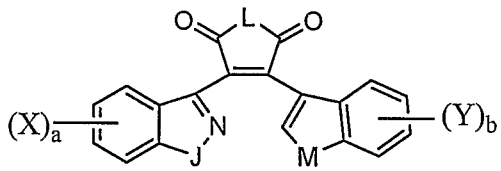
In a further embodiment, the present invention relates to a compound of formula **I** and the attendant definitions, wherein Y is Cl and b is 2.

In a further embodiment, the present invention relates to a compound of formula **I** and the attendant definitions, wherein L is NH and J is NH.

In a further embodiment, the present invention relates to a compound of formula **I** and the attendant definitions, wherein L is NH, J is NH, and a is 0.

In a further embodiment, the present invention relates to a compound of formula **I** and the attendant definitions, wherein L is NH, J is NH, a is 0, Y is Cl and b is 2.

In part, the present invention relates to a compound of formula **II**:



II

wherein, independently for each occurrence

L is O, S, or NR;

M is O, S, or NR;

J is O, S, or NR;

R is H, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or $-(CH_2)_n-R_2$

wherein,

n is an integer from 1 to 6 inclusive;

R_2 is -OH, $-NH_2$, -NH(alkyl), $-N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), $-N(aryl)_2$, or one or more saccharide units;

X and Y are -OH, halide, -NO_2, carboxylic, ketone, aldehyde, -NH_2, -NH(alkyl), -$N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), or -$N(aryl)_2$;

a is an integer from 1 to 4 inclusive; and

b is an integer from 1 to 5 inclusive.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein L is NH.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein J is NH.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein J is NCH_3.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein M is NH.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein M is NCH_3.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein M is $N(CH_2)_3OH$.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein M is $N(CH_2)_3N(CH_3)_2$.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein M is $N(CH_2)_3NHCH_3$.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein M is $N(CH_2)_3NH_2$.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein a is 0.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein b is 0.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein Y is Cl and b is 1.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein L is NH, J is NH, M is NH, a is 0, and b is 0.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein L is NH, J is NH, M is NCH_3, a is 0, and b is 0.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein L is NH, J is NH, M is $N(CH_2)_3OH$, a is 0, and b is 0.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein L is NH, J is NH, M is $N(CH_2)_3N(CH_3)_2$, a is 0, and b is 0.

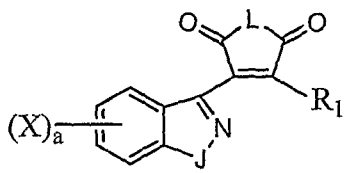
In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein L is NH, J is NH, M is $N(CH_2)_3NHCH_3$, a is 0, and b is 0.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein L is NH, J is NH, M is $N(CH_2)_3NH_2$, a is 0, and b is 0.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein L is NH, J is NCH_3, M is NCH_3, a is 0, and b is 0.

In a further embodiment, the present invention relates to a compound of formula **II** and the attendant definitions, wherein L is NH, J is NH, M is NCH_3, a is 0, Y is Cl, and b is 1.

In part, the present invention relates to a compound of formula **III**:



III

wherein, independently for each occurrence

L is O, S, or NR;

M is O, S, or NR;

J is O, S, or NR;

R is H, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or $-(CH_2)_n-R_2$

wherein,

n is an integer from 1 to 6 inclusive;

R_2 is -OH, $-NH_2$, -NH(alkyl), $-N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), $-N(aryl)_2$, or one or more saccharide units;

X is -OH, halide, $-NO_2$, carboxylic, ketone, aldehyde, $-NH_2$, -NH(alkyl), $-N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), or $-N(aryl)_2$;

R_1 is heteroaryl or heterocycloalkyl; and

a is an integer from 1 to 4 inclusive.

In a further embodiment, the present invention relates to compound of formula **III** and the attendant definitions, wherein L is NH.

In a further embodiment, the present invention relates to a compound of formula **III** and the attendant definitions, wherein J is NH.

In a further embodiment, the present invention relates to a compound of formula **III** and the attendant definitions, wherein R_1 is 3-indazolyl.

In a further embodiment, the present invention relates to a compound of formula **III** and the attendant definitions, wherein a is 0.

In a further embodiment, the present invention relates to a compound of formula **III** and the attendant definitions, wherein L is NH and J is NH.

In a further embodiment, the present invention relates to a compound of formula **III** and the attendant definitions, wherein L is NH, J is NH, and R_1 is 3-indazolyl.

In a further embodiment, the present invention relates to a compound of formula **III** and the attendant definitions, wherein L is NH, J is NH, R_1 is 3-indazolyl, and a is 0.

In another embodiment, the present invention relates to a pharmaceutical composition comprising a compound of formula **I**, **II**, or **III** and a pharmaceutically acceptable excipient.

In cases in which the compounds of formula **I**, **II**, or **III** have unsaturated carbon-carbon double bonds, both the cis (Z) and trans (E) isomers are within the scope of this invention. In cases wherein the compounds may exist in tautomeric forms, such as keto-enol tautomers, such as  and , each tautomeric form is contemplated as being included within this invention, whether existing in equilibrium or locked in one form by appropriate substitution with R'. The meaning of any substituent at any one occurrence is independent of its meaning, or any other substituent's meaning, at any other occurrence.

Also included in the protein kinase inhibitor compounds of the present invention are prodrugs of the compounds of formula **I**, **II**, or **III**. Pharmaceutically acceptable prodrugs of the compounds of this invention include, without limitation, esters, amino acid esters, phosphate esters, metal salts and sulfonate esters.

Pharmaceutically acceptable salts of the compounds of this invention 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, palmoate, 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 pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g., sodium and potassium), alkaline earth metal (e.g., magnesium), ammonium and $N^+(C_{1-4}\ alkyl)_4$ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization.

The compounds of this invention may exist in unsolvated as well as in solvated forms with pharmaceutically acceptable solvents such as water, ethanol and the like. In

general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of this invention.

In another embodiment, the present invention relates to a compound of formula **I**, **II**, or **III**, wherein the compound reduces the activity of GSK-3 by greater than about 20%. In a further embodiment, the compound of formula **I**, **II**, or **III** reduces the activity of GSK-3 by greater than about 40%. In a further embodiment, the compound of formula **I**, **II**, or **III** reduces the activity of GSK-3 by greater than about 60%. In a further embodiment, the compound of formula **I**, **II**, or **III** reduces the activity of GSK-3 by greater than about 80%. In a further embodiment, the compound of formula **I**, **II**, or **III** reduces the activity of GSK-3 by greater than about 90%. In a further embodiment, the compound of formula **I**, **II**, or **III** reduces the activity of GSK-3 by greater than about 95%. In a further embodiment, the compound of formula **I**, **II**, or **III** reduces the activity of GSK-3 by greater than about 98%.

In accordance with the present invention, a compound of the present invention may be prepared as pharmaceutical compositions that are particularly useful for the treatment of neurodegenerative, cancerous, or diabetic diseases. Such compositions comprise a compound of the present invention with pharmaceutically acceptable carriers and/or excipients.

For example, these compositions may be prepared as medicines to be administered orally, parenterally, rectally, transdermally, bucally, or nasally. Suitable forms for oral administration include tablets, compressed or coated pills, dragees, sachets of powder for reconstitution, hard or gelatin capsules, sub-lingual tablets, syrups and suspensions. Suitable forms for parenteral administration include an aqueous or non-aqueous solution or emulsion, while for rectal administration suitable forms include suppositories with hydrophilic or hydrophobic vehicles. For topical application the invention provides ointments or aerosol formulations known in the art; for transdermal delivery there are provided suitable delivery systems as known in the art. For nasal delivery there are provided suitable aerosol delivery systems known in the art.

In another aspect of the present invention, the pharmaceutical compositions of the present invention may be used in the manufacture of a medicament to treat neurodegenerative, cancerous, or diabetic disorders. In certain embodiments, the present invention is directed to a method for formulating compositions of the present invention in a

pharmaceutically acceptable carrier.

In another aspect, the present invention also provides for kits containing at least one dose of a subject composition, and often many doses, and other materials for a treatment regimen. For example, in one embodiment, a kit of the present invention contains sufficient subject composition for from five to thirty days and optionally equipment and supplies necessary to measure one or more indices relevant to the treatment regiment. In another embodiment, kits of the present invention contain all the materials and supplies, including subject compositions, for carrying out any methods of the present invention. In still another embodiment, kits of the present invention, as described above, additionally include instructions for the use and administration of the subject compositions.

The dosage may be selected to assuage the disorder in a subject in such a way as to provide at least partial relief if not complete relief. The skilled artisan may identify this amount as provided herein as well as by using other methods known in the art.

The amount of the protein kinase inhibitor that may be combined with the carrier materials to produce a single dosage form will vary depending upon the patient treated and 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 inhibitor 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 the inhibitor will also depend upon the particular compound in the composition.

In another embodiment, the present invention relates to a method of inhibiting a protein kinase in a mammal comprising administering to the mammal a compound of formula **I**, **II**, or **III**. In a further embodiment, the mammal is a primate, equine, canine, or feline. In a further embodiment, the mammal is a human. In a further embodiment the protein kinase is GSK-3.

In another embodiment, the present invention relates to a method of inhibiting a protein kinase in a mammal comprising administering to the mammal a compound of

formula **I**, **II**, or **III**, wherein the compound is administered orally. In a further embodiment, the compound is administered intravenously, sublingually, ocularly, transdermally, rectally, vaginally, topically, intramuscularly, subcutaneously, buccally, or nasally. In a further embodiment the protein kinase is GSK-3.

In another embodiment, the present invention relates to a method of treating a mammal suffering from any disease or other deleterious condition or state in which GSK-3 is known to play a role comprising administering a therapeutically effective amount of a compound of formula **I**, **II**, or **III**. Such diseases or conditions include, without limitation, diabetes, Alzheimer's disease, Huntington's Disease, Parkinson's Disease, AIDS-associated dementia, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), schizophrenia, cardiomycete hypertrophy, reperfusion/ischemia, and baldness. In a further embodiment, the mammal is a primate, equine, canine, or feline. In a further embodiment, the mammal is a human. In a further embodiment, the compound is administered orally, intravenously, sublingually, ocularly, transdermally, rectally, vaginally, topically, intramuscularly, subcutaneously, buccally, or nasally.

Depending upon the particular protein kinase-mediated condition to be treated or prevented, additional therapeutic agents, which are normally administered to treat or prevent that condition, may be administered together with the inhibitors of this invention. For example, in the treatment of diabetes other anti-diabetic agents may be combined with the GSK-3 inhibitors of this invention to treat diabetes. These agents include, without limitation, insulin or insulin analogues, in injectable or inhalation form, glitazones, alpha glucosidase inhibitors, biguanides, insulin sensitizers, and sulfonyl ureas.

Other examples of agents the inhibitors of this invention may also be combined with include, without limitation, chemotherapeutic agents or other anti-proliferative agents such as adriamycin, dexamethasone, vincristine, cyclophosphamide, fluorouracil, topotecan, taxol, interferons, and platinum derivatives; anti-inflammatory agents such as corticosteroids, TNF blockers, IL-1 RA, azathioprine, cyclophosphamide, and sulfasalazine; immunomodulatory and immunosuppressive agents such as cyclosporin, tacrolimus, rapamycin, mycophenolate mofetil, interferons, corticosteroids, cyclophophamide, azathioprine, and sulfasalazine; neurotrophic factors such as acetylcholinesterase inhibitors, MAO inhibitors, interferons, anti-convulsants, ion channel blockers, riluzole, and anti-Parkinsonian agents; agents for treating cardiovascular disease such as beta-blockers, ACE

inhibitors, diuretics, nitrates, calcium channel blockers, and statins; agents for treating liver disease such as corticosteroids, cholestyramine, interferons, and anti-viral agents; agents for treating blood disorders such as corticosteroids, anti-leukemic agents, and growth factors; and agents for treating immunodeficiency disorders such as gamma globulin.

Those additional agents may be administered separately from the protein kinase inhibitor-containing composition, as part of a multiple dosage regimen. Alternatively, those agents may be part of a single dosage form, mixed together with the protein kinase inhibitor of this invention in a single composition.

As explained herein in greater detail, the invention will readily enable the design and implementation of trials in warm-blooded animals, including humans and mammals, necessary for easily determining or tailoring the form and dose for any composition of the present invention.

These embodiments of the present invention, other embodiments, and their features and characteristics, will be apparent from the description, drawings and claims that follow.

Brief Description of the Figures

Figure 1 depicts the differentiation inhibitory effect in murine embryonic stem (ES) cells of GSK-3 inhibitor 3-(3-indazolyl)-4-(3-indolyl)-1*H*-pyrrole-2,5-dione (JS 340).

Figure 2 depicts the differentiation inhibitory effect in murine ES cells of GSK-3 inhibitor 3-(3-indazolyl)-4-(3-indolyl)-1*H*-pyrrole-2,5-dione (JS 340) as visualized by green fluorescence.

Figure 3 depicts the differentiation inhibitory effect in murine ES cells of GSK-3 inhibitor 3,4-(3-indolyl)-1*H*-pyrrole-2,5-dione (JS 343).

Figure 4 depicts the differentiation inhibitory effect in murine ES cells of GSK-3 inhibitor 3,4-(3-indolyl)-1*H*-pyrrole-2,5-dione (JS 343) as visulized by green fluorescence.

Figure 5 depicts the differentiation inhibitory effect in murine ES cells of GSK-3 inhibitor 3-(3-indazolyl)-4-[(1-methyl)-3-indolyl]-1*H*-pyrrole-2,5-dione (JS 349).

Figure 6 depicts the differentiation inhibitory effect in murine ES cells of GSK-3 inhibitor 3-(3-indazolyl)-4-[(1-methyl)-3-indolyl]-1*H*-pyrrole-2,5-dione (JS 349) as visualized by green fluorescence.

Figure 7 depicts the effects on the development of frog embryos of GSK-3 inhibitors 3-(3-indazolyl)-4-(3-indolyl)-1*H*-pyrrole-2,5-dione (JS 340), 3,4-(3-indolyl)-1*H*-pyrrole-2,5-dione (JS 343), and 3-(3-indazolyl)-4-[(1-methyl)-3-indolyl]-1*H*-pyrrole-2,5-dione (JS 349).

Figure 8 depicts cellular changes in mesencephalic neurons after treatment with MPTP and GSK-3β inhibitors. Expression levels of PHF-Tau (**a**), α-synuclein (**b**) and p-GSK3β (**c**) were assessed by Western blots in the control and MPP^+-treated mesencephalic neurons, in the presence or absence of specific GSK-3β inhibitors. Results in (**d**) show cell viability, measured by the MTT cell viability assay. Three independent experiments were performed with embryonic rat ventral mesencephalic neuronal cultures. One timed-pregnant rat at gestational day 16 was used per experiment. Mesencephalic neurons were treated with 50 μM of MPP^+ for 48 h or with vehicle (0.2% (vol/vol) DMSO), in the presence or absence of the GSK-3β inhibitors (1 μM added for the last 16 hrs of MPP^+ exposure). The lower panel in each individual figure shows the quantitation of the results, which were obtained by measuring the optical density of each band, and these are expressed as the ratio of the densities between: PHF-Tau and total Tau (**a**); α-synuclein and β-actin (**b**); p-GSK-3β(Y216) and GSK-3β (**c**). Values from each treatment are expressed as the mean ± s.d. The significance of difference, as compared to the vehicle-treated respective groups (which were set at 100%) was determined with a *t*-test (*, $p \leq 0.05$ for immunoblot analysis; *, $p \leq 0.01$ for MTT assay).

Figure 9 shows that GSK-3β hyperphosphorylation of Tau is dependent on the presence of both DAT and α-Syn. Immunoblot analysis of the expression levels of Tau phosphorylated at Ser 396/404 (**a**), α–Syn (**b**), and p-GSK3β (**c**), evaluated from cell lysates of vehicle- or MPP^+- treated SHSY5Y neuroblastoma cells stably transfected with wild type alpha-synuclein (SHα-Syn) and additionally transiently co-transfected with the human dopamine transporter cDNA (hDAT; SHα-Syn/hDAT), in the presence or absence of specific GSK-3β inhibitors. Results in (**d**) show cell viability, measured by the MTT reduction assay. Experimental design, figures arrangements, and statistical analysis (n=3) were performed as described in Figure 3, except that $p \leq 0.01$ was chosen to denote statistical significance (*) using the *t*-test for the statistical analysis of the MTT assay results in **d** (n = 3 in triplicate).

Detailed Description of the Invention

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "ED_{50}" means the dose of a drug which produces 50% of its maximum response or effect. Alternatively, the dose which produces a pre-determined response in 50% of test subjects or preparations.

The term "LD_{50}" means the dose of a drug which is lethal in 50% of test subjects.

The term "therapeutic index" refers to the therapeutic index of a drug defined as LD_{50}/ED_{50}.

The term "structure-activity relationship (SAR)" refers to the way in which altering the molecular structure of drugs alters their interaction with a receptor, enzyme, etc.

The term "agonist" refers to a compound that mimics the action of natural transmitter or, when the natural transmitter is not known, causes changes at the receptor complex in the absence of other receptor ligands.

The term "antagonist" refers to a compound that binds to a receptor site, but does not cause any physiological changes unless another receptor ligand is present.

The term "inverse agonist" refers to a compound that binds to a constitutively active receptor site and reduces its physiological function.

The term "competitive antagonist" refers to a compound that binds to a receptor site; its effects can be overcome by increased concentration of the agonist.

The term "partial agonist" refers to a compound that binds to a receptor site but does not produce the maximal effect regardless of its concentration.

The term "ligand" refers to a compound that binds at the receptor site.

The term "GSK-3-mediated condition" or "disease", as used herein, means any

disease or other deleterious condition or state in which GSK-3 is known to play a role. Such diseases or conditions include, without limitation, diabetes, Alzheimer's disease, Huntington's Disease, Parkinson's Disease, AIDS-associated dementia, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), schizophrenia, cardiomycete hypertrophy, reperfusion/ischemia, and baldness.

A "pharmaceutically acceptable derivative or prodrug" means any pharmaceutically acceptable salt, ester, salt of an ester or other derivative of a compound of this invention which, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an inhibitorily active metabolite or residue thereof. Particularly favored derivatives or prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a patient (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) relative to the parent species.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are boron, nitrogen, oxygen, phosphorus, sulfur and selenium.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure. The term "alkyl" is also defined to include halosubstituted alkyls.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. Preferred alkyl groups are lower alkyls. In preferred embodiments, a substituent designated herein as alkyl is a lower alkyl.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "aryl" as used herein includes 5-, 6- and 7-membered single-ring aromatic groups such as, for example, benzene, and the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls. Examples of such aryl rings include naphthalene, anthracene, 1,2,3,4-tetrahydronaphthalene, thianthrene, isobenzofuran, chromene, xanthene, phenoxathiin, phenothiazine, phenoxazine, and the like.

Aryl groups having heteroatoms in the ring structure are referred to as "heteroaryl." Heteroaryls may have from 1 to 4 heteroatoms in the ring. The heteroaromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, $-CF_3$, -CN, or the like. Examples of heteroaryls include pyrrole, furan, thiophene, imidazole, oxazole, isoxazole, thiazole, isothiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine, furazan, pyrimidine, and the like. The term "heteroaryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls. Examples of such rings include quinoline, indole, indazole, purine, isoquinoline, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, phenanthridine, acridine, phenanthroline, phenazine, phthalazine, carboline, isoindole, phenarsazine, indolizine, naphthyridine, and the like.

The terms *ortho*, *meta* and *para* apply to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and *ortho*-dimethylbenzene are synonymous.

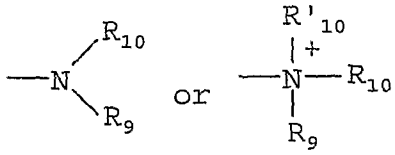
The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclyl groups include, for example, azetidine, azepine, pyran, quinolizine, pyrrolidine, oxolane, thiolane, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

The terms "polycyclyl" or "polycyclic group" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

The term "carbocycle", as used herein, refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

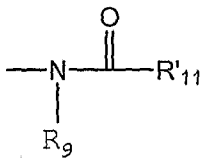
As used herein, the term "nitro" means $-NO_2$; the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means $-SO_2-$.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:



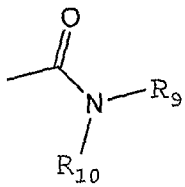
wherein R_9, R_{10} and R'_{10} each independently represent a group permitted by the rules of valence.

The term "acylamino" is art-recognized and refers to a moiety that can be represented by the general formula:



wherein R_9 is as defined above, and R'_{11} represents a hydrogen, an alkyl, an alkenyl or $-(CH_2)_m-R_8$, where m and R_8 are as defined above.

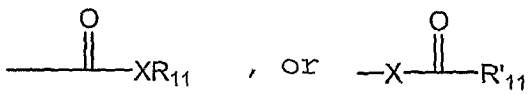
The term "amido" is art recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:



wherein R_9, R_{10} are as defined above. Preferred embodiments of the amide will not include imides which may be unstable.

The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In preferred embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, -S-alkynyl, and $-S-(CH_2)_m-R_8$, wherein m and R_8 are defined above. Representative alkylthio groups include methylthio, ethyl thio, and the like.

The term "carbonyl" is art recognized and includes such moieties as can be represented by the general formula:



wherein X is a bond or represents an oxygen or a sulfur, and R_{11} represents a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R_8$ or a pharmaceutically acceptable salt, R'_{11} represents a hydrogen, an alkyl, an alkenyl or $-(CH_2)_m-R_8$, where m and R_8 are as defined above. Where X is an oxygen and R_{11} or R'_{11} is not hydrogen, the formula represents an "ester". Where X is an oxygen, and R_{11} is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R_{11} is a hydrogen, the formula represents a "carboxylic acid". Where X is an oxygen, and R'_{11} is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiolcarbonyl" group. Where X is a sulfur and R_{11} or R'_{11} is not hydrogen, the formula represents a "thiolester." Where X is a sulfur and R_{11} is hydrogen, the formula represents a "thiolcarboxylic acid." Where X is a sulfur and R_{11}' is hydrogen, the formula represents a "thiolformate." On the other hand, where X is a bond, and R_{11} is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R_{11} is hydrogen, the above formula represents an "aldehyde" group.

The terms "alkoxyl" or "alkoxy" as used herein refers to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, $-O-(CH_2)_m-R_8$, where m and R_8 are described above.

The abbreviations Me, Et, Ph, Tf, Nf, Ts, Ms represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, *p*-toluenesulfonyl and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the *Journal of Organic Chemistry*; this list is typically presented in a table entitled Standard List of Abbreviations. The abbreviations contained in said list, and all abbreviations utilized by organic chemists of ordinary skill in the art are hereby incorporated by reference.

Analogous substitutions can be made to alkenyl and alkynyl groups to produce, for example, aminoalkenyls, aminoalkynyls, amidoalkenyls, amidoalkynyls, iminoalkenyls, iminoalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

As used herein, the definition of each expression, e.g. alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

The phrase "protecting group" as used herein means temporary substituents which protect a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991).

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, it may be isolated using chiral chromatography methods, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof (e.g., functioning as analgesics), wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound in binding to opioid receptors. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, inside cover.

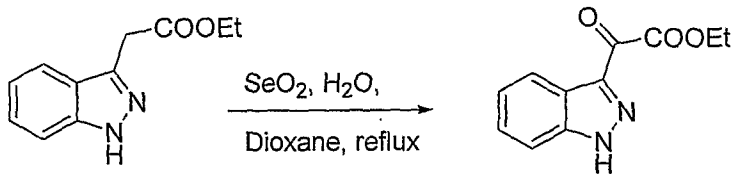
Synthesis of Compounds

The compounds of the invention may be prepared by any conventional method useful for the preparation of analogous compounds and as described in the examples below. Starting materials for the processes described in the present patent application are known or can be prepared by known processes from commercially available materials. A compound of the invention can be converted to another compound of the invention using conventional methods. The products of the reactions described herein are isolated by conventional means such as extraction, crystallization, distillation, chromatography, and the like.

Useful starting materials for the preparation of compounds of the present invention are indazolyl-3-glyoxylates, the preparation of which is depicted in scheme 1 for ethyl

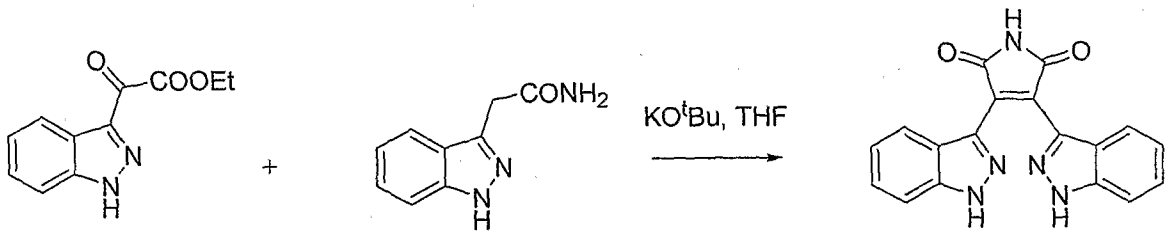
indazolyl-3-glyoxylate.

Scheme 1. General synthesis of indazolyl-3-glyoxylates.



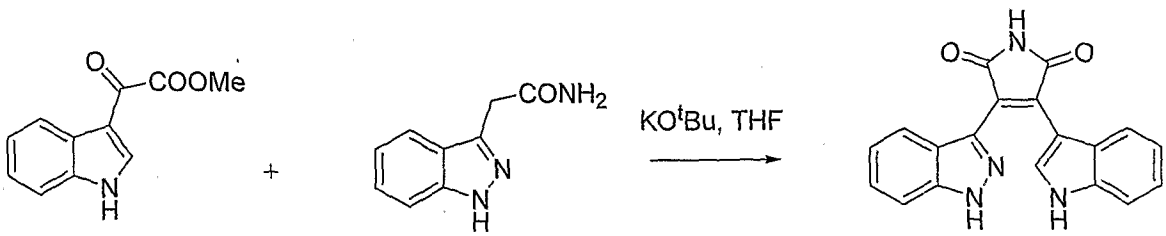
From these indazolyl-3-glyoxylates, symmetrical 3-indazolyl-1*H*-pyrrole-2,5,-diones of the present invention can be prepared by coupling with indazole-3-acetamides as depicted in scheme 2.

Scheme 2. Preparation of 3,4,-(3-Indazolyl)-1*H*-pyrrole-2,5-diones.



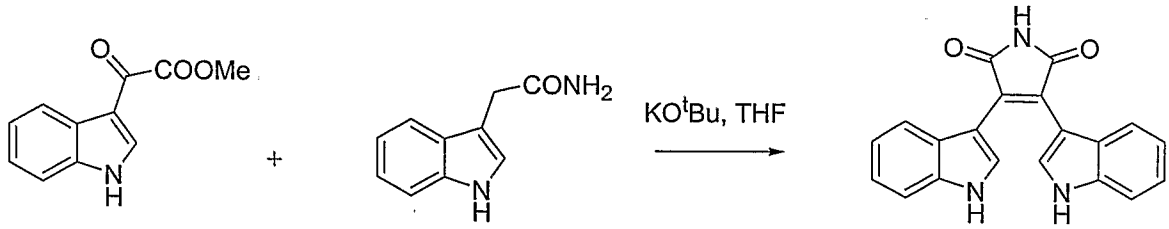
Non-symmetrical 3-indazolyl-3-indolyl-1*H*-pyrrole-2,5-diones of the present invention are prepared by substituting the ethyl indazolyl-3-glyoxylate in scheme 2 with indolyl-3-glyoxylate as depicted in scheme 3.

Scheme 3. Preparation of 3-(3-indazolyl)-4-(3-indolyl)-1*H*-pyrrole-2,5-dione.



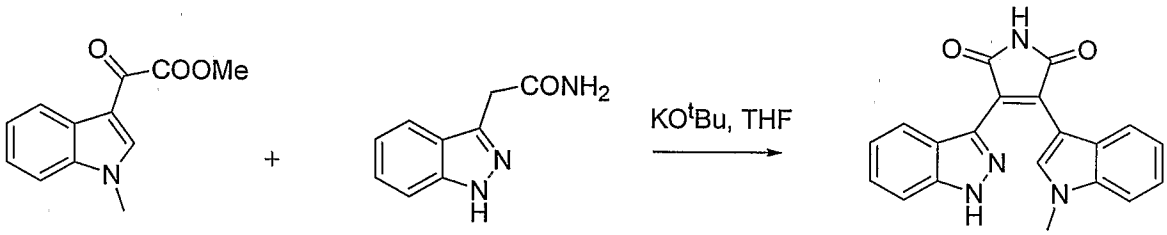
Preparation of 3,4,-(3-Indolyl)-1*H*-pyrrole-2,5-diones are prepared similarly but substituting the indazolyl acetamide with an indolyl acetamide as depicted in scheme 4.

Scheme 4. Preparation of 3,4,-(3-Indolyl)-1*H*-pyrrole-2,5-diones.

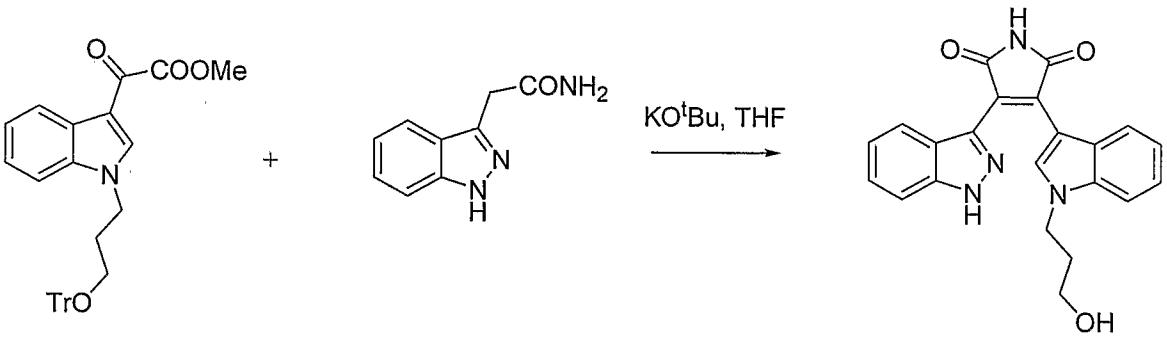


N-substituted 3-(3-indazolyl)-4-(3-indolyl)-1*H*-pyrrole-2,5-diones are prepared by starting with the appropriately substituted indolyl-3-glyoxylate. Schemes 5 and 6 depict the synthesis of 1-methyl and 1-(3-hydroxypropyl) substituted analogs of 3-(3-indazolyl)-4-(3-indolyl)-1*H*-pyrrole-2,5-dione, respecively.

Scheme 5. Preparation of 3-(3-indazolyl)-4-[(1-methyl)-3-indolyl]-1*H*-pyrrole-2,5-dione.

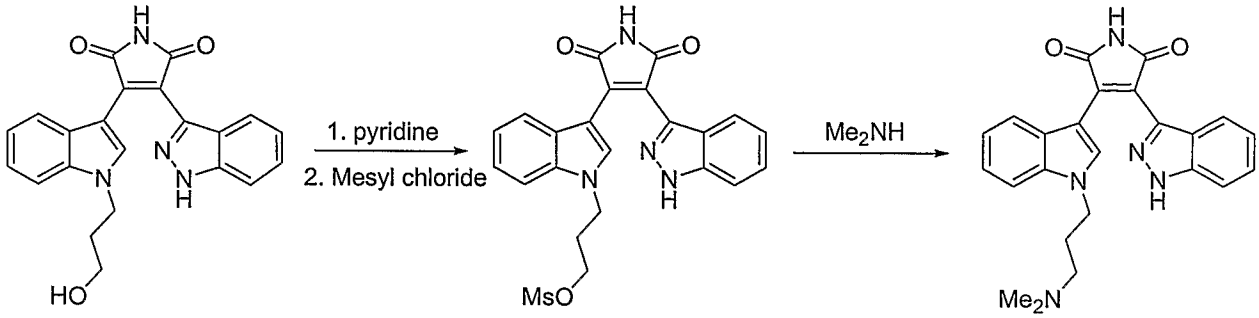


Scheme 6. Preparation of 3-(3-indazolyl)-4-[1-(3-hydroxypropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione (Tr = triphenylmethyl).



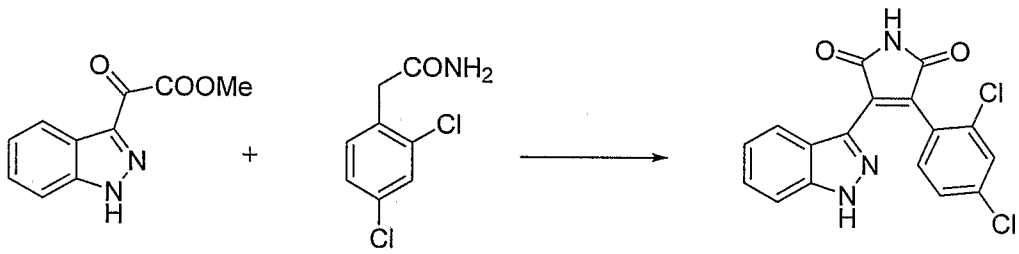
The hydroxy group is readily substituted for an amine group in the manner depicted in scheme 7.

Scheme 7. Substitution of the hydroxy group in 3-(3-indazolyl)-4-[1-(3-hydroxypropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione for an amine group.



Aryl analogs of the indazolyl-1*H*-pyrrole-2,5-diones of the present invention are prepared by reacting indazolyl-3-glyoxylate with the appropriate aryl acetamide. Scheme 8 depicts an example of this synthesis with the preparation of 3-(2,4-dichlorophenyl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione.

Scheme 8. Preparation of 3-(2,4-dichlorophenyl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione.

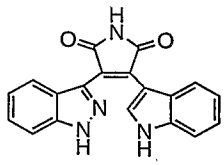
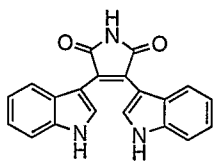
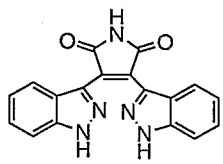
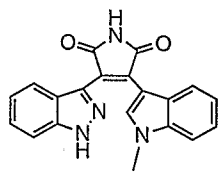


Binding Affinity Assays

The binding affinities of the inhibiting compounds were determined by testing the inhibitors against a panel of 30 protein kinases. Table 1 describes the percentage of kinase activity exhibited by a particular protein kinase after treatment with an inhibitor concentration of 10 μM relative to the control incubation. The first column for each inhibitor is the percentage of kinase activity in the presence of the inhibitor. The second column is a +/- SEM (standard error measurement). If the value is low then the molecule is considered to be a good inhibitor for that kinase. It can be seen that cpd JS340 inhibits S6K1, GSK3b and DYRK1a to a greater degree than it does the other protein kinases. It can also be seen that cpd JS343 inhibits many kinases, namely MAPKAP-K1a, PKCa, PDK1, GSK3b, AMPK, CHK1, PHOS.KINASE, and DYRK1, to a good extent as well. Cpd JS349, which is an indole N-methyl derivative of cpd JS340, shows excellent inhibition for GSK3b and because its inhibition for other kinases is considerably less than it

is for GSK3b, one may conclude that this molecule is selective. Based on the results for cpds JS340 and JS349, these two inhibitors are used as models to design ligands which have the potential for greater selectivity and potency than cpd JS349. For more information on the nature of this type of experiment see (1) Bain, J.; McLaughlan. H.; Elliott, M.; and Cohen, P. Biochem. J., (2003), 371, 199-204; (2) Davies, S. P.; Reddy, H.; Caivano, M.; and Cohen, P. Biochem. J., (2000), 351, 95-105.

Table 1.

	cpd JS340 10μM	**cpd JS343** 10μM	**cpd JS345** 10μM	**cpd JS349** 10μM
				

	cpd 340		**cpd 343**		**cpd 345**		**cpd 349**	
MKK1	**40**	2	**24**	4	**103**	10	**79**	3
MAPK2/ERK2	**60**	6	**52**	5	**101**	1	**74**	9
JNK/SAPK1c	**87**	2	**71**	3	**98**	5	**111**	1
SAPK2a/p38	**79**	5	**83**	3	**90**	6	**99**	9
SAPK2b/p38b2	**81**	5	**85**	3	**69**	12	**98**	6
SAPK3/p38d	**85**	4	**76**	4	**97**	3	**88**	5
SAPK4/p38d	**95**	3	**82**	0	**97**	13	**96**	6
MAPKAP-K1a	**30**	1	**6**	0	**58**	11	**39**	2
MAPKAP-K2	**60**	2	**75**	9	**84**	6	**72**	6
MSK1	**31**	5	**22**	2	**71**	9	**52**	2
PRAK	**58**	4	**72**	5	**75**	0	**68**	7
PKA	**87**	4	**67**	13	**101**	9	**65**	3
PKCa	**54**	1	**13**	1	**94**	1	**31**	0
PDK1	**53**	2	**8**	1	**86**	2	**75**	15
PKBΔph	**62**	2	**98**	1	**71**	15	**59**	4
SGK	**42**	5	**34**	1	**81**	7	**69**	11
S6K1	**14**	1	**108**	5	**71**	5	**50**	1
GSK3b	**14**	2	**5**	0	**77**	6	**2**	1
ROCK-II	**34**	9	**50**	9	**92**	14	**91**	6
AMPK	**47**	4	**12**	6	**84**	5	**73**	7
CHK1	**35**	4	**5**	0	**81**	2	**69**	6
CK2	**83**	10	**91**	3	**87**	11	**96**	2
PHOS.KINASE	**54**	2	**6**	2	**62**	2	**63**	2
Lck	**53**	1	**43**	2	**85**	10	**59**	11
CSK	**103**	2	**100**	11	**100**	2	**97**	11

CDK2/cyclin A	**62**	12	**31**	1	**101**	4	**31**	1
CK1	**62**	1	**88**	1	**91**	2	**84**	5
DYRK1a	**12**	3	**5**	1	**53**	4	**43**	7
NEK6	**28**	4	**86**	5	**103**	9	**65**	13
PP2A	**79**	16	**79**	19	**88**	1	**96**	2

The inhibitor concentration is 10 μM. The results are presented as kinase activity as a percentage of that in control incubations.

Figures 1-6 depict the differentiation results of compounds JS340, JS3343, and JS349, of murine embryonic stem (ES) cells by looking at the inhibition of green fluorescence protein (GFP), which is expressed from the muscle-specific myosin heavy chain-alpha promoter. ES cells were plated either undifferentiated, partially induced (d2 embryoid bodies, Ebs) or more completely induced (d4 Ebs). Figures 1 and 2 show the results for JS340. Figures 3 and 4 show the results for JS343, and Figures 5 and 6 show the results for JS349. Figures 1, 3, and 5 show brightfield images of dose responses of ligands. Mouse ES cells were either plated straight (naïve) or pre-differentiated by culturing in aggregates without leukemia inhibitory factor (LIF) for 2 or 4 days. Four days is particularly effective at promoting differentiation towards multiple lineages. JS343 maintained cells in smooth foci of tightly compacted cells (see Figure 3) and prevented cardiomyocyte differentiation as visualized by green fluorescence of GFP under transcriptional control of the myosin heavy chain alpha (MHCalpha) promoter (see Figure 4).

Wnt signaling is known to enhance proliferation and maintenance of certain stem cell populations, such as hematopoietic stem cells. Consistent with these data, our data show that blocking Wnt signaling initiates cardiogenesis in very early embryos (that is, in the absence of Wnt, GSK-3 is produced in the cell which promotes differentiation). The first column is Figures 2, 4, and 6 which represent untreated samples show this result through detection of the GFP. Inhibiting GSK-3 with a compound of the present invention promotes potency of the stem cells rather than differentiation (see the second and third columns of Figures 2, 4, and 6 where detection of GFP is less). Currently, the natural product protein LIF is used to maintain murine ES cells in totipotent state. LIF is costly and does not work on human ES cells, so an alternative means would have value, moreover if it were to work on human ES cells.

Figure 7 depicts the effect GSK-3 inhibiting compounds JS340, JS343, and JS349

have on the development of frog embryos. Consistent with the above discussion, one can see the retardation in development due to non-differentiation of the stem cells. The inhibitory effect compounds JS340, JS343, and JS349 have on GSK-3 maintains stem cell potency.

Dosages

The dosage of any compositions of the present invention will vary depending on the symptoms, age and body weight of the patient, the nature and severity of the disorder to be treated or prevented, the route of administration, and the form of the subject composition. Any of the subject formulations may be administered in a single dose or in divided doses. Dosages for the compositions of the present invention may be readily determined by techniques known to those of skill in the art or as taught herein.

In certain embodiments, the dosage of the subject compounds will generally be in the range of about 0.01 ng to about 10 g per kg body weight, specifically in the range of about 1 ng to about 0.1 g per kg, and more specifically in the range of about 100 ng to about 10 mg per kg.

An effective dose or amount, and any possible affects on the timing of administration of the formulation, may need to be identified for any particular composition of the present invention. This may be accomplished by routine experiment as described herein, using one or more groups of animals (preferably at least 5 animals per group), or in human trials if appropriate. The effectiveness of any subject composition and method of treatment or prevention may be assessed by administering the composition and assessing the effect of the administration by measuring one or more applicable indices, and comparing the post-treatment values of these indices to the values of the same indices prior to treatment.

The precise time of administration and amount of any particular subject composition that will yield the most effective treatment in a given patient will depend upon the activity, pharmacokinetics, and bioavailability of a subject composition, physiological condition of the patient (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage and type of medication), route of administration, and the like. The guidelines presented herein may be used to optimize the treatment, e.g., determining the optimum time and/or amount of administration, which will require no more than routine experimentation consisting of monitoring the subject and adjusting the dosage

and/or timing.

While the subject is being treated, the health of the patient may be monitored by measuring one or more of the relevant indices at predetermined times during the treatment period. Treatment, including composition, amounts, times of administration and formulation, may be optimized according to the results of such monitoring. The patient may be periodically reevaluated to determine the extent of improvement by measuring the same parameters. Adjustments to the amount(s) of subject composition administered and possibly to the time of administration may be made based on these reevaluations.

Treatment may be initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage may be increased by small increments until the optimum therapeutic effect is attained.

The use of the subject compositions may reduce the required dosage for any individual agent contained in the compositions because the onset and duration of effect of the different agents may be complimentary.

Toxicity and therapeutic efficacy of subject compositions may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} and the ED_{50}.

The data obtained from the cell culture assays and animal studies may be used in formulating a range of dosage for use in humans. The dosage of any subject composition lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For compositions of the present invention, the therapeutically effective dose may be estimated initially from cell culture assays.

Formulation

The compositions of the present invention may be administered by various means, depending on their intended use, as is well known in the art. For example, if compositions of the present invention are to be administered orally, they may be formulated as tablets, capsules, granules, powders or syrups. Alternatively, formulations of the present invention may be administered parenterally as injections (intravenous, intramuscular or subcutaneous), drop infusion preparations or suppositories. For application by the

ophthalmic mucous membrane route, compositions of the present invention may be formulated as eyedrops or eye ointments. These formulations may be prepared by conventional means, and, if desired, the compositions may be mixed with any conventional additive, such as an excipient, a binder, a disintegrating agent, a lubricant, a corrigent, a solubilizing agent, a suspension aid, an emulsifying agent or a coating agent.

In formulations of the subject invention, wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants may be present in the formulated agents.

Subject compositions may be suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal, aerosol and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of composition that may be combined with a carrier material to produce a single dose vary depending upon the subject being treated, and the particular mode of administration.

Methods of preparing these formulations include the step of bringing into association compositions of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association agents with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia), each containing a predetermined amount of a subject composition thereof as an active ingredient. Compositions of the present invention may also be administered as a bolus, electuary, or paste.

In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the subject composition is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or

any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the subject composition moistened with an inert liquid diluent. Tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the subject composition, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Suspensions, in addition to the subject composition, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan

esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing a subject composition with one or more suitable non-irritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the body cavity and release the active agent. Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for transdermal administration of a subject composition includes powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active component may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to a subject composition, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays may contain, in addition to a subject composition, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays may additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Compositions of the present invention may alternatively be administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the compound. A non-aqueous (e.g., fluorocarbon propellant) suspension could be used. Sonic nebulizers may be used because they minimize exposing the agent to shear, which may result in degradation of the compounds contained in the subject compositions.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or

suspension of a subject composition together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular subject composition, but typically include non-ionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise a subject composition in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

Kits

This invention also provides kits for conveniently and effectively implementing the methods of this invention. Such kits comprise any subject composition, and a means for facilitating compliance with methods of this invention. Such kits provide a convenient and effective means for assuring that the subject to be treated takes the appropriate active in the correct dosage in the correct manner. The compliance means of such kits includes any means which facilitates administering the actives according to a method of this invention. Such compliance means include instructions, packaging, and dispensing means, and combinations thereof. Kit components may be packaged for either manual or partially or wholly automated practice of the foregoing methods. In other embodiments involving kits, this invention contemplates a kit including compositions of the present invention, and

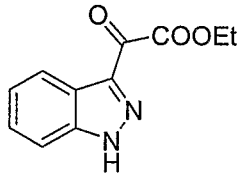
optionally instructions for their use.

Exemplification

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1

Ethyl indazolyl-3-glyoxylate



To a solution of ethyl 3-indazoleacetate (100 mg, 0.49 mmol) in dioxan (2 mL) was added Selenium dioxide (110 mg, 0.95 mmol) and then refluxed for 8 hours (monitored by GC-MS). The mixture was filtered and the filtrate evaporated *in vacou* to obtain a residue, which was purified by chromatography on silica gel employing 1:4 ethyl acetate:hexane as eluant to give the product as a light yellow solid (91 mg, 85%). ^{1}HNMR ($CDCl_3$, 300 MHz) 1.45 (t, J = 7.2 Hz, 3H), 4.51 (q, J = 7.2 Hz, 2H), 7.41 (t, J = 6.9 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.64 (d, J = 8.4 Hz, 1H), 8.35 (d, J = 8.1 Hz, 1H), 11.08 (bs, 1H) ^{13}CNMR ($CDCl_3$, 75 MHz) 13.9, 62.6, 111.0, 121.8, 122.6, 124.6, 127.9, 140.0, 141.1, 163.7, 180.8 MS (EI), *m/z*: 218 (M^+).

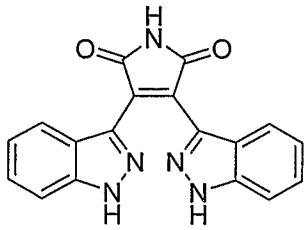
Example 2

General procedure for the preparation of maleimides

To a suspension of indazole-3-acetamide and methyl indole-3-acetamide in tetrahydrofuran at 0 °C was added 1.0M potassium *tert*-butoxide in tetrahydrofuran. The reaction was allowed to come to room temperature and stirred for 5 h. The reaction was quenched, extracted and purified (for conditions see individual examples).

Example 3

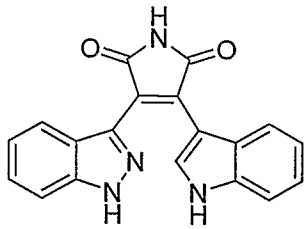
3,4-(3-Indazolyl)-1*H*-pyrrole-2,5-dione



The general procedure was followed using indazole-3-acetamide (20 mg, 0.12 mmol) and ethyl indazolyl-3-glyoxylate (30 mg, 0.14 mmol) in tetrahydrofuran with 1.0 M potassium *tert*-butoxide in tetrahydrofuran (0.35 mL, 0.34 mmol). The reaction was quenched with concentrate HCl, diluted with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, filtered and concentrate. The residue purified by column chromatography on silicagel using 1;19 methanol:chloroform as eluant to give the product as a dark yellow solid (31 mg, 84%). ^{1}HNMR (DMSO-*d6*, 300 MHz) 6.97 (t, J = 7.5 Hz, 1H), 7.24-7.33 (m, 2H), 7.53 (d, J = 8.4 Hz, 1H), 11.35 (s, 1H), 13.5 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 100.7, 111.6, 122.3, 123.0, 127.4, 141.8, 172.2, MS (EI), *m/z*: 329 (M^+).

Example 4

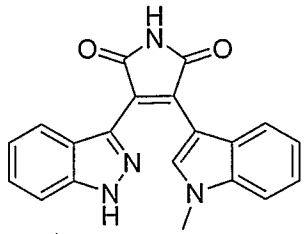
3-(3-Indazolyl)-4-(3-indolyl)-1*H*-pyrrole-2,5-dione



The general procedure was followed using indazole-3-acetamide (20 mg, 0.11 mmol) and methyl indolyl-3-glyoxylate (26 mg, 0.11 mmol) in tetrahydrofuran with 1.0 M potassium *tert*-butoxide in tetrahydrofuran (0.35 mL, 0.34 mmol). The reaction was quenched with concentrate HCl, diluted with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, filtered and concentrate. The residue purified by column chromatography on silicagel using 1;19 methanol:chloroform as eluant to give the product as a orange solid (30 mg, 81%). ^{1}HNMR (DMSO-*d6*, 300 MHz) 6.31 (d, J = 7.8 Hz, 1H), 6.60 (t, J = 7.2 Hz, 1H), 6.96-7.06 (m, 2H), 7.30-7.38 (m, 2H), 7.55 (d, J = 8.7 Hz, 1H), 8.12 (d, J = 3 Hz, 1H), 11.12 (s, 1H), 11.86 (s, 1H), 13.39 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 105.3, 110.4, 112.1, 119.9, 120.8, 121.5, 122.0, 122.6, 124.9, 126.3, 131.6, 136.5, 140.6, 172.2, 172.4. MS (EI), *m/z*: 328 (M^+).

Example 5

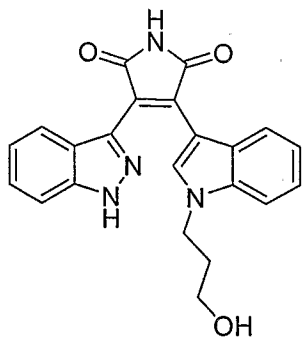
3-(3-Indazolyl)-4-[(1-methyl)-3-indolyl]-1*H*-pyrrole-2,5-dione



The general procedure was followed using indazole-3-acetamide (20 mg, 0.11 mmol) and ethyl (1-methylindolyl-3-glyoxylate (26 mg, 0.13 mmol) in tetrahydrofuran with 1.0 M potassium *tert*-butoxide in tetrahydrofuran (0.35 mL, 0.34 mmol). The reaction was quenched with concentrate HCl, diluted with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, filtered and concentrate. The residue purified by column chromatography on silicagel using 1;19 methanol:chloroform as eluant to give the product as a orange-red solid (32 mg, 82%). ^{1}HNMR (DMSO-*d6, 300 MHz*) 3.88 (s, 3H), 6.21 (d, J = 8.1 Hz, 1H), 6.62 (t, J = 8.1 Hz, 1H), 7.02-7.08 (m, 2H), 7.34 (t, J = 8.1 Hz, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.54-7.59 (m, 2H), 8.45 (s, 1H), 11.14 (s, 1H), 13.38 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 33.2, 104.3, 110.6, 120.3, 120.9, 121.5, 122.2, 125.3, 126.4, 135.3, 137.1, 172.2. MS (EI), *m/z*: 342 (M^+).

Example 6

3-(3-Indazolyl)-4-[1-(3-hydroxypropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione

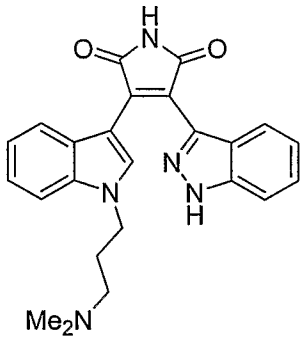


To a suspension of indazole-3-acetamide (185 mg, 0.37 mmol) and methyl 1-(3-O'-triphenylmethylpropyl)indole-3-acetamide (59 mg, 34 mmol) in tetrahydrofuran at 0 °C was added 1.0M potassium *tert*-butoxide in tetrahydrofuran (1.01 mL, 1.01 mmol). The reaction was allowed to come to room temperature and stirred for 5 h. The reaction was quenched

with concentrated HCl (0.5 mL), heated to reflux for 1 h and diluted with ethyl acetate. The organic layer was washed with water and saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, filtered and concentrate. The residue was purified by column chromatography on silicagel using 1:19 methanol:chloroform as eluant to give the product as a orange-red solid (120 mg, 91%). ^{1}HNMR (DMSO-*d6*, 300 MHz) 1.80-1.95 (m, 2H), 3.4-3.43 (m, 2H), 4.33 (t, J = 6.9 Hz, 2H), 4.68 (t, J = 4.8 Hz, 1H), 6.29 (d, J = 8.1 Hz, 1H), 6.64 (t, J = 7.5 Hz, 1H), 7.05 (t, J = 8.1 Hz, 2H), 7.34 (t, J = 8.4 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.54-7.59 (m, 3H), 8.15 (s, 1H), 11.14 (s, 1H), 13.41 (s, 1H) ^{13}CNMR (DMSO-*d6*, 75 MHz) 32.8, 43.1, 57.7, 104.5, 110.4, 110.6, 120.2, 120.9, 121.1, 121.5, 122.1, 122.5, 125.4, 126.4, 134.5, 172.1, 172.4 MS(EI) *m/z*: 386 (M^+).

Example 7

3-(3-Indazolyl)-4-[1-(3-dimethylaminopropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione

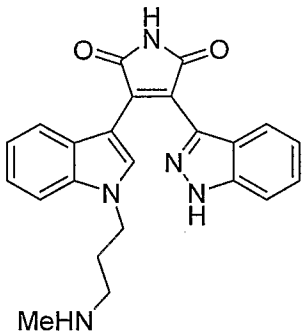


A suspension of 3-(3-Indazolyl)-4-[1-(3-hydroxypropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione (31 mg, 0.081 mmol) in dichloromethane (0.3 mL) was treated with pyridine (20 μL, 0.24 mmol) and methanesulfonyl chloride (7 μL, 0.089 mmol) and stirred for 4 h at room temperature. The reaction was then quenched with aqueous 1N HCl and the organic layer was washed with water, saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated to give the mesylate. A suspension of the mesylate in tetrahydrofuran (1 mL) was treated with 40% aqueous solution of dimethylamine (0.5 mL) to give a clear solution which was stirred at room temperature for 30 h. The reaction was diluted with ethyl acetate and the organic layer was washed with water. The solvent was removed in vacuo to give a residue which was purified by preparative TLC using 40:10:1 ethyl acetate:methanol:30% aqueous NH_3 to give the product as a orange-red solid (21 mg, 63%). ^{1}HNMR (DMSO-*d6*, 300 MHz) 1.87-1.92 (m, 2H), 2.14 (s, 6H), 3.32-3.38 (m, 2H),

4.27-4.32 (m, 2H), 6.31 (d, J = 8.1 Hz, 1H), 6.64 (t, J = 7.5 Hz, 1H), 6.9-7.1 (m, 2H), 7.34 (t, J = 8.4 Hz, 1H), 7.47-7.57 (m, 3H), 8.14 (s, 1H), 11.14 (s, 1H), 13.41 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 27.5, 29.1, 43.9, 45.2, 55.7, 104.4, 110.5, 120.1, 120.8, 121.1, 121.4, 122.1, 122.5, 123.1, 125.4, 126.3, 134.5, 136.3, 140.7, 172.1, 172.4.

Example 8

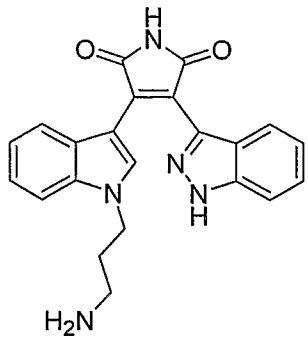
3-(3-Indazolyl)-4-[1-(3-methylaminopropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione



A suspension of 3-(3-Indazolyl)-4-[1-(3-hydroxypropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione (30 mg, 0.078 mmol) in dichloromethane (0.3 mL) was treated with pyridine (18 μL, 0.23 mmol) and methanesulfonyl chloride (7 μL, 0.093 mmol) and stirred for 4 h at room temperature. The reaction was then quenched with aqueous 1N HCl and the organic layer was washed with water, saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated to give the mesylate. A suspension of the mesylate in tetrahydrofuran (1 mL) was treated with 40% aqueous solution of methylamine (0.5 mL) to give a clear solution which was stirred at room temperature for 30 h. The reaction was diluted with ethyl acetate and the organic layer was washed with water. The solvent was removed in vacuo to give a residue which was purified by preparative TLC using 40:10:1 ethyl acetate:methanol:30% aqueous NH_3 to give the product as a orange-red solid (23 mg, 74%). ^{1}HNMR (CD_3OD, 300 MHz) 1.9-2.05 (m, 2H), 2.35 (s, 3H), 2.54 (t, J = 7.2 Hz, 2H), 4.3 (t, J = 6.6 Hz, 2H), 6.29 (d, J = 8.1 Hz, 1H), 6.56-6.61 (m, 1H), 6.89-6.94 (m, 1H), 7.00-7.05 (m, 1H), 7.28-7.39 (m, 3H), 7.52 (d, J = 8.4 Hz, 1H), 7.90 (s, 1H), 8.10 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 24.4, 30.5, 36.0, 45.6, 106.5, 111.2, 111.4, 121.6, 122.2, 122.5, 122.6, 122.8, 123.6, 124.0, 127.2, 128.0, 135.5, 136.1, 137.5, 138.0, 142.4, 172.9.

Example 9

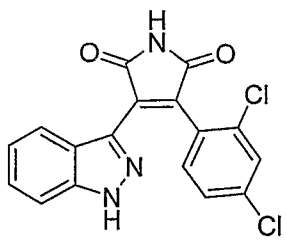
3-(3-Indazolyl)-4-[1-(3-dimethylaminopropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione



A suspension of 3-(3-Indazolyl)-4-[1-(3-hydroxypropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione (30 mg, 0.078 mmol) in dichloromethane (0.3 mL) was treated with pyridine (18 μL, 0.23 mmol) and methanesulfonyl chloride (7 μL, 0.093 mmol) and stirred for 4 h at room temperature. The reaction was then quenched with aqueous 1N HCl and the organic layer was washed with water, saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated to give the mesylate. A suspension of the mesylate in tetrahydrofuran (1 mL) was treated with 33% aqueous solution of ammonia (0.5 mL) to give a clear solution which was stirred at room temperature for 30 h. The reaction was diluted with ethyl acetate and the organic layer was washed with water. The solvent was removed in vacuo to give a residue which was purified by preparative TLC using 40:10:1 ethyl acetate:methanol:30% aqueous NH_3 to give the product as a orange-red solid (21 mg, 70%). ^{1}HNMR (DMSO-*d6*, 300 MHz) 2.07 (m, 2H), 2.74 (t, J = 7.2 Hz, 2H), 4.34 (t, J = 7.2 Hz, 2H), 6.27 (d, J = 8.1 Hz, 1H), 6.56-6.61 (m, 1H), 6.92-6.97 (m, 1H), 7.01-7.06 (m, 1H), 7.29-7.34 (m, 1H), 7.39-7.42 (m, 2H), 7.53 (d, J = 8.4 Hz, 1H), 8.09 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 32.4, 39.4, 45.2, 106.5, 111.2, 111.4, 121.7, 122.3, 122.5, 122.6, 123.6, 124.0, 127.3, 128.1, 135.30, 136.6, 138.0, 142.4, 173.8.

Example 10

3-(2,4-Dichloro-phenyl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione

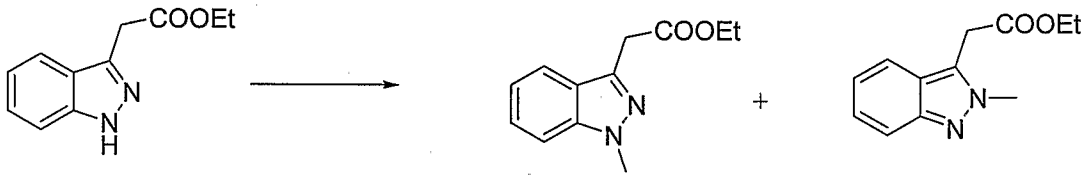


The general procedure was followed using ethyl (1-methylindolyl-3-glyoxylate (20 mg, 0.11 mmol) and 2-(2,4-dichloro-phenyl)-acetamide (26 mg, 0.11 mmol) in

tetrahydrofuran with 1.0 M potassium *tert*-butoxide in tetrahydrofuran (0.35 mL, 0.34 mmol). The reaction was quenched with concentrate HCl, diluted with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, filtered and concentrate. The residue purified by column chromatography on silicagel using 1:10 methanol:chloroform as eluant to give the product. ^{1}HNMR (DMSO-*d6*, 300 MHz) 7.18 (t, *J*=7.5 Hz, 1), 7.36-7.42 (m, 2H), 7.51-7.58 (m, 2H), 7.70(s, 1H), 7.88(d, *J*=8.4 Hz, 1H), 11.48 (s, 1H), 13.72 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 110.6, 121.6, 121.9, 122.0, 126.6, 127.2, 127.3, 128.4, 128.7 129.0, 132.0, 133.3, 133.5, 133.7, 134.5, 134.7, 140.8, 170.7, 170.8.

Example 11

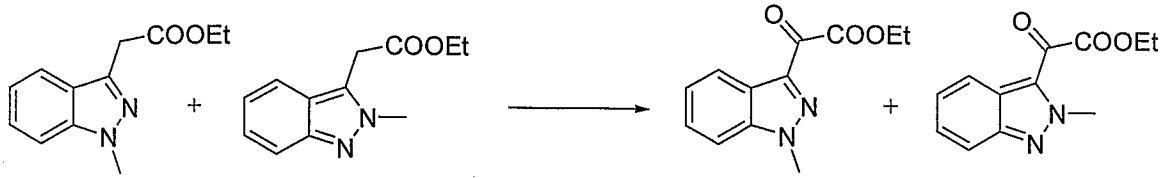
(1-Methyl-1*H*-indazol-3-yl)-acetic acid ethyl ester



To a solution of (1*H*-Indazol-3-yl)-acetic acid ethyl ester(200 mg, 0.98 mmol) in anhydrous dimethylformamide (2 mL) was added sodium hydride (45 mg of 60% suspension, 1.17 mmol), followed by iodomethane (0.12 mL, 1.96 mmol). After 15 min the reaction mixture was poured into ice-water; 1N Hcl was added to adjust the pH to about 4, and the solution was extracted with ethylacetate. The ethylacetate extract was washed with water, dried over anhydrous sodium sulfate and concentrated. The residue purified by column chromatography on silica gel using 4:1 hexane:ethylacetate to give the inseparable alkylated products (190 mg). The products were subjected to further reaction without separation (N1methyl:N2methyl = 5:1).

Example 12

(1-Methyl-1*H*-indazol-3-yl)-oxo-acetic acid ethyl ester

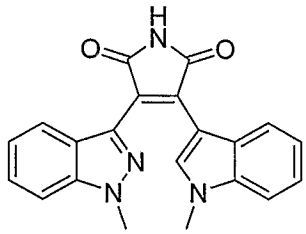


To a solution of (1-Methyl-1*H*-indazol-3-yl)-acetic acid ethyl ester (50 mg, 0.23 mmol) in dioxane (2 mL) was added selenium dioxide (50 mg, 0.46 mmol) and water (8

μL, 0.46 mmol). The reaction mixture was refluxed 7 h (monitored by GC-MS). The mixture was filtered and the filtrate evaporated *in vacou* to obtain a residue, which was purified by chromatography on silica gel employing 1:4 ethyl acetate:hexane as eluant to give the product as a solid (43 mg, 81%). ^{1}HNMR ($CDCl_3$, 300 MHz) 1.43 (t, *J*=7.5 Hz, 3H), 4.18 (s, 3H), 4.85 (q, *J*=7.5 Hz, 2H), 7.36-7.41 (m, 1H), 7.43-7.50 (m, 2H), 8.31 (d, *J*=8.1 Hz, 1H).

Example 13

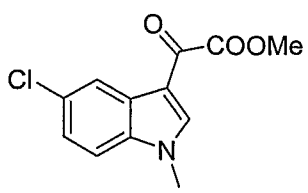
3-(1-Methyl-1H-indazol-3-yl)-4-(1-methyl-1H-indol-3-yl)-pyrrole-2,5-dione



The general procedure was followed using (1-Methyl-1*H*-indazol-3-yl)-oxo-acetic acid ethyl ester (30 mg, 0.11 mmol) and ethyl (1-methyl)indolyl-3-acetamide (20 mg, 0.13 mmol) in tetrahydrofuran with 1.0 M potassium *tert*-butoxide in tetrahydrofuran (0.35 mL, 0.34 mmol). The reaction was quenched with concentrate HCl, diluted with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, filtered and concentrate. The residue purified by column chromatography on silicagel using 1:19 methanol:chloroform as eluant to give the product as a orange-red solid (22 mg, 60%). ^{1}HNMR (DMSO-*d6*, 300 MHz) 3.90 (s, 3H), 3.98 (s, 3H), 6.20 (d, *J*=7.5 Hz, 1H), 6.67 (t, *J*=7.2 Hz, 1H), 7.06-7.11 (m, 2H), 7.37-7.46 (m, 2H), 7.57 (d, *J*=8.4 Hz, 1H), 7.68 (d, *J*=9.0 Hz,1H), 8.20 (s, 1H), 11.15 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 33.2, 35.8, 104.3, 110.0, 110.5, 120.0, 121.0, 121.3, 121.7, 122.2, 123.2, 125.2, 126.4, 134.5, 135.5, 137.0, 140.4, 172.1, 172.4.

Example 14

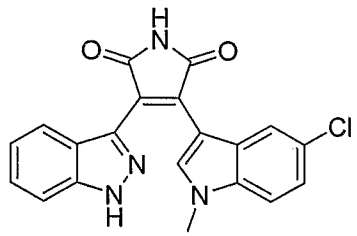
(5-Chloro-1-methyl-1H-indol-3-yl)-oxo-acetic acid methyl ester



A solution of 5-chloro-1-methyl-1H-indole (0.1 g, 0.65 mmol) in tetrahydrofuran (1 mL) was cooled to 0°C and oxalyl chloride (56 μL, 0.65 mmol) was added dropwise. The reaction was then stirred for 0.5 h at 0°C. It was then cooled to -60°C and 30% solution of sodium methoxide in methanol was added, after which the reaction mixture was allowed to warm to room temperature. The reaction was quenched by the addition of water and diluted with ethyl acetate. The organic layer was separated, dried over anhydrous sodium sulfate and concentrated. The residue purified by column chromatography using 1:9 ethyl acetate:hexane as eluant to obtain the product in a yield of 0.14 g (88%). ^{1}HNMR ($CDCl_3$, 300 MHz) 3.80 (s, 3H), 3.94 (s, 3H), 7.14-7.22 (m, 2H), 8.21 (s, 1H), 8.277 (d, *J*=1.5 Hz, 1H). ^{13}CNMR ($CDCl_3$, 75 MHz) 33.8, 52.7, 110.9, 112.0, 121.8, 124.2, 127.7, 129.2, 135.4, 141.0, 162.8, 176.4.

Example 15

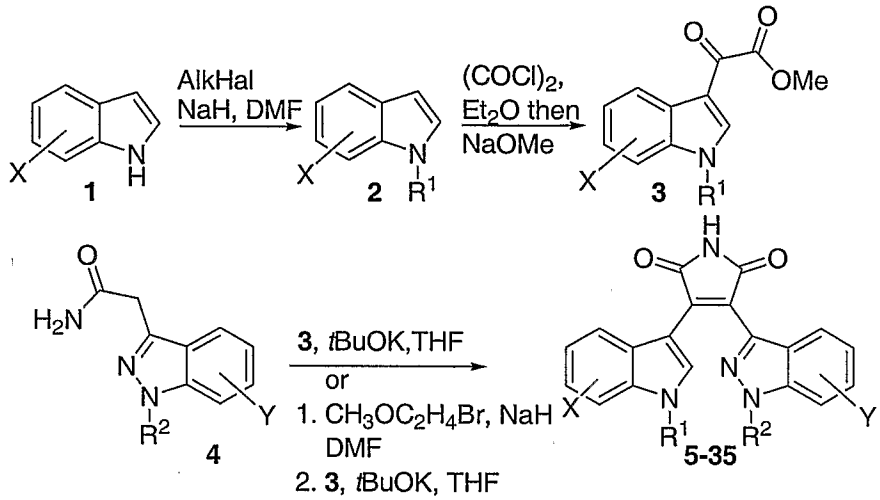
3-(5-Chloro-1-methyl-1H-indol-3-yl)-4-(1H-indazol-3-yl)-pyrrole-2,5-dione



The general procedure was followed using indazole-3-acetamide (20 mg, 0.11 mmol) and (5-Chloro-1-methyl-1H-indol-3-yl)-oxo-acetic acid methyl ester (32 mg, 0.13 mmol) in tetrahydrofuran with 1.0 M potassium *tert*-butoxide in tetrahydrofuran (0.35 mL, 0.34 mmol). The reaction was quenched with concentrate HCl, diluted with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, filtered and concentrate. The residue purified by column chromatography on silicagel using 1:19 methanol:chloroform as eluant to give the product as a orange-red solid (22 mg, 76%). ^{1}HNMR (DMSO-*d6*, 300 MHz) 3.88 (s, 3H), 6.12 (d, *J*=1.8 Hz, 1H), 7.03-7.09 (m, 2H), 7.36 (t, *J*=7.5 Hz, 1H), 7.47 (d, *J*=8.7 Hz, 1H), 7.56-7.60 (m, 2H), 8.21 (s, 1H), 8.315 (d, *J*=0.3 Hz, 1H), 11.18 (s, 1H), 13.49 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 33.4, 103.9, 110.4, 112.1, 120.5, 121.0, 121.3, 121.9, 122.6, 123.5, 124.9, 126.3, 126.5, 133.5, 135.5, 136.5, 140.7, 172.0, 172.2.

Example 16

Synthesis of Certain Compounds of the Invention



3-(Indol-3-yl)-4-(1H-indazol-3-yl)maleimide-based compounds **5-35** were prepared by condensation of the indolyl-3-glyoxylate esters **3** and the indazolyl-3-acetamides **4**. N-Alkylation of indoles **1** with various alkyl halides in the presence of sodium hydride followed by acylation of the resulting indoles **2** with oxalyl chloride and then ester formation afforded the precursors **3**. The required reaction partners, the indazolyl-3-acetamides **4**, were prepared from the appropriate *o*-nitrobenzaldehyde according to a known procedure. Appendage of an alkyl side chain onto the indazole ring (as in compound **11**) was achieved by treatment of the acetamides **4** with 2-bromoethyl methyl ether in the presence of sodium hydroxide followed by column chromatography.

General procedure for the preparation of maleimides ***5-35.*** The following method represents a typical procedure for the synthesis of the 3-indolyl-4-indazolylmalemide-based ligands. One of ordinary skill in the art will ready recognize how to apply the method to related starting materials to arrive the other compounds reported in this Example.

3-[5-Chloro-1-(2-methoxy-ethyl)-1*H*-indol-3-yl]-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (17).

To a solution of 5-chloro-indole (500 mg, 3.30 mmol) in dry DMF (6 mL) cooled with an ice bath was added NaH (55% suspension in mineral oil, 290 mg, 6.60 mmol), followed by 2-bromoethyl methyl ether (500 mg, 3.60 mmol) after which the reaction mixture was allowed to warm to room temperature. After 6 h the reaction mixture was poured into ice-water; 1N HCl was added to adjust the pH to about 4, and the solution was extracted with

ethyl acetate. The ethyl acetate extract was washed with water and brine, then dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was filtered through silica gel (ethyl acetate:hexane; 1:4). The product was subjected to further reaction without additional purification. To a solution of 5-chloro-1-(2-methoxy-ethyl)-1H-indole in Et_2O (10 mL) cooled to 0 °C a 1.0 M solution of oxalyl chloride in THF (6.60 mL) was added dropwise. The reaction was then stirred for 0.5 h at 0 °C and allowed to warm to room temperature and stirred overnight. It was then cooled to -60 °C and a 21% solution of NaOMe in MeOH (3.50 mL, 13.19 mmol) was added, after which the reaction mixture was allowed to warm to room temperature. The reaction was quenched by the addition of water and diluted with ethyl acetate. The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (ethyl acetate:hexane; 1:3) to give a product (570 mg, 58%). ^{1}H NMR ($CDCl_3$, 360 MHz) 3.28 (s, 3H), 3.67 (t, *J*=5.4 Hz, 2H), 3.94 (s, 3H), 4.24 (t, *J*=5.4 Hz, 2H), 6.42 (d, *J*=2.9 Hz, 1H), 7.13 (dd, *J*=2.0, 8.2 Hz, 1H), 7.16 (d, *J*=2.9 Hz, 1H), 7.25 (d, *J*=8.2 Hz, 1H), 7.57 (d, *J*=2.0, 1H).

The indazolyl-3-acetamides **(4)** were prepared from 2-nitrobenzaldehyde and 5-chloro-2-nitrobenzaldehyde.

To a suspension of indazolyl-3-acetamide **(4)** (Y = H, R^2=H; 42 mg, 0.24 mmol) and indolyl-3-glyoxylate **(3)** (X = 5-Cl, R^1 = 3-methoxyethyl; 92 mg, 0.31 mmol) in dry THF (2.5 mL) at 0 °C was added dropwise a 1.0 M solution of *tert*-BuOK in THF (1.0 mL), and the reaction mixture allowed to stir at room temperature overnight. The reaction mixture was quenched with 12 N HCl and diluted with EtOAc. The organic solution was washed with saturated $NaHCO_3$, brine, then dried over Na_2SO_4, evaporated *in vacuo* and purified by preparative TLC (MeOH:$CHCl_3$; 1:9) to afford product (53 mg, 53%) as an orange solid. ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.19 (s, 3H), 3.63 (t, *J*=5.4 Hz, 2H), 4.39 (t, *J*=5.4 Hz, 2H), 6.20 (d, *J*=8.6 Hz, 1H), 7.02-7.06 (m, 2H), 7.34 (t, *J*=7.2 Hz, 1H), 7.50-7.60 (m, 3H), 8.19 (s, 1H), 11.20 (s, 1H), 13.48 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 46.8, 58.9, 71.4, 104.8, 111.1, 113.0, 121.3, 121.6, 122.0, 122.5, 123.1, 124.5, 125.5, 127.0, 127.1, 134.6, 125.7, 136.6, 141.4, 172.6, 172.9. FAB-HRMS calcd for $C_{22}H_{17}ClN_4O_3 + Na^+$: 443.0887; found: 443.0872. HPLC purity: 96%.

3-(3-Indazolyl)-4-(3-indolyl)-1*H*-pyrrole-2,5-dione (5). ^{1}H NMR (DMSO-*d6*, 300 MHz) 6.31 (d, *J*=7.8 Hz, 1H), 6.60 (t, *J*=7.2 Hz, 1H), 6.96-7.06 (m, 2H), 7.30-7.38 (m, 2H), 7.55

(d, *J*=8.7 Hz, 1H), 8.12 (d, *J*=3.0 Hz, 1H), 11.12 (s, 1H), 11.86 (s, 1H), 13.39 (s, 1H). ^{13}CNMR (DMSO-*d6*, 75 MHz) 105.3, 110.4, 112.1, 119.9, 120.8, 121.5, 122.0, 122.6, 123.1, 124.9, 126.3, 131.6, 134.9, 135.7, 136.5, 140.6, 172.2, 172.4. FAB-HRMS calcd for $C_{19}H_{12}N_4O_2 + Na^+$: 351.0857; found: 351.0852. HPLC purity: 97%.

3-(3-Indazolyl)-4-[(1-methyl)-3-indolyl]-1*H*-pyrrole-2,5-dione (6). ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.88 (s, 3H), 6.21 (d, *J*=8.1 Hz, 1H), 6.62 (t, *J*=8.1 Hz, 1H), 7.02-7.08 (m, 2H), 7.34 (t, *J*=8.1 Hz, 1H), 7.43 (d, *J*=8.1 Hz, 1H), 7.54-7.59 (m, 2H), 8.45 (s, 1H), 11.14 (s, 1H), 13.38 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 33.2, 104.3, 110.6, 120.3, 120.9, 121.5, 122.2, 125.3, 126.4, 135.3, 137.1, 172.2, 172.4. FAB-HRMS calcd for $C_{20}H_{14}N_4O_2 + Na^+$: 365.1014; found: 365.1014. HPLC purity: 95%.

3-(3-1*H*-Indazol-3-yl)-4-[1-(3-trityloxy-propyl)-1*H*-indol-3-yl]-pyrrole-2,5-dione (7). ^{1}H NMR (DMSO-*d6*, 360 MHz) 1.80-1.95 (m, 2H), 3.4-3.43 (m, 2H), 4.33 (t, *J* =6.9Hz, 2H), 4.68 (t, *J*=4.8 Hz, 1H), 6.29 (d, *J*=8.1 Hz, 1H), 6.64 (t, *J*=7.5 Hz, 1H), 7.05 (t, *J*=8.1 Hz, 2H), 7.34 (t, *J*=8.4 Hz, 1H), 7.48 (d, *J*=8.4 Hz, 1H), 7.54-7.59 (m, 2H), 8.15 (s, 1H), 11.14 (s, 1H), 13.41 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 32.8, 43.1, 57.7, 104.5, 110.4, 110.6, 120.2, 120.9, 121.1, 121.5, 122.1, 122.5, 125.4, 126.4, 134.5, 172.1, 172.4. FAB-HRMS calcd for $C_{41}H_{32}N_4O_3 + Na^+$: 651.2372; found: 651.2386. HPLC purity: 98%.

3-(3-Indazolyl)-4-[1-(3-hydroxypropyl)-3-indolyl]-1*H*-pyrrole-2,5-dione (8). ^{1}H NMR (DMSO-*d6*, 360 MHz) 1.80-1.95 (m, 2H), 3.4-3.43 (m, 2H), 4.33 (t, *J*=6.9 Hz, 2H), 4.68 (t, *J*=4.8 Hz, 1H), 6.29 (d, *J*=8.1 Hz, 1H), 6.64 (t, *J*=7.5 Hz, 1H), 7.05 (t, *J*=8.1 Hz, 2H), 7.34 (t, *J*=8.4 Hz, 1H), 7.48 (d, *J*=8.4 Hz, 1H), 7.54-7.59 (m, 2H), 8.15 (s, 1H), 11.14 (s, 1H), 13.41 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 32.8, 43.1, 57.7, 104.5, 110.4, 110.6, 120.2, 120.9, 121.1, 121.5, 122.1, 122.5, 125.4, 126.4, 134.5, 172.1, 172.4. FAB-HRMS calcd for $C_{22}H_{18}N_4O_3 + Na^+$: 409.1276; found: 409.1270. HPLC purity: 96%.

3-(1*H*-indazol-3-yl)-4-[1-(2-methoxy-ethyl)-1*H*-indol-3-yl]-pyrrole-2,5-dione (9). ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.21 (s, 3H), 3.65 (t, *J*=5.0 Hz, 2H), 4.41 (t, *J*=5.0 Hz, 2H), 6.28 (d, *J*=7.9 Hz, 1H), 6.62 (t, *J*=7.2 Hz, 1H), 7.00-7.05 (m, 2H), 7.33 (t, *J*=7.2 Hz, 1H), 7.47-7.59 (m, 3H), 8.13 (s, 1H), 11.11 (s, 1H), 13.36 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 46.8, 58.9, 71.4, 104.8, 111.1, 113.0, 121.3, 121.6, 122.0, 122.5, 123.1, 124.5, 125.5, 127.0, 127.1, 134.6, 125.7, 136.6, 141.4, 172.6, 172.9. FAB-HRMS calcd for $C_{22}H_{18}N_4O_3 + Na^+$: 409.1285; found: 409.1276. HPLC purity: 95%.

3-(1-Methyl-1*H*-indazol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-pyrrole-2,5-dione (10). ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.90 (s, 3H), 3.98 (s, 3H), 6.20 (d, *J*=7.5 Hz, 1H), 6.67 (t, *J*=7.2 Hz, 1H), 7.06-7.11 (m, 2H), 7.37-7.46 (m, 2H), 7.57 (d, *J*=8.4 Hz, 1H), 7.68 (d, *J*=9.0 Hz, 1H), 8.20 (s, 1H), 11.15 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 33.2, 35.8, 104.3, 110.0, 110.5, 120.0, 121.0, 121.3, 121.7, 122.2, 123.2, 125.2, 126.4, 134.5, 135.5, 137.0, 140.4, 172.1, 172.4. FAB-HRMS calcd for $C_{21}H_{16}N_4O_2 + Na^+$: 379.1170; found: 379.1166. HPLC purity: 96%.

3-[1-(2-methoxy-ethyl)-1*H*-indazol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-pyrrole-2,5-dione (11). ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.14 (s, 3H), 3.51 (t, *J*=5.0 Hz, 2H), 3.88 (s, 3H), 4.48 (t, *J*=5.0 Hz, 2H), 6.24 (d, *J*=7.9 Hz, 1H), 6.64 (t, *J*=7.2 Hz, 1H), 7.04-7.09 (m, 2H), 7.35 (t, *J*=7.2 Hz, 1H), 7.43 (d, *J*=7.9 Hz, 1H), 7.59 (d, *J*=8.2 Hz, 1H), 7.67 (d, *J*=8.2 Hz, 1H), 8.20 (s, 1H), 11.13 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 33.8, 49.0, 56.6, 71.3, 104.9, 111.0, 111.1, 120.9, 121.6, 121.9, 122.4, 122.7, 122.8, 123.8, 125.8, 127.0, 135.1, 135.6, 136.2, 141.3, 172.8, 173.0. FAB-HRMS calcd for $C_{23}H_{20}N_4O_3 + Na^+$: 423.1433; found: 423.1432. HPLC purity: 96%.

3-[4-(1*H*-indazol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1-methyl-1*H*-indole-5-carbonitrile (12). ^{1}H NMR (DMSO-*d6*, 360 MHz) 6.75 (s, 1H), 7.05 (t, *J*=4.9 Hz, 1H), 7.31-7.42 (m, 2H), 7.53-7.60 (m, 3H), 8.28 (s, 1H), 12.34 (s, 1H), 13.57 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 102.3, 106.0, 110.7, 113.8, 120.3, 121.3, 121.6, 122.6, 124.9, 125.0, 125.5, 126.7, 126.8, 133.8, 134.1, 135.5, 138.6, 141.0, 172.1, 172.4, FAB-HRMS calcd for $C_{20}H_{11}N_5O_2 + Na^+$: 376.0811; found: 376.0801. HPLC purity: 95%.

3-(1*H*-Indazol-3-yl)-4-(1-methyl-5-nitro-1*H*-indol-3-yl)-pyrrole-2,5-dione (13). ^{1}H NMR (MeOD, 360 MHz) 3.32 (s, 3H), 7.05 (t, *J* = 6.9 Hz, 1H), 7.21 (d, *J* = 2.0 Hz, 1H), 7.34 (t, *J* = 6.9 Hz, 1H), 7.57 (m, 2H), 7.66 (d, *J* = 9.1 Hz), 7.95 (dd, *J* = 9.1 Hz, 2.0 Hz, 1H), 8.37 (s, 1H), 11.29 (s, 1H), 13.50 (s, 1H). ^{13}C NMR (MeOD, 75 MHz) 34.3, 107.0, 111.2, 111.9, 117.7, 118.9, 121.75, 122.0, 123.0, 125.2, 126.0, 127.17, 133.5, 135.8, 138.8, 141.5, 141.7, 172.4, 172.7. FAB-HRMS calcd for $C_{20}H_{13}N_5O_4 + Na^+$: 410.0865; found: 410.0862. HPLC purity: 97%.

3-(1*H*-Indazol-3-yl)-4-[1-(2-methoxy-ethyl)-5-nitro-1*H*-indol-3-yl]-pyrrole-2,5-dione (14). ^{1}H NMR (MeOD, 360 MHz) 3.97 (s, 3H), 3.74 (t, *J*=5.0 Hz, 2H), 4.47 (t, *J*=5.0 Hz, 1H), 6.93 (t, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 2.1 Hz, 1H), 7.28-7.36 (m, 2H), 7.53 (d, *J* = 8.2

Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 7.90 (d, J = 9.0 Hz), 8.31 (s, 1H). ^{13}C NMR (MeOD, 75 MHz) 47.0, 58.1, 71.2, 107.1, 110.5, 110.7, 117.2, 118.2, 120.9, 121.3, 122.7, 125.2, 126.9, 137.59, 140.0, 141.95, 172.2. FAB-HRMS calcd for $C_{22}H_{17}N_5O_5$ + Na^+: 454.1127; found: 454.1124. HPLC purity: 96%.

3-(5-Chloro-1-methyl-1*H*-indol-3-yl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (15). ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.87 (s, 3H), 6.19 (d, J=8.0 Hz, 1H), 6.65 (t, J=7.2 Hz, 1H), 7.06 (t, J=7.2 Hz, 1H), 7.35 (dd, J=1.8, 8.8 Hz, 1H), 7.44 (d, J=8.0 Hz, 1H), 7.59 (d, J=8.8 Hz, 1H), 7.70 (d, J=1.8 Hz, 1H), 8.13 (s, 1H), 11.14 (s, 1H), 13.53 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 33.8, 104.8, 1111.2, 112.9, 120.9, 121.5, 121.6, 122.8, 122.9, 124.0, 125.8, 125.9, 127.4, 135.2, 136.0, 136.2, 137.8, 139.9, 172.8, 173.0. FAB-HRMS calcd for $C_{20}H_{13}ClN_4O_2$ + Na^+: 399.0624; found: 399.0618. HPLC purity: 97%.

3-[5-Chloro-1-(3-hydroxy-propyl)-1*H*-indol-3-yl]-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (16). ^{1}H NMR (DMSO-*d6*, 360 MHz) 1.87 (m, 2H), 3.34 (t, J=6.8 Hz, 2H), 4.30 (t, J=6.8 Hz, 2H), 4.66 (bs, 1H), 6.31 (d, J=8.6 Hz, 1H), 6.70 (dd, J=1.8, 8.6 Hz, 1H), 7.02 (t, J=7.9 Hz, 1H), 7.32 (t, J=7.9 Hz, 1H), 7.54 (d, J=8.6 Hz, 2H), 8.13 (s, 1H), 11.15 (s, 1H), 13.43 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 33.4, 43.8, 58.3, 106.3, 111.2, 120.9, 121.6, 122.2, 123.0, 123.1, 124.8, 124.9, 127.0, 127.6, 134.3, 135.8, 136.0, 137.5, 141.4, 172.8, 173.0. FAB-HRMS calcd for $C_{22}H_{17}ClN_4O_3$ + Na^+: 443.0887; found: 443.0887. HPLC purity: 95%.

3-(5-Flouro-1*H*-indol-3-yl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (18). ^{1}H NMR (DMSO-*d6*, 400 MHz) 5.93 (dd, J=2.0, 11.2 Hz, 1H), 6.85 (ddd, J=2.0, 9.0, 11.2 Hz, 1H), 7.02 (t, J=8.0 Hz, 1H), 7.33-7.39 (m, 2H), 7.55-7.59 (m, 2H), 8.19 (s, 1H), 11.16 (s, 1H), 11.98 (s, 1H), 13.48 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 105.6, 105.7, 106.1, 106.3, 110.3, 110.6, 110.7, 113.3, 113.4, 121.2, 121.7, 122.8, 123.6, 125.7, 125.8, 126.7, 133.4, 133.5, 134.9, 135.9, 140.9, 156.1, 158.4, 172.3, 172.6. FAB-HRMS calcd for $C_{19}H_{11}FN_4O_2$ + H^+: 347.0944; found: 347.0952. HPLC purity: 97%.

3-(5-Flouro-1-methyl-1*H*-indol-3-yl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (19). ^{1}H NMR (DMSO-*d6*, 400 MHz) 3.88 (s, 3H), 5.87 (dd, J=2.4, 9.0 Hz, 1H), 6.92 (td, J=2.0, 9.0 Hz, 1H), 7.06 (t, J=7.6 Hz, 1H), 7.36 (t, J=7.6 Hz, 1H), 7.45 (dd, J=4.5, 9.0 Hz, 1H), 7.58 (m, 2H), 8.22 (s, 1H), 11.15 (s, 1H), 13.47 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 31.7, 105.6, 105.7, 106.1, 106.3, 110.3, 110.6110.7, 113.3, 113.4, 121.2, 121.7, 122.8, 123.6,

125.7, 125.8, 126.7, 133.4, 133.5, 134.9, 135.9, 140.9, 156.1, 158.4, 172.3, 172.6. FAB-HRMS calcd for $C_{20}H_{13}FN_4O_2 + Na^+$: 383.0921; found: 383.0923. HPLC purity: 96%.

3-[5-Fluoro-1-(2-methoxy-ethyl)-1*H*-indol-3-yl]-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (20). ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.36 (s, 3H), 3.65 (t, *J*=5.0 Hz, 2H), 4.42 (t, *J*=5.0 Hz, 2H), 5.91 (dd, *J*=2.5, 9.0 Hz, 1H), 6.91 (td, *J*=2.5, 9.0 Hz, 1H), 7.06 (t, *J*=7.5 Hz, 1H), 7.35 (t, *J*=7.5 Hz, 1H), 7.51 (q, *J*= 9.0 Hz, 1H), 7.56-7.59 (m, 2H), 8.21 (s, 1H), 11.16 (s, 1H), 13.45 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 46.9, 58.9, 71.4, 105.2, 105.3, 106.7, 107.0, 110.6, 110.9, 111.1, 112.5, 112.6, 121.6, 122.1, 123.1, 124.0, 126.4, 126.5, 127.1, 133.9, 134.7, 136.2, 136.9, 141.3, 156.5, 159.1, 172.7, 172.9. FAB-HRMS calcd for $C_{22}H_{17}FN_4O_3 + Na^+$: 427.1182; found: 427.1183. HPLC purity: 97%.

3-(5-Bromo-1*H*-indol-3-yl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (21). ^{1}H NMR (DMSO-*d6*, 400 MHz) 6.36 (d, *J*=1.4 Hz, 1H), 7.05 (t, *J*=7.5 Hz, 1H), 6.11 (dd, *J*=1.5, 8.5 Hz, 1H), 7.33-7.37 (m, 2H), 7.55 (d, *J*= 8.5 Hz, 1H), 7.58 (d, *J*= 8.5 Hz, 1H), 8.17 (s, 1H), 11.16 (s, 1H), 12.02 (s, 1H), 13.50 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 105.1, 110.7, 112.9, 114.2, 121.2, 121.5, 122.8, 123.8, 124.1, 124.7, 126.7, 126.9, 133.0, 134.8, 135.4, 135.8, 141.0, 172.3, 172.5. FAB-HRMS calcd for $C_{19}H_{11}BrN_4O_2 + Na^+$: 428.9963; found: 428.9955. HPLC purity: 98%.

3-(5-Bromo-1-methyl-1*H*-indol-3-yl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (22). ^{1}H NMR (DMSO-*d6*, 400 MHz) 3.87 (s, 3H), 6.25 (d, *J*=1.4 Hz, 1H), 7.06 (t, *J*=7.2 Hz, 1H), 7.17 (dd, *J*=1.4, 8.6 Hz, 1H), 7.36 (t, *J*=7.2 Hz, 1H), 7.42 (d, *J*=8.6 Hz, 1H), 7.58 (m, 2H), 8.20 (s, 1H), 11.18 (s, 1H), 13.49 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 33.6, 104.1, 110.7, 112.8, 113.3, 121.2, 121.5, 122.9, 123.9, 124.0, 124.7, 126.6, 127.2, 134.3, 135.8, 136.0, 136.6, 141.0, 172.3, 172.5. FAB-HRMS calcd for $C_{20}H_{13}BrN_4O_2 + Na^+$: 443.0120; found: 443.0126. HPLC purity: 97%.

3-(5-Cloro-1*H*-indazol-3-yl)-4-[5-fluoro-1-(2-methoxy-ethyl)-1*H*-indol-3-yl]-pyrrole-2,5-dione (23). ^{1}H NMR (MeOD, 360 MHz) 3.32 (s, 3H), 3.74 (t, *J*=5.0 Hz, 2H), 4.33 (t, *J*=5.0 Hz, 2H), 5.99 (dd, *J*=2.5, 9.0 Hz, 1H), 6.82 (td, *J*=2.5, 9.0 Hz, 1H), 7.22-7.27 (m, 2H), 7.35 (dd, *J*=0.7, 1.8 Hz, 1H), 7.46 (dd, *J*= 0.7, 9.0 Hz, 1H), 8.11 (s, 1H). ^{13}C NMR (MeOD), 75 MHz) 47.0, 59.0, 71.0, 105.0, 105.1, 106.7, 107.0, 110.6, 110.7, 111.0, 111.6, 119.7, 120.6, 122.2, 123.4, 126.2, 126.3, 126.9, 127.4, 133.2, 134.5, 135.3, 135.8, 139.4, 156.3, 159.3, 171.7, 171.9. FAB-HRMS calcd for $C_{22}H_{16}ClFN_4O_3 + Na^+$: 461.0792; found:

461.0790. HPLC purity: 96%.

3-(5-Benzyloxy-1-methyl-1*H*-indol-3-yl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (24). ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.88 (s, 3H), 3.94 (s, 2H), 5.58 (d, J = 2.4 Hz, 1H), 6.73 (dd, J = 8.7 Hz, 2.4 Hz, 1H), 7.06-7.15 (m, 3H), 7.23-7.39 (m, 5H), 7.59 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 8.4 Hz), 8.14 (s, 1H), 11.12 (s, 1H), 13.43 (s, 1H). FAB-HRMS calcd for $C_{27}H_{20}N_4O_3 + Na^+$: 471.1433; found: 471.1421. HPLC purity: 97%.

3-[5-Benzyloxy-1-(3-hydroxy-propyl)-1*H*-indole-3-yl]-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (25). ^{1}H NMR (MeOD, 360 MHz) 2.06 (m, 2H), 3.58 (t, J=6.8 Hz, 2H), 3.94 (s, 2H), 4.34 (t, J=6.8 Hz, 2H), 5.69 (s, 1H), 6.70 (dd, J=2.4, 8.6 Hz, 1H), 7.03-7.11 (m, 3H), 7.23-7.38 (m, 5H), 7.52-7.57 (m, 2H), 8.13 (s, 1H). ^{13}C NMR (MeOD, 75 MHz) 32.7, 43.4, 58.4, 69.4, 104.0, 105.0, 111.0, 113.5, 121.3, 121.6, 123.3, 126.5, 127.2, 127.3, 127.6, 128.2, 132.1, 134.9, 135.9, 137.4, 153.9, 172.8, 172.9. FAB-HRMS calcd for $C_{29}H_{24}N_4O_4 + Na^+$: 515.1695; found: 515.1704. HPLC purity: 98%.

3-[5-Benzyloxy-1-(2-methoxy-ethyl)-1*H*-indole-3-yl]-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (26). ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.11 (s, 3H), 3.65 (t, J=5.0 Hz, 2H), 3.91 (s, 2H), 4.39 (t, J=5.0 Hz, 1H), 6.68 (dd, J=2.5, 9.0 Hz, 1H), 7.02-7.04 (m, 2H), 7.09-7.13 (m, 2H), 7.22-7.40 (m, 6H), 7.56 (d, J=8.4 Hz, 1H), 7.68 (d, J=8.2 Hz, 1H), 8.11 (s, 1H), 11.10 (s, 1H), 13.41 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 46.8, 58.9, 69.1, 71.5, 104.5, 105.0, 121.6, 122.2, 122.5, 123.5, 127.2, 128.0, 128.2, 128.7, 128.8, 129.0, 132.3, 135.4,135.8, 137.5, 141.5, 153.5, 172.9, 173.0. FAB-HRMS calcd for $C_{29}H_{24}N_4O_4 + Na^+$: 515.1695; found: 515.1700. HPLC purity: 98%.

3-[5-Benzyloxy-1-(3-methoxy-ethyl)-1*H*-indole-3-yl]-4-(5-chloro-1*H*-indazol-3-yl)-pyrrole-2,5-dione (27). ^{1}H NMR (MeOD, 360 MHz) 3.32 (s, 3H), 3.74 (t, J=5.0 Hz, 2H), 4.06 (s, 2H), 4.38 (t, J=5.0 Hz, 2H), 5.68 (2, J=2.3, 1H), 6.73 (dd, J=2.3, 8.6 Hz, 1H), 7.08-7.21 (m, 2H), 7.22-7.33 (m, 5H), 7.50-7.53 (m, 2H), 8.13 (s, 1H). ^{13}C NMR (MeOD, 75 MHz) 58.2, 69.5, 71.3, 104.0, 104.9, 111.1, 111.6, 113.5, 120.9, 127.1, 127.6, 127.7, 128.2, 132.3, 135.5, 137.3, 153.9, 172.6, 172.7. FAB-HRMS calcd for $C_{29}H_{23}N_4O_4 + Na^+$: 549.1306; found: 549.1309. HPLC purity: 96%.

3-(1*H*-Indazol-3-yl)-4-(1-methyl-6-nitro-1*H*-indol-3-yl)-pyrrole-2,5-dione (28). ^{1}H NMR (MeOD, 360 MHz) 4.00 (s, 3H), 6.50 (d, J=9.0 Hz, 1H), 7.05 (t, J = 7.9 Hz, 1H), 7.33 (t, J = 7.9 Hz, 1H), 7.48-7.57 (m, 2H), 7.62 (d, J = 8.3 Hz, 1H), 8.41 (s, 1H), 8.45 (s,

1H). ^{13}C NMR (MeOD, 75 MHz) 34.3, 105.6, 108.3, 111.2, 115.4, 121.8, 121.9, 122.3, 122.9, 126.6, 127.1, 1130.7, 133.4, 135.8, 136.3, 140.8, 141.4, 143.0, 172.7, 172.9. FAB-HRMS calcd for $C_{20}H_{13}N_5O_4 + Na^+$: 410.0865; found: 410.0865. HPLC purity: 97%.

3-(6-Chloro-1-methyl-1*H*-indol-3-yl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (29). ^{1}H NMR (MeOD, 360 MHz) 3.82 (s, 3H), 6.12 (d, *J*=8.6 Hz, 1H), 6.51 (dd, *J*=1.8, 8.6, 1H), 6.96 (t, *J*=7.5 Hz, 1H), 7.31-7.40 (m, 3H), 7.53 (d, *J*=8.6 Hz, 1H), 8.04 (s, 1H). (MeOD, 75 MHz) 32.5, 105.1, 110.0, 110.2, 120.6, 121.2, 121.3, 122.1, 124.5, 127.0, 128.3, 136.0, 138.2, 272.0, 172.1. FAB-HRMS calcd for $C_{20}H_{13}ClN_4O_2 + Na^+$: 399.0625; found: 399.0627. HPLC purity: 96%.

3-(6-Flouro-1*H*-indol-3-yl)-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (30). ^{1}H NMR (DMSO-*d6*, 360 MHz) 6.31 (dd, *J*=4.1, 6.8 Hz, 1H), 6.50 (ddd, *J*=1.8, 7.2, 8.8 Hz, 1H), 7.02 (t, *J*=5.6 Hz, 1H), 7.16 (dd, *J*=1.8, 7.2 Hz, 1H), 7.32 (t, *J*=5.6 Hz, 1H), 7.54 (m, 2H), 8.09 (s, 1H), 11.14 (s, 1H), 11.88 (s, 1H), 13.42 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 98.3, 98.6, 105.6, 108.4, 108.7, 110.7, 121.2, 121.8, 121.9, 123.1, 124.1, 126.6, 132.4, 134.7, 135.8, 136.7, 136.8, 140.9, 172.3, 172.6. FAB-HRMS calcd for $C_{19}H_{11}FN_4O_2 + Na^+$: 369.0764; found: 369.0774. HPLC purity: 95%.

3-(5-Cloro-1*H*-indazol-3-yl)-4-[1-(2-methoxy-ethyl)-6-methyl-1*H*-indol-3-yl]-pyrrole-2,5-dione (31). ^{1}H NMR (MeOD, 360 MHz) 2.30 (s, 1H), 3.31 (s, 3H), 3.71 (t, *J*=5.4 Hz, 2H), 4.26 (t, *J*=5.4 Hz, 2H), 6.27 (d, *J*=8.2 Hz, 1H), 6.51 (d, *J*=8.2 Hz, 1H), 7.05 (s, 1H), 7.21 (dd, *J*=1.6, 9.0 Hz, 1H), 7.36 (d, *J*=1.6 Hz, 1H), 7.43 (d, *J*=9.0 Hz, 1H), 8.00 (s, 1H), 8.51 (br s, 1H). ^{13}C NMR (MeOD), 75 MHz) 22.0, 47.0, 59.4, 71.2, 105.4, 110.1, 110.2, 112.1, 121.3, 123.6, 123.7, 123.9, 127.3, 127.6, 128.0, 133.1, 134.8, 137.5, 139.7, 171.3, 171.4. FAB-HRMS calcd for $C_{23}H_{19}ClN_4O_3 + Na^+$: 457.1043; found: 457.1057. HPLC purity: 97%.

3-[6-Benzyloxy-1-(2-methoxy-ethyl)-1*H*-indole-3-yl]-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (32). ^{1}H NMR (MeOD, 360 MHz) 3.28 (s, 3H), 3.69 (t, *J*=5.4 Hz, 2H), 4.32 (t, *J*=5.4 Hz, 2H), 5.01 (s, 2H), 6.10 (d, *J*=8.6 Hz, 1H), 6.31 (dd, *J*=2.1, 8.6 Hz, 1H), 6.95 (t, *J*=7.5 Hz, 1H), 6.97 (d, *J*=2.1 Hz, 1H), 7.25-7.39 (m, 7H), 7.53 (d, *J*=8.6 Hz, 1H), 8.01 (s, 1H). ^{13}C NMR (MeOD, 75 MHz) 46.7, 58.8, 67.9, 71.5, 104.3, 106.0, 121.6, 122.4, 122.5, 123.2, 127.5, 128.0, 128.2, 128.6, 128.8, 129.0, 132.6, 135.4,135.8, 138.0, 141.5, 153.7, 172.8, 172.9. FAB-HRMS calcd for $C_{29}H_{24}N_4O_4 + Na^+$: 515.1695; found: 515.1690. HPLC purity:

98%.

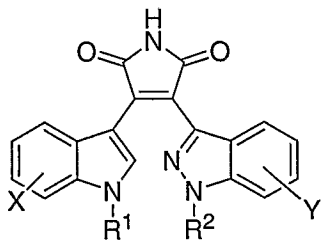
3-[7-Benzyloxy-1-(2-methoxy-ethyl)-1*H*-indole-3-yl]-4-(1*H*-indazol-3-yl)-pyrrole-2,5-dione (33). ^{1}H NMR (MeOD, 360 MHz) 3.29 (s, 3H), 3.62 (t, *J*=5.4 Hz, 2H), 4.58 (t, *J*=5.4 Hz, 2H), 5.13 (s, 2H), 5.90 (d, *J*=7.8 Hz, 1H), 6.47 (t, *J*=7.8 Hz, 1H), 6.64 (d, *J*=7.8 Hz, 1H), 7.28-7.52 (m, 8H), 7.92 (s, 1H). ^{13}C NMR (MeOD, 75 MHz) 42.5, 58.6, 70.0, 105.0, 105.9, 111.0, 113.7, 121.1, 121.6, 123.3, 126.0, 127.2, 127.3, 128.0, 128.2, 132.1, 135.0, 135.9, 137.6, 153.2, 172.7, 172.8. FAB-HRMS calcd for $C_{29}H_{24}N_4O_4 + H^+$: 493.1876; found: 493.1882. HPLC purity: 98%.

3-(5-Chloro-1*H*-indazol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-pyrrole-2,5-dione (34). ^{1}H NMR (DMSO-*d6*, 360 MHz) 3.87 (s, 3H), 6.29 (d, *J*=8.0 Hz, 1H), 6.65 (t, *J*=7.2 Hz, 1H), 7.06 (t, *J*=7.2 Hz, 1H), 7.35 (dd, *J*=1.9, 8.6 Hz, 1H), 7.44 (d, *J*=8.0 Hz, 1H), 7.59 (d, *J*=8.8 Hz, 1H), 7.70 (d, *J*=1.9 Hz, 1H), 8.13 (s, 1H), 11.14 (s, 1H), 13.53 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 33.8, 104.8, 111.2, 112.9, 120.9, 121.5, 121.6, 122.8, 122.9, 124.0, 125.8, 125.9, 127.4, 135.3, 136.0, 136.2, 137.8, 139.9, 172.8, 173.0. FAB-HRMS calcd for $C_{20}H_{13}ClN_4O_2 + Na^+$: 399.0625; found: 399.0631. HPLC purity: 96%.

3-(5-Chloro-1*H*-indazol-3-yl)-4-[1-(3-hydroxy-propyl)-1*H*-indol-3-yl]-pyrrole-2,5-dione (35). ^{1}H NMR (DMSO-*d6*, 360 MHz) 1.80 (m, 2H), 3.39 (m, 2H), 4.32 (m, 2H), 6.25 (d, *J*=8.0 Hz, 1H), 6.64 (t, *J*=7.5 Hz, 1H), 7.04 (t, *J*=7.5 Hz, 1H), 7.33 (d, *J*=8.6 Hz, 1H), 7.48 (d, *J*=8.0 Hz, 2H), 7.56-7.61 (m, 2H), 8.13 (s, 1H), 11.14 (s, 1H), 13.56 (s, 1H). ^{13}C NMR (DMSO-*d6*, 75 MHz) 33.5, 43.7, 58.35, 105.0, 111.3, 112.9, 120.9, 121.5, 121.7, 122.8, 123.1, 124.0, 125.9, 126.0, 127.3, 135.2, 136.2, 237.0, 139.8, 172.7, 172.9. FAB-HRMS calcd for $C_{22}H_{17}ClN_4O_3 + Na^+$: 443.0887; found: 443.0903. HPLC purity: 97%.

Example 17

In Vitro Inhibition of GSK-3



5-35

The maleimides (**5-35**) of the previous Example were screened for their potency to inhibit GSK-3β. Briefly, recombinant human His6-GSK-3β (53 nM) produced in our laboratories or commercially available human GSK-3β (21 nM) was assayed for its ability to phosphorylate the pGS peptide substrate (RRRPASVPPSPSLSRHSSHQRR; 10 μM) in the presence of 0-50 μM of the maleimides. For comparison purposes, we also determined the K_i values of the known GSK-3β inhibitors SB-415286 and SB-216763. The K_i values are tabulated below.

GSK-3β inhibition by substituted maleimides, the SB ligands, and LiCl.

Compound	**X**	**Y**	**R^1**	**R^2**	**IC_{50} (μM)**	**K_i (nM)**
LiCl					2,000	3.33 $\times 10^6$
SB-415286					1.3	180
SB-216763					0.050	35
bis-indole-maleimide					1.3	225 *
5	H	H	H	H	12.0	2,250 *
6	H	H	CH_3	H	2.6	433
7	H	H	$(CH_2)_3OTr$	H	27	4,500
8	H	H	$(CH_2)_3OH$	H	1.8	300
9	H	H	$(CH_2)_2OCH_3$	H	2.5	417
10	H	H	CH_3	CH_3	0.120	80
11	H	H	CH_3	$(CH_2)_2OCH_3$	0.049	33
12	CN	H	H	H	0.081	54
13	5-NO_2	H	CH_3	H	0.46	57 *
14	5-NO_2	H	$(CH_2)_2OCH_3$	H	0.052	35
15	5-Cl	H	CH_3	H	1.1	183
16	5-Cl	H	$(CH_2)_3OH$	H	5.0	833
17	5-Cl	H	$(CH_2)_2OCH_3$	H	0.38	41 *
18	5-F	H	H	H	0.0114	7.6
19	5-F	H	CH_3	H	0.049	33
20	5-F	H	$(CH_2)_2OCH_3$	H	0.036	24
21	5-Br	H	H	H	0.850	567
22	5-Br	H	CH_3	H	0.0035	2.3

23	5-F	5-Cl	$(CH_2)_2OCH_3$	H	0.165	110
24	5-OBn	H	CH_3	H	1.2	200
25	5-OBn	H	$(CH_2)_3OH$	H	0.100	67
26	5-OBn	H	$(CH_2)_2OCH_3$	H	0.130	87
27	5-OBn	5-Cl	$(CH_2)_2OCH_3$	H	0.750	500
28	6-NO_2	H	CH_3	H	1.3	200
29	6-Cl	H	CH_3	H	3.1	517
30	6-F	H	H	H	0.535	357
31	6-CH_3	5-Cl	$(CH_2)_2OCH_3$	H	2.4	1,600
32	6-OBn	H	$(CH_2)_2OCH_3$	H	0.450	300
33	7-OBn	H	$(CH_2)_2OCH_3$	H	0.400	267
34	H	5-Cl	CH_3	H	3.1	517
35	H	5-Cl	$(CH_2)_3OH$	H	3.6	600

*K_i values are the average of experiments performed at both 10 and 100 μM ATP. The apparent equilibrium dissociation constants of the inhibitors (K_is) were estimated using the Cheng-Prusoff equation and a K_m for ATP equal to 20 μM.

Example 18

Kinase Assays

Kinase assays were performed essentially as described by Welsh and coworkers. GSK-3 activity was measured as the ability to transfer [γ^{32}P] from [γ^{32}P]-ATP to the primed Glycogen Synthase peptide substrate (RRRPASVPPSPSLSRHSSHQRR, where the **S** is the designated primed phosphoserine). The ability of recombinant human His_6-GSK-3β (His_6-GSK-3β/pET29b, 4-53 nM, or 21 nM, EMD Biosciences) to phosphorylate the pGSM peptide substrate (RRRPASVPPSPSLSRHSSHQRR with the priming phosphoserine underlined, 10 μM final concentration) was assayed in the presence of 10 μM or 100 μM ATP (specific activity 1.3μCi of [γ-^{32}P] ATP/nM). After incubation for 30 minutes at 30°C, 25 μl of the samples were spotted on 2.5 cm P81 Whatman filters, dried for 30 seconds and immediately transferred into a beaker containing 0.75% phosphoric acid. The filters were dried and counted in 3 mL ScintiSafe (FisherScientific, Hanover Park, IL) cocktail in a Beckman LS6000IC scintillation counter (Beckman Coulter, Fullerton, CA).

Example 19

Whole Cell Assays

Cell culture and transfection

Human neuroblastoma SH-SY5Y cells stably transfected with human wild-type *α-Syn* cDNA (SHα-Syn) were grown in DMEM/F12 medium, containing 10% FBS, 2 mM L-glutamine, 100U/ml penicillin, and 100μg/ml streptomycin. Cells were transiently transfected with human dopamine transporter (hDAT; 2 μg DNA /1.0 x 10^5 cells for 12 well dishes and 4 μg DNA /1.2 x 10^6 cells for 6- well dishes) at 80 % confluency by the Lipofectamine reagent, accordingly to manufacture's protocol (Invitrogen), and further grown for 48 hours after transfection to allow expression of the transgenes.

Primary mesencephalic neuronal cultures were prepared from the ventral mesencephalon of gestational 16–18-day-old rat embryos, and grown in Neurobasal medium (Invitrogen) supplemented with 2 % (vol/vol) B27 Supplement (Invitrogen), 10 U/ml of a mixture of penicillin/streptomycin and 25 μM of β-mercaptoethanol at 37 °C and 5% CO_2.

Cell treatment

MPP^+ iodide was prepared at a concentration of 50 μM, and then added directly to the medium in the 6-or 12 well-dishes. Cells were exposed to MPP^+ for 48 h. Concentrated stock solutions of chemically synthesized inhibitors of GSK-3β (**14**, **16**, **8**, **19**, **18** and **22**) were prepared in DMSO at a concentration of 10 mM. On the day of each experiment, each inhibitor was added to the wells to a final concentration of 1 μM. GSK-3 inhibitors were applied for the last 16 hours of MPP^+ treatment.

Sample preparation and immunoblot analysis

Due different biochemical properties of analyzed proteins, two separate protocols were used to check protein expression levels of α-Syn and GSK-3β (protocol I) and phosphorylation pattern of Tau (protocol II) by Western blot.

Protocol I. Tissue or cells were collected by gentle scraping, washed three times with DPBS, and lysed in buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EDTA) containing 0.1% Nonidet P-40, 0.1% Triton X100, 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail tablets (Complete Mini, EDTA-free (Roche

Diagnostics GmbH, Germany). The cellular material was left for 20 min on ice. The lysate was then centrifuged for 10 min at 14,000×*g*, and the supernatant was collected. Protein concentrations were measured using the Bradford assay (Bio-Rad, Richmond, CA).

Protocol II. Cells were washed 2 times with ice-cold PBS and collected with 200 µl 2X-Stop solution (500 mM Tris–Cl (pH 6.8), 10% sodium dodecyl sulfate (SDS), 100 mM EDTA, 100 mM EGTA, 10% glycerol) containing a protease inhibitor mixture and 1 mM the phosphatase inhibitor, Na-orthovanadate. Samples were sonicated and protein concentrations were determined using the DC Protein assay (Bio-Rad, Richmond, CA), for measurements of proteins following detergent solubilizations. The samples were diluted in 2x Laemmli stop buffer and the indicated amounts of proteins were resolved on 10% SDS-polyacrylamide gels (SDS-PAGE). Blots were probed with the antibodies to: Tau, which include the phosphorylation-independent antibodiy TAU-5 (1:1,000; Chemicon International, Inc.; MAB 361;) and PHF-1 (1:500; gift from Dr. P. Davies, Albert Einstein College of Medicine, Bronx, NY, USA that recognizes phosphorylated forms of Tau at Ser396/404); α-synuclein (1:1,000; mouse monoclonal antibodiy; BD Transduction Laboratories, Cat.#610786); GSK-3β (1:1,000; Chemicon International, Inc.; AB8687); GSK-3(pY216; 1:1,000; BD Transduction Laboratories, Cat.#612312). To confirm equal protein loading, blots were reprobed with anti-β-Actin antibody (1/500; Santa Cruz Biotechnology, sc-1616).

Cell viability

Cell viability assay was performed by the MTT test. Briefly, after cell treatment in 12 wells, cells were carefully washed twice with D-PBS and incubated for 2 h at 37 °C and 5% CO_2 in appropriate medium without serum, containing 0.5 mg/ml of MTT (Sigma). After two careful washes with D-PBS, formazan salts were solubilized with 1 ml/12 well of pure ethanol, and the absorbance at 564 nm was measured by visible spectrophotometry against an ethanol blank.

Statistical analysis

Cell viability results are expressed as o.d.564nm ± s.d., in % of control, vehicle-treated cells. Statistical significance was obtained by a Student's *t*-test.

Incorporation By Reference

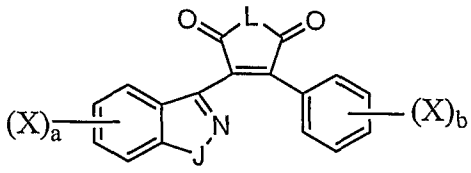
All of the U.S. patents and U.S. patent application publications cited herein are hereby incorporated by reference.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We Claim:

1. A compound of formula **I**:



I

wherein, independently for each occurrence

L is O, S, or NR;

J is O, S, or NR;

R is H, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or $-(CH_2)_n-R_2$

wherein,

n is an integer from 1 to 6 inclusive;

R_2 is -OH, $-NH_2$, -NH(alkyl), $-N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), $-N(aryl)_2$, or one or more saccharide units;

X is -OH, halide, $-NO_2$, carboxylic, ketone, aldehyde, $-NH_2$, -NH(alkyl), $-N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), or $-N(aryl)_2$;

a is an integer from 1 to 4 inclusive; and

b is an integer from 1 to 5 inclusive; or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein L is NH.

3. The compound of claim 1, wherein J is NH.

4. The compound of claim 1, wherein a is 0.

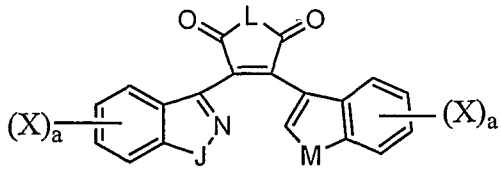
5. The compound of claim 1, wherein Y is Cl and b is 2.

6. The compound of claim 1, wherein L is NH and J is NH.

7. The compound of claim 1, wherein L is NH, J is NH, and a is 0.

8. The compound of claim 1, wherein L is NH, J is NH, a is 0, Y is Cl and b is 2.

9. A compound of formula **II**:



II

wherein, independently for each occurrence

L is O, S, or NR;

M is O, S, or NR;

J is O, S, or NR;

R is H, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or $-(CH_2)_n-R_2$

wherein,

n is an integer from 1 to 6 inclusive;

R_2 is -OH, $-NH_2$, -NH(alkyl), $-N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), $-N(aryl)_2$, or one or more saccharide units;

X is -OH, halide, $-NO_2$, carboxylic, ketone, aldehyde, $-NH_2$, -NH(alkyl), $-N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), or $-N(aryl)_2$; and

a is an integer from 1 to 4 inclusive; or a pharmaceutically acceptable salt thereof.

10. The compound of claim 9, wherein L is NH.

11. The compound of claim 9, wherein J is NH.

12. The compound of claim 9, wherein J is NCH_3.

13. The compound of claim 9, wherein M is NH.

14. The compound of claim 9, wherein M is NCH_3.

15. The compound of claim 9, wherein M is $N(CH_2)_3OH$.

16. The compound of claim 9, wherein M is $N(CH_2)_3N(CH_3)_2$.

17. The compound of claim 9, wherein M is $N(CH_2)_3NHCH_3$.

18. The compound of claim 9, wherein M is $N(CH_2)_3NH_2$.

19. The compound of claim 9, wherein a is 0.

20. The compound of claim 9, wherein b is 0.

21. The compound of claim 9, wherein Y is Cl and b is 1.

22. The compound of claim 9, wherein L is NH, J is NH, M is NH, a is 0, and b is 0.

23. The compound of claim 9, wherein L is NH, J is NH, M is NCH_3, a is 0, and b is 0.

24. The compound of claim 9, wherein L is NH, J is NH, M is $N(CH_2)_3OH$, a is 0, and b is 0.

25. The compound of claim 9, wherein L is NH, J is NH, M is $N(CH_2)_3N(CH_3)_2$, a is 0, and b is 0.

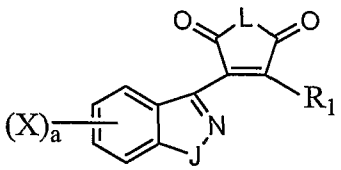
26. The compound of claim 9, wherein L is NH, J is NH, M is $N(CH_2)_3NHCH_3$, a is 0, and b is 0.

27. The compound of claim 9, wherein L is NH, J is NH, M is $N(CH_2)_3NH_2$, a is 0, and b is 0.

28. The compound of claim 9, wherein L is NH, J is NCH_3, M is NCH_3, a is 0, and b is 0.

29. The compound of claim 9, wherein L is NH, J is NH, M is NCH_3, a is 0, Y is Cl, and b is 1.

30. A compound of formula **III**:



III

wherein, independently for each occurrence

L is O, S, or NR;

M is O, S, or NR;

J is O, S, or NR;

R is H, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or $-(CH_2)_n-R_2$

wherein,

n is an integer from 1 to 6 inclusive;

R_2 is -OH, -NH_2, -NH(alkyl), -$N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), -$N(aryl)_2$, or one or more saccharide units;

X is -OH, halide, -NO_2, carboxylic, ketone, aldehyde, -NH_2, -NH(alkyl), -$N(alkyl)_2$, -NHCO(alkyl), -NHCO(aryl), -NH(aryl), or -$N(aryl)_2$;

R_1 is heteroaryl or heterocycloalkyl; and

a is an integer from 1 to 4 inclusive; or a pharmaceutically acceptable salt thereof.

31. The compound of claim 30, wherein L is NH.

32. The compound of claim 30, wherein J is NH.

33. The compound of claim 30, wherein R_1 is 3-indazolyl.

34. The compound of claim 30, wherein a is 0.

35. The compound of claim 30, wherein L is NH and J is NH.

36. The compound of claim 30, wherein L is NH, J is NH, and R_1 is 3-indazolyl.

37. The compound of claim 30, wherein L is NH, J is NH, R_1 is 3-indazolyl, and a is 0.

38. The compound of claim 1, 9, or 30, wherein the compound reduces the activity of GSK-3 by greater than about 20%.

39. The compound of claim 1, 9, or 30, wherein the compound reduces the activity of GSK-3 by greater than about 40%.

40. The compound of claim 1, 9, or 30, wherein the compound reduces the activity of GSK-3 by greater than about 60%.

41. The compound of claim 1, 9, or 30, wherein the compound reduces the activity of GSK-3 by greater than about 80%.

42. The compound of claim 1, 9, or 30, wherein the compound reduces the activity of GSK-3 by greater than about 90%.

43. The compound of claim 1, 9, or 30, wherein the compound reduces the activity of GSK-3 by greater than about 95%.

44. The compound of claim 1, 9, or 30, wherein the compound reduces the activity of

GSK-3 by greater than about 98%.

45. A pharmaceutical composition comprising a compound of claim 1, 9, or 30 and a pharmaceutically acceptable carrier.

46. A method of modulating the activity of a protein kinase in a mammal, comprising administering to the mammal a therapeutically active amount of a compound of claim 1, 9, or 30.

47. The method of claim 46, wherein the protein kinase is selected from one of the following: MKK1, MAPK2/ERK2, JNK/SAPK1c, SAPK2a/p38, SAPK2b/p38b2, SAPK3/p38d, SAPK4/p38d, MAPKAP-K1a, MAPKAP-K2, MSK1, PRAK, PKA, PKCa, PDK1, PKBΔph, SGK, S6K1, GSK-3, ROCK-II, AMPK, CHK1, CK2, PHOS.KINASE, Lck, CSK, CDK2/cyclin A, CK1, DYRK1a, or NEK6.

48. The method of claim 47, wherein the protein kinase is GSK-3.

49. The method of claim 46, wherein the mammal is primate, equine, canine, or feline.

50. The method of claim 46, wherein the mammal is human.

51. The method of claim 46, wherein the compound is administered orally.

52. The method of claim 46, wherein the compound is administered intravenously.

53. The method of claim 46, wherein the compound is administered sublingually.

54. The method of claim 46, wherein the compound is administered ocularly.

55. The method of claim 46, wherein the compound is administered transdermally.

56. The method of claim 46, wherein the compound is administered rectally.

57. The method of claim 46, wherein the compound is administered vaginally.

58. The method of claim 46, wherein the compound is administered topically.

59. The method of claim 46, wherein the compound is administered intramuscularly.

60. The method of claim 46, wherein the compound is administered subcutaneously.

61. The method of claim 46, wherein the compound is administered bucally.

62. The method of claim 46, wherein the compound is administered nasally.

63. A method of treating a mammal suffering from diabetes, Alzheimer's disease,

Huntington's Disease, Parkinson's Disease, AIDS-associated dementia, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), schizophrenia, cardiomycete hypertrophy, reperfusion/ischemia, or baldness comprising administering to the mammal a therapeutically effective amount of a compound of claim 1, 9, or 30.

64. The method of claim 63, wherein the mammal is a primate, equine, canine, or feline.

65. The method of claim 63, wherein the mammal is human.

66. The method of claim 63, wherein the compound is administered orally.

67. The method of claim 63, wherein the compound is administered intravenously.

68. The method of claim 63, wherein the compound is administered sublingually.

69. The method of claim 63, wherein the compound is administered ocularly.

70. The method of claim 63, wherein the compound is administered transdermally.

71. The method of claim 63, wherein the compound is administered rectally.

72. The method of claim 63, wherein the compound is administered vaginally.

73. The method of claim 63, wherein the compound is administered topically.

74. The method of claim 63, wherein the compound is administered intramuscularly.

75. The method of claim 63, wherein the compound is administered subcutaneously.

76. The method of claim 63, wherein the compound is administered bucally.

77. The method of claim 63, wherein the compound is administered nasally.

78. A kit comprising a compound of claim 1, 9, or 30 and instructions for the use thereof.

Figure 1

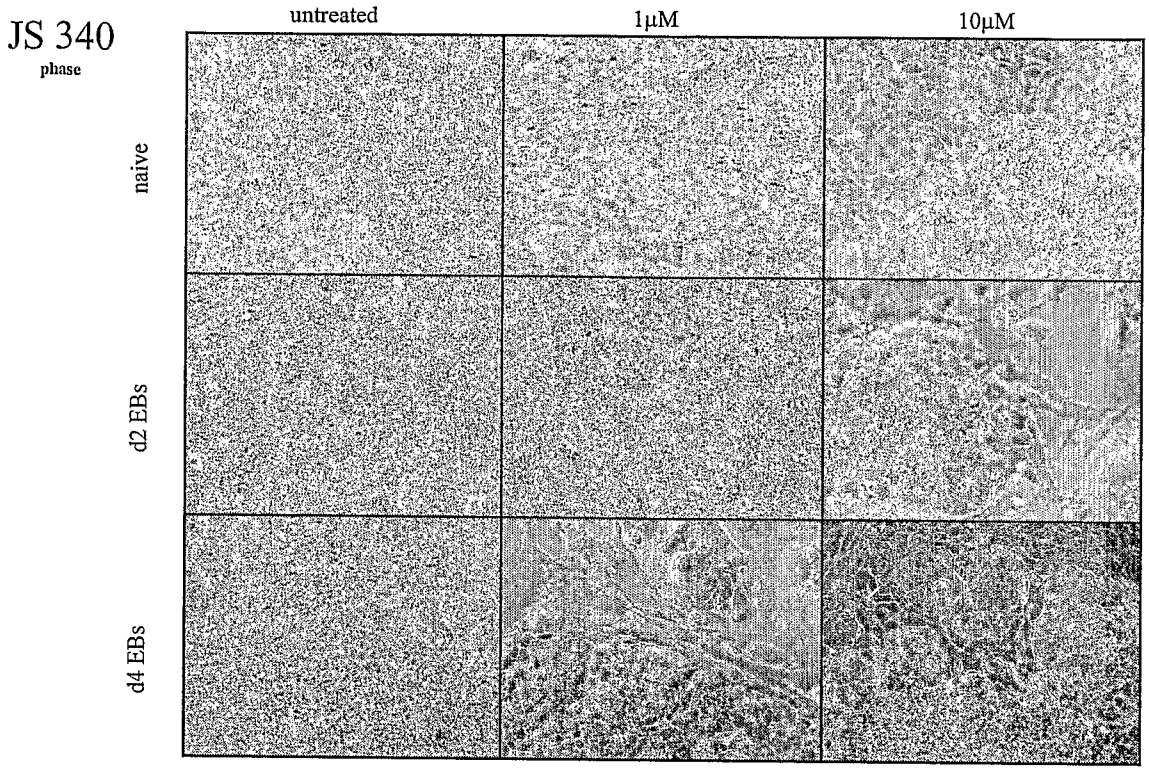


Figure 2

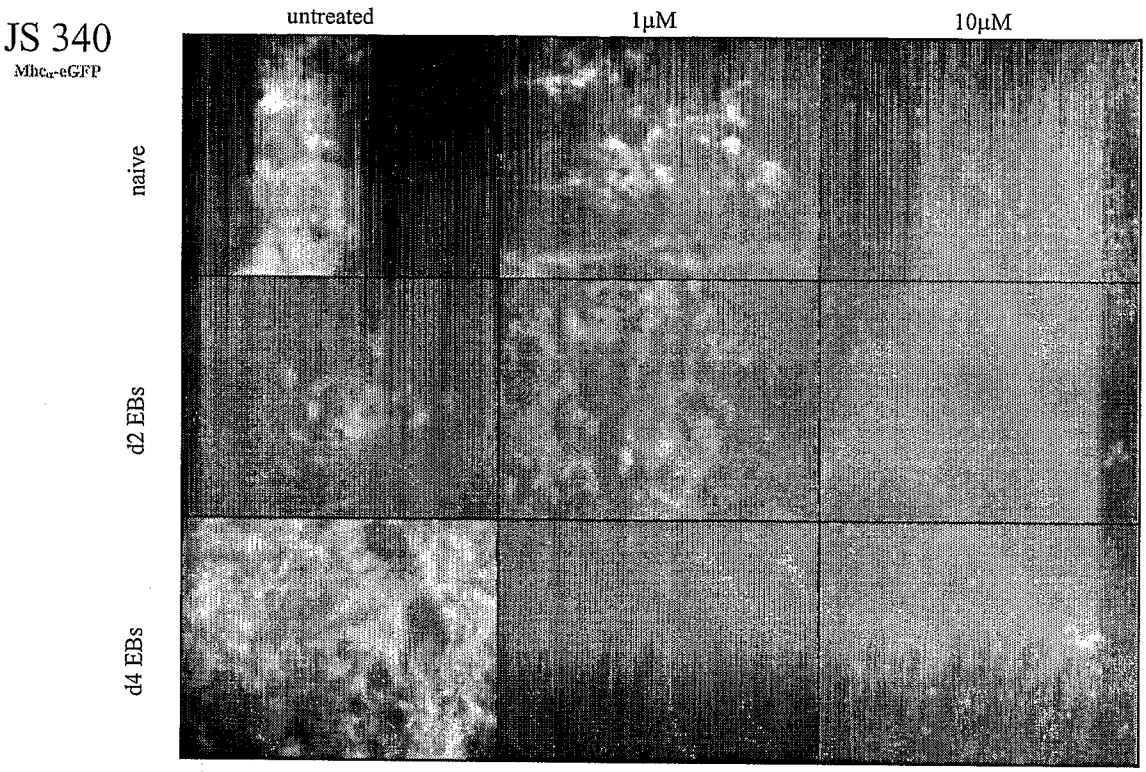


Figure 3

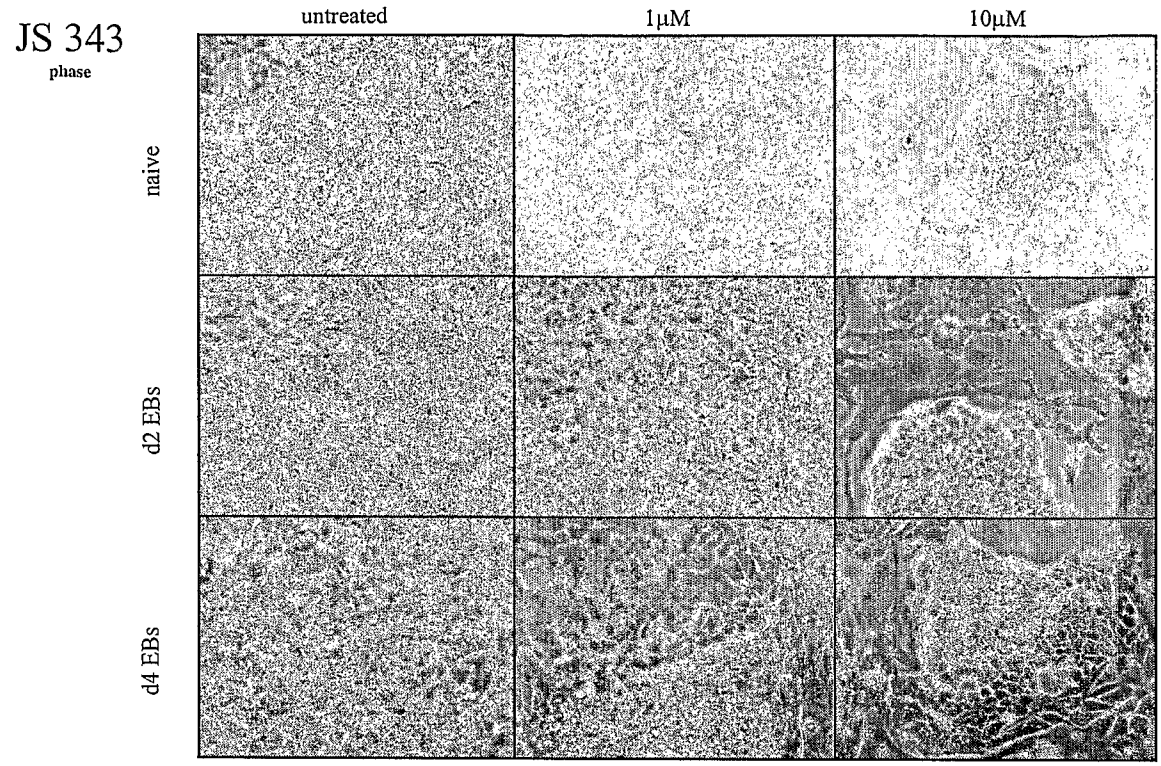


Figure 4

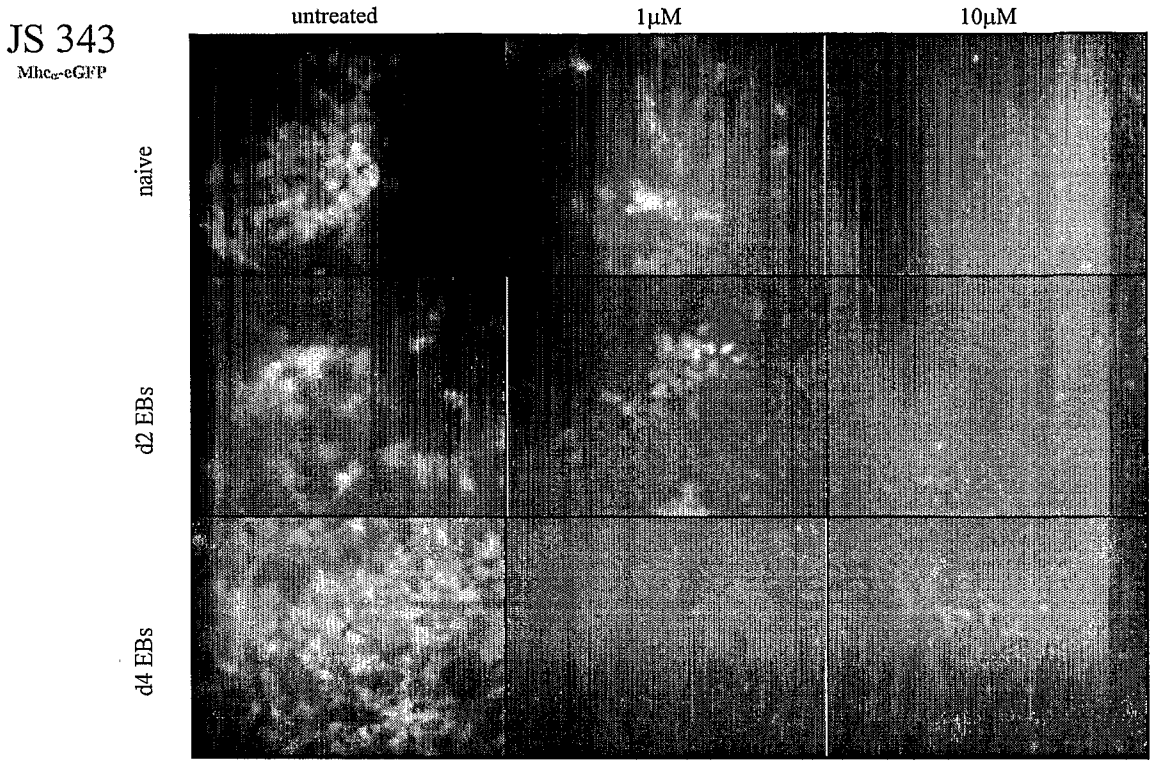


Figure 5

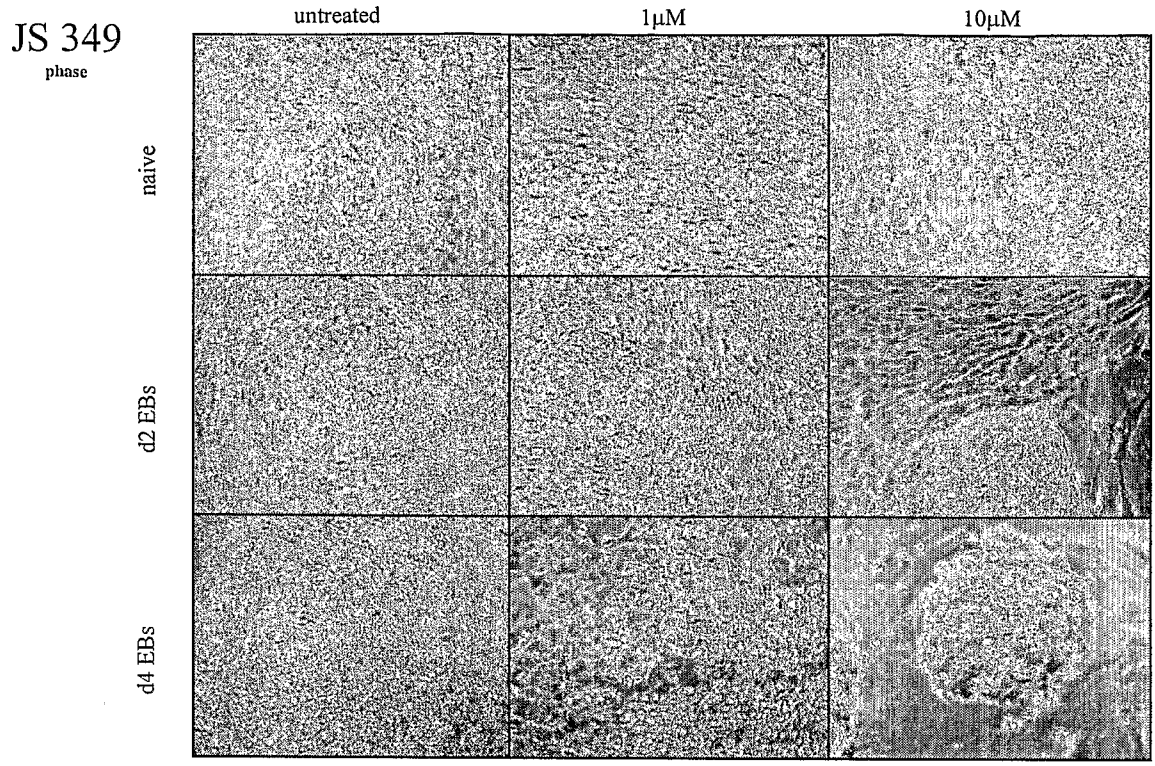


Figure 6

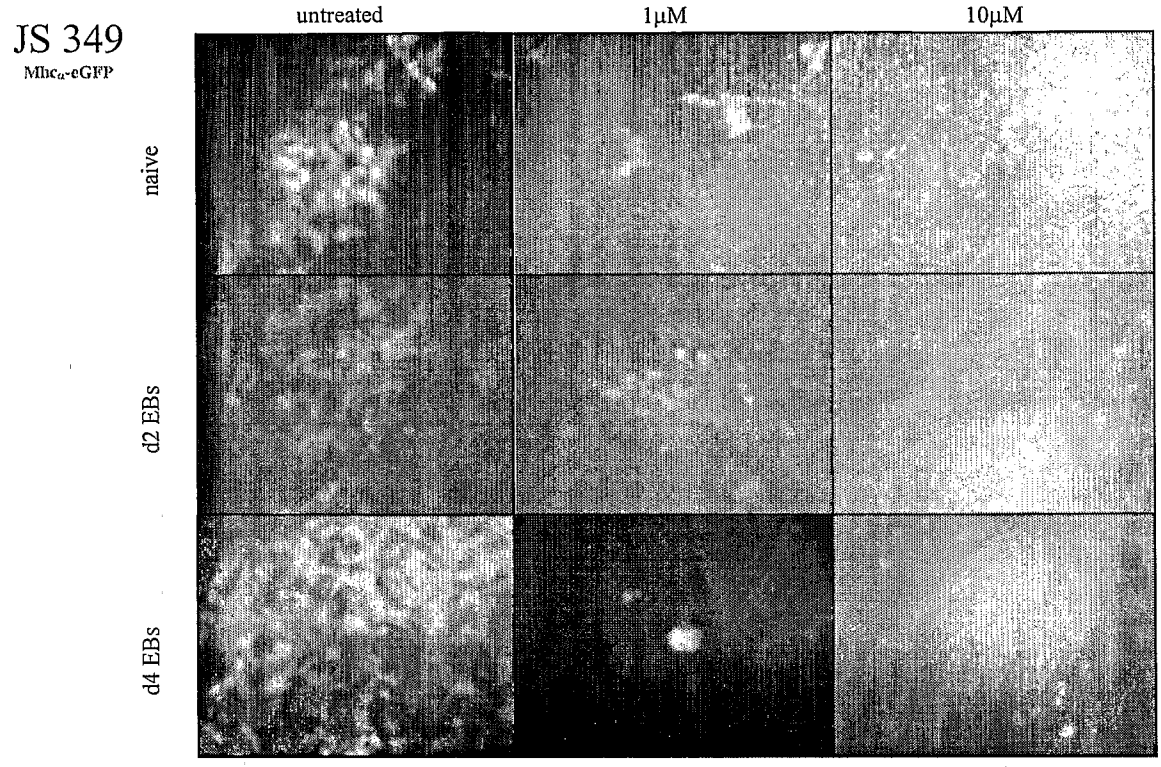


Figure 7

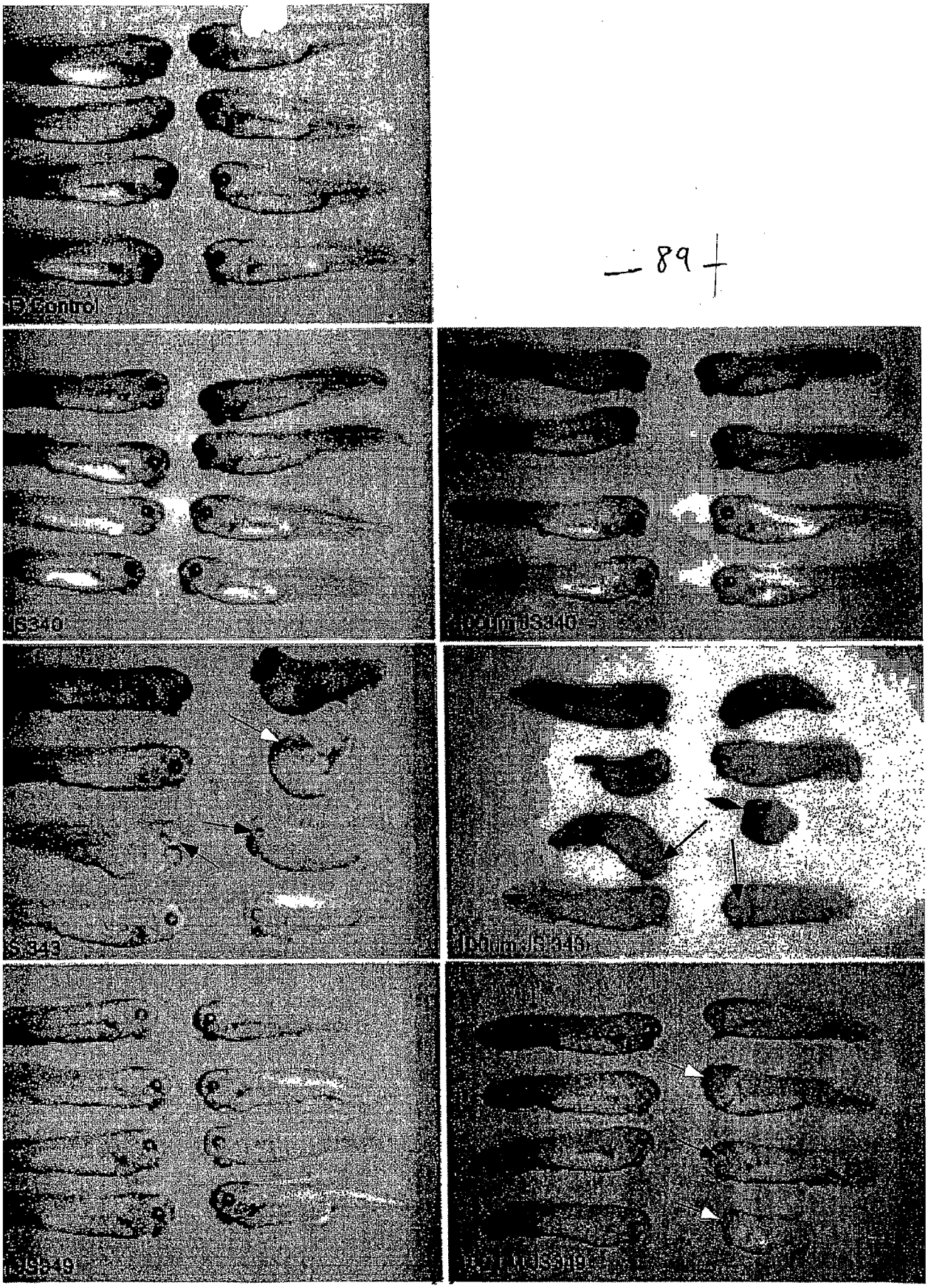


Figure 8

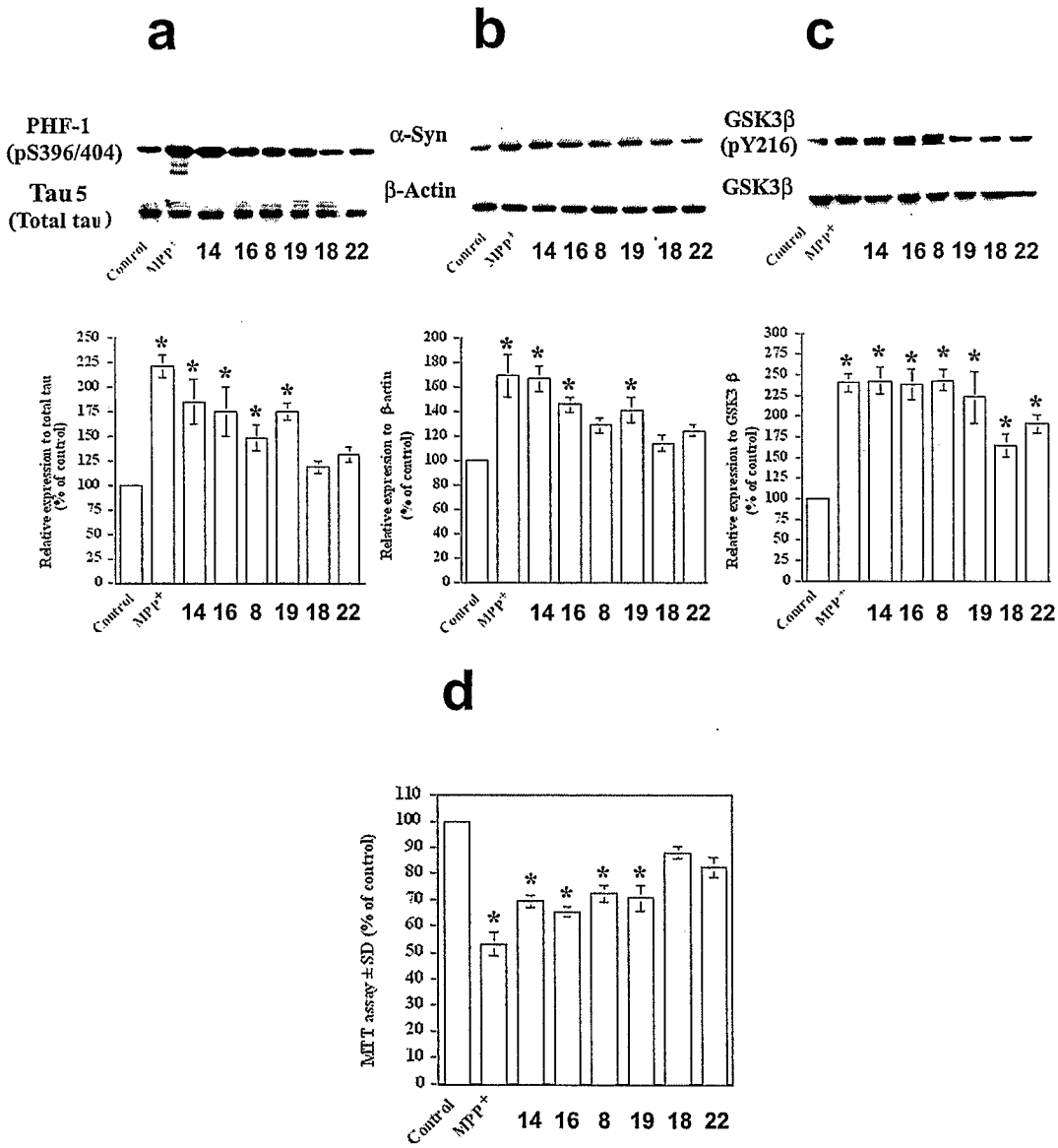
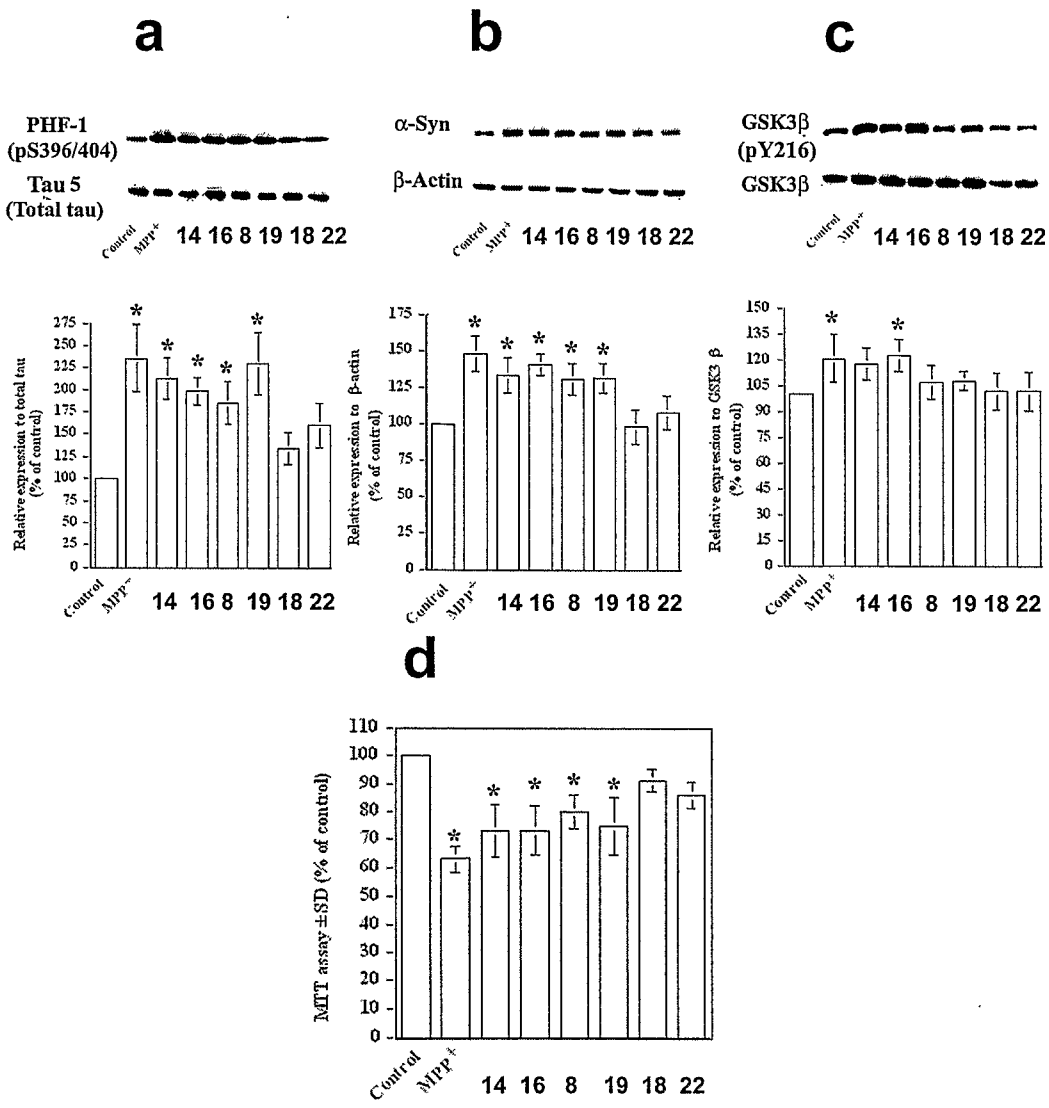

a
b
c
PHF-1
(pS396/404)
Tau 5
(Total tau)
α-Syn
β-Actin
GSK3β
(pY216)
GSK3β
Control
MPP+
14 16 8 19 18 22
Relative expression to total tau (% of control)
Relative expression to β-actin (% of control)
Relative expression to GSK3 β (% of control)
d
MTT assay ± SD (% of control)

Figure 9


a
b
c
PHF-1
(pS396/404)
Tau 5
(Total tau)
α-Syn
β-Actin
GSK3β
(pY216)
GSK3β
Control
MPP+
14 16 8 19 18 22
Relative expression to total tau
(% of control)
Relative expression to β-actin
(% of control)
Relative expression to GSK3 β
(% of control)
d
MTT assay ±SD (% of control)