Title: INHIBITORS OF GLYCOGEN SYNTASE KINASE-3

Abstract: The present invention relates generally to inhibitors of the serine/threonine kinase GSK3, and more particularly to triarylimidazole compounds of the following formula (I):

![Chemical Structure](image-url)
INHIBITORS OF GLYCOGEN SYNTHASE KINASE-3

FIELD OF THE INVENTION

The present invention relates generally to inhibitors of the serine/threonine kinase GSK3, and more particularly to novel triarylimidazole compounds.

BACKGROUND OF THE INVENTION

Glycogen synthase kinase (GSK3) is a proline-directed serine/threonine kinase. Originally, GSK3 was identified as a kinase that inhibits glycogen synthase by direct phosphorylation. Upon insulin activation, GSK3 is inactivated, thereby allowing the activation of glycogen synthase and possibly other insulin-dependent events. Thus, agents that inhibit GSK3 activity are useful in the treatment of disorders that are mediated by GSK3 activity.

For example, type II diabetes, otherwise known as Non-Insulin Dependent Diabetes Mellitus (NIDDM), is initially characterized by decreased sensitivity to insulin (insulin resistance) and a compensatory elevation in circulating insulin concentrations. Increased insulin levels are caused by increased secretion from the pancreatic beta cells in an attempt to overcome the insulin resistance. The resulting hyperinsulinemia is associated with a variety of cardiovascular complications.

As insulin resistance worsens, the demand on the pancreatic beta cells steadily increases until the pancreas can no longer provide adequate levels of insulin, thereby resulting in elevated levels of glucose in the blood. Thus, diabetes causes impaired glucose transport into skeletal muscle and increased hepatic glucose production, in addition to inadequate insulin response. The disorders and conditions associated with hyperglycemia and hyperlipidemia include cardiovascular disease, renal failure, and blindness.
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Despite the utility of various anti-diabetic agents, there is a need for new and improved drugs for the treatment and prevention of diabetes.

As described above, GSK3 inhibition stimulates insulin-dependent processes and is consequently useful in the treatment of diseases and conditions, such as type 2 diabetes, that are mediated by GSK3 activity, or, more specifically, characterized by a need for the inhibition of GSK3. For example, Klein et al., *PNAS* 93:8455-9 (1996) report that lithium ion inhibits GSK3 activity. Lithium has been reported to have anti-diabetic effects such as reduction of plasma glucose levels, increased glycogen uptake, potentiation of insulin, and stimulation of glycogen synthesis in skin, muscle, and fat cells. Lithium, however, effects molecular targets other than GSK3, and is, therefore, not a widely accepted therapy for diabetics.

Other examples of GSK3 mediated diseases or conditions include obesity, various CNS disorders such as Alzheimer’s Disease and schizophrenia, neurotraumatic injuries such as acute stroke, immune potentiation, baldness or hair loss, and cancer. See, for example, published PCT application WO 00/38675, the background of which is herein incorporated by reference.

**SUMMARY OF THE INVENTION**

The present invention includes compounds of formula (I):

![Chemical structure](image-url)
including salts, solvates, and physiologically functional derivatives thereof.

A further aspect of the invention includes pharmaceutical compositions that contain, among other things, a compound of the present invention, namely, 4-[2-(2-bromophenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl]pyridine, and a pharmaceutically acceptable carrier. A further aspect of the present invention includes use of a compound of the present invention as an active therapeutic substance. A further aspect of the invention includes use of a compound of the present invention for inhibiting GSK3 in a subject in need thereof. Accordingly, another aspect of the present invention includes use of a compound of the present invention for the treatment or prophylaxis of diabetes. A further aspect of the present invention includes use of a compound of the present invention in the manufacture of a medicament for use in the inhibition of GSK3, and in the treatment or prophylaxis of diabetes. Another aspect of the present invention includes a method for the treatment or prophylaxis of a disease or condition that are mediated by GSK3 activity, by administering to a subject in need of such treatment a therapeutically effective amount of a compound of the present invention. Thus, another aspect of the present invention includes a method for the treatment or prophylaxis of diabetes by administering to a subject in need of such treatment a therapeutically effective amount of a compound of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, the term "effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal or human that is being sought, for instance, by a researcher or clinician. Furthermore, the term “therapeutically effective amount” means any
amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function.

A therapeutically effective amount of a compound of the present invention will depend upon a number of factors. For example, the age and weight of the animal, the precise condition requiring treatment and its severity, the nature of the formulation, and the route of administration are all factors to be considered. The therapeutically effective amount ultimately should be at the discretion of the attendant physician or veterinarian. Regardless, an effective amount of a compound of the present invention for the treatment of disorders or conditions mediated by GSK3 activity, such as diabetes, generally should be in the range of about 0.1 to 100 mg/kg body weight of recipient (mammal) per day. Thus, for a 70 kg adult mammal the actual amount per day would usually be from about 7 to 700 mg. This amount may be given in a single dose per day or in a number (such as two, three, four, five, or more) of sub-doses per day such that the total daily dose is the same. An effective amount of a salt or solvate, or physiologically functional derivative thereof, may be determined as a proportion of the effective amount of the compound of the present invention per se. Similar dosages should be appropriate for treatment of the other conditions referred to above. The subject or mammal requiring treatment with a compound of the present invention is typically a human being.

As used herein, the term "physiologically functional derivative" refers to any pharmaceutically acceptable derivative of a compound of the present invention; for
example, an ester or an amide, which upon administration to a mammal is capable of providing (directly or indirectly) a compound of the present invention or an active metabolite thereof. Such derivatives will be clear to those skilled in the art, without undue experimentation, and with reference to the teaching of Burger’s Medicinal Chemistry And Drug Discovery, 5th Edition, Vol 1: Principles and Practice, which is incorporated herein by reference to the extent that it teaches physiologically functional derivatives.

As used herein, the term “solvate” refers to a complex of variable stoichiometry formed by a solute (a compound of the present invention, or a salt or physiologically functional derivative thereof) and a solvent. Solvents, for the purpose of the invention, should not interfere with the biological activity of the solute. Non-limiting examples of suitable solvents include, but are not limited to water, methanol, ethanol, and acetic acid. Preferably the solvent used is a pharmaceutically acceptable solvent. Non-limiting examples of suitable pharmaceutically acceptable solvents include water, ethanol, and acetic acid. Most preferably the solvent used is water.

Typically, the salts of the present invention are pharmaceutically acceptable salts. Salts encompassed within the scope of the present invention include non-toxic salts of the compounds of this invention. Salts of the compounds of the present invention may comprise acid addition salts. Representative salts include acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinolate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, laurate, malate, maleate,
mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, monopotassium maleate, mucate, napsylate, nitrate, N-methylglucamine, oxalate, pamoate (embonate), palmitate, pantothenate, phosphate/diphosphate, polygalacturonate, potassium, salicylate, sodium, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, trimethylammonium, and valerate salts. Other salts, that may or may not be pharmaceutically acceptable, may be useful, for example in the preparation of pharmaceutically acceptable compounds, and these form a further aspect of the invention.

For use in therapy, therapeutically effective amounts of a compound of the present invention, as well as salts, solvates and physiological functional derivatives thereof, may be administered as the raw chemical, or the active ingredient may be presented as a pharmaceutical composition. Accordingly, the invention further provides pharmaceutical compositions that include therapeutically effective amounts of compounds of the present invention and salts, solvates and physiological functional derivatives thereof, and one or more pharmaceutically acceptable carriers, diluents, or excipients. The compounds of the present invention and salts, solvates and physiologically functional derivatives thereof, are as described above. The carrier(s), diluent(s) or excipient(s) must be acceptable, in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient of the pharmaceutical composition. In accordance with another aspect of the invention there is also provided a process for the preparation of a pharmaceutical formulation including admixing a compound of the formula (I), or salts, solvates and physiological functional derivatives thereof, with one or more pharmaceutically acceptable carriers, diluents or excipients.
Pharmaceutical formulations may be presented in unit dose forms containing a predetermined amount of active ingredient per unit dose. Such a unit may contain, as a non-limiting example, 0.5mg to 1g of a compound of the present invention, depending on the condition being treated, the route of administration, and the age, weight, and condition of the patient. Preferred unit dosage formulations are those containing a daily dose or sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient. Such pharmaceutical formulations may be prepared by any of the methods well known in the pharmacy art.

Pharmaceutical formulations may be adapted for administration by any appropriate route, for example by an oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal, or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such formulations may be prepared by any method known in the art of pharmacy, for example by bringing into association the active ingredient with the carrier(s) or excipient(s).

Pharmaceutical formulations adapted for oral administration may be presented as discrete units such as capsules or tablets; powders or granules; solutions or suspensions, each with aqueous or non-aqueous liquids; edible foams or whips; or oil-in-water liquid emulsions or water-in-oil liquid emulsions.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Generally, powders are prepared by comminuting the compound to a suitable fine size and mixing with an appropriate pharmaceutical carrier such as an edible carbohydrate, as,
for example, starch or mannitol. Flavorings, preservatives, dispersing agents, and coloring agents can also be present.

Capsules are made by preparing a powder, liquid, or suspension mixture and encapsulating with gelatin or some other appropriate shell material. Glidants and lubricants such as colloidal silica, talc, magnesium stearate, calcium stearate or solid polyethylene glycol can be added to the mixture before the encapsulation. A disintegrating or solubilizing agent such as agar-agar, calcium carbonate or sodium carbonate can also be added to improve the availability of the medicament when the capsule is ingested.

Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents, and coloring agents can also be incorporated into the mixture. Examples of suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like.

Lubricants useful in these dosage forms include, for example, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like. Tablets are formulated, for example, by preparing a powder mixture, granulating or slugging, adding a lubricant and disintegrant, and pressing into tablets. A powder mixture is prepared by mixing the compound, suitably comminuted, with a diluent or base as described above, and optionally, with a binder such as carboxymethylcellulose, an alginate, gelatin, or polyvinyl pyrrolidone, a solution retardant such as paraffin, a resorption accelerator such as a quaternary salt and/or an absorption agent such as bentonite, kaolin or
dicalcium phosphate. The powder mixture can be granulated by wetting with a binder such as syrup, starch paste, acadia mucilage or solutions of cellulosic or polymeric materials, and forcing through a screen. As an alternative to granulating, the powder mixture can be run through the tablet machine and the result is imperfectly formed slugs broken into granules. The granules can be lubricated to prevent sticking to the tablet forming dies by means of the addition of stearic acid, a stearate salt, talc or mineral oil. The lubricated mixture is then compressed into tablets. The compounds of the present invention can also be combined with a free flowing inert carrier and compressed into tablets directly without going through the granulating or slugging steps. A clear or opaque protective coating consisting of a sealing coat of shellac, a coating of sugar or polymeric material, and a polish coating of wax can be provided. Dyes can be added to these coatings, for example, to distinguish different unit dosages.

Oral fluids such as solutions, syrups and elixirs can be prepared in dosage unit form so that a given quantity contains a predetermined amount of the compound. Syrups can be prepared, for example, by dissolving the compound in a suitably flavored aqueous solution, while elixirs are prepared through the use of a non-toxic alcoholic vehicle. Suspensions can be formulated generally by dispersing the compound in a non-toxic vehicle. Solubilizers and emulsifiers such as ethoxylated isostearyl alcohols and polyoxyethylene sorbitol ethers, preservatives; flavor additives such as peppermint oil, or natural sweeteners, saccharin, or other artificial sweeteners; and the like can also be added.

Where appropriate, dosage unit formulations for oral administration can be microencapsulated. The formulation can also be prepared to prolong or sustain the
release as for example by coating or embedding particulate material in polymers, wax or the like.

The compounds of the present invention and salts, solvates and physiological functional derivatives thereof, can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

The compounds of the present invention and salts, solvates and physiologically functional derivatives thereof may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide–phenol, polyhydroxyethylaspartamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug; for example, polylactic acid, polyeplson caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates, and cross-linked or amphipathic block copolymers of hydrogels.

Pharmaceutical formulations adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis as generally described in Pharmaceutical Research, 3(6), 318 (1986), incorporated herein by reference as related to such delivery systems.
Pharmaceutical formulations adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols, or oils.

For treatments of the eye or other external tissues, for example mouth and skin, the formulations are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water cream base or a water-in-oil base.

Pharmaceutical formulations adapted for topical administrations to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent.

Pharmaceutical formulations adapted for topical administration in the mouth include lozenges, pastilles, and mouthwashes.

Pharmaceutical formulations adapted for rectal administration may be presented as suppositories or as enemas.

Pharmaceutical formulations adapted for nasal administration, where the carrier is a solid, include a coarse powder having a particle size for example in the range 20 to 500 microns. The powder is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient.
Pharmaceutical formulations adapted for administration by inhalation include fine particle dusts or mists, which may be generated by means of various types of metered, dose pressurized aerosols, nebulizers, or insufflators.

Pharmaceutical formulations adapted for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulations.

Pharmaceutical formulations adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations may include other agents conventional in the art having regard to the type of formulation in question. For example, formulations suitable for oral administration may include flavoring agents.

The compounds of this invention may be made by a variety of methods, including standard synthetic methods. Furthermore, the methods disclosed in International Publication WO 93/14081, may be useful in synthesizing the compounds of the present invention, such methods are herein incorporated by reference.
Illustrative general synthetic methods are set out below and then specific compounds of the invention are prepared in the Examples.

The following represents a general reaction scheme for the preferred embodiment of the present invention:

Certain embodiments of the present invention will now be illustrated by way of example only. The physical data given for the compounds exemplified is consistent with the assigned structure of those compounds.

**EXAMPLES**

As used herein the symbols and conventions used in these processes, schemes and examples are consistent with those used in the contemporary scientific literature, for example, the *Journal of the American Chemical Society* or the *Journal of Biological Chemistry*. Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification. Specifically, the
following are examples of abbreviations that may be used throughout the present specification:

\[
\begin{align*}
g \text{ (grams);} & \quad mg \text{ (milligrams);} \\
L \text{ (liters);} & \quad mL \text{ (milliliters);} \\
\mu L \text{ (microliters);} & \quad M \text{ (molar);} \\
h \text{ (hour(s));} & \quad mol \text{ (moles);} \\
mmol \text{ (millimoles);} & \quad RT \text{ (room temperature);} \\
\text{min (minutes);} & \quad h \text{ (hours);} \\
mp \text{ (melting point);} & \quad TLC \text{ (thin layer chromatography);} \\
\text{THF (tetrahydrofuran);} & \quad DMSO \text{ (dimethylsulfoxide);} \\
\text{DMF (N,N-dimethylformamide);} & \quad \text{AcOH (acetic acid);}
\end{align*}
\]

All references to ether are to diethyl ether; brine refers to a saturated aqueous solution of NaCl. Unless otherwise indicated, all temperatures are expressed in degrees Centigrade (°C). All reactions were conducted under an inert atmosphere at room temperature unless otherwise noted.

\(^1\)H NMR spectra were recorded on a Varian VXR-300, a Varian Unity-300, a Varian Unity-400 instrument, or a General Electric QE-300. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), bs (broad singlet).

Mass spectra (MS) were obtained on Micromass Platform II mass spectrometers from Micromass Ltd., Altrincham, UK, using Electrospray Ionization (ESI).
Example 1

4-[2-(2-bromophenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl]pyridine

A. Preparation of 1-(4-fluorophenyl)-2-(4-pyridinyl)-ethanone:

To a 1-L flask was added 4-picoline (27.8 g, 300 mmol), 4-fluoroethylbenzoate (50.2 g, 300 mmol) and THF (400 mL), and the resulting solution cooled in an ice-water bath and stirred with a magnetic stir bar. Lithium bis(trimethylsilyl)amide (100.0 g, 600 mmol) was added portion wise over 1 h resulting in a yellow precipitate and the reaction mixture was allowed to warm to room temperature and stirred for 1.5 h. Reaction was quenched with water (200 mL) and neutralized by dropwise addition of concentrated hydrochloric acid. Diluted with ether (400 mL) and organic phase separated. The aqueous phase was washed twice with ether (200 mL) and the organic phases combined, dried over magnesium sulfate and concentrated to yield a yellow solid. Product was slurried in ethyl acetate/hexane and filtered and washed with hexane to yield product as a yellow solid in 62.2 g yield.

$^1$H NMR (DMSO-d$_6$): δ 8.53 (d, 2H), 8.16 (dd, 2H), 7.42 (t, 2H), 7.31 (d, 2H), 4.51 (s, 2H); ESI-MS m/z 216 (M+H)$^+$. 
B. Preparation of (4-fluorophenyl)-4-pyridinyl-ethanedione 2-oxime:

![Chemical Structure]

In a 1-L flask 1-(4-fluorophenyl)-2-(4-pyridinyl)-ethanone (50.2 g, 233 mmol) was dissolved in acetic acid (230 mL) and water (25 mL) and the resulting solution cooled in an ice-water bath and stirred with a magnetic stir bar. A slurry of sodium nitrite (24.2 g, 351 mmol) in water (20 mL) was added and stirring continued for 15 min., during which time a yellow precipitate deposited. The precipitate was filtered off, washed with water and dried to yield 53.5 g of product.

^1H NMR (DMSO-d_6): δ 12.42 (s, 1H), 8.60 (d, 2H), 7.91 (dd, 2H), 7.38-7.45 (m, 4H); ESI-MS m/z 245 (M+H)^+.

C. Preparation of 4-[4-(4-fluorophenyl)-1-hydroxy-2-[2-bromophenyl]-1H-imidazol-5-yl]-pyridine:

![Chemical Structure]
In a 1-L flask was added (4-fluorophenyl)-4-pyridinyl-ethanedione 2-oxime (30.6 g, 122 mmol), 2-bromobenzaldehyde (34.5 g, 186 mmol), ammonium acetate (61.2 g, 796 mmol) and acetic acid (400 mL). The reaction mixture was heated to reflux at which time all solid went into solution and refluxed for 16 h. Solution was then quenched with water (2 L) and neutralized by drop-wise addition of aqueous ammonia. The resulting yellow precipitate was filtered off, washed with water and dried to yield 36.4 g of product. A second crop was obtained by partitioning the residual semi-solid between ethyl acetate and hydrochloric acid, separating the acid phase and adjusting to pH 7 with 2N aqueous sodium hydroxide solution and dissolving the precipitated solid in ethyl acetate and dichloromethane. On standing a solid deposited which was filtered off, washed with ethyl acetate and dried to give 20.8 g of additional product. \(^1\)H NMR (DMSO-d6): \(\delta\) 11.7 (bs, 1H), 8.6 (bs, 2H), 7.77 (d, 1H), 7.30-7.66 (m, 7H), 7.14 (t, 2H); ESI-MS \(m/z\) 410, 412 (M+H)^+. 

D. Preparation of 4-[2-(2-bromophenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl]pyridine:

![Chemical Structure]

In a 1-L flask was dissolved 4-[4-(4-fluorophenyl)-1-hydroxy-2-[2-bromophenyl]-1H-imidazol-5-yl]-pyridine (32 g, 78 mmol) and triethyl phosphite (13 g, 78 mmol) in DMF (350 mL). The reaction mixture was heated to 100°C for 6 h.
with stirring. The solution was then quenched with water (2 L), neutralized by addition of aqueous ammonia and extracted with 1:1 ethyl acetate/dichloromethane (1 L). The organic solution was separated and extracted with 1N hydrochloric acid (500 mL). The acidic phase was separated, washed with ethyl acetate and neutralized by addition of aqueous sodium hydroxide solution. The resulting yellow precipitate was filtered off, washed with water and dried to yield 28.7 g of product. $^1$H NMR (DMSO-$d_6$): $\delta$ 12.9 (s, 1H), 8.43 (d, 2H), 7.73 (d, 1H), 7.69 (dd, 1H), 7.51 (dd, 2H), 7.46 (t, 1H), 7.41 (d, 2H), 7.35 (t, 1H), 7.25 (t, 2H); ESI-MS m/z 394, 396 (M+H)$^+$. 

BIOLOGICAL DATA

The present invention elicits important and measurable pharmacological responses. In evaluating those responses, the present invention also demonstrated unexpected advantageous biological and pharmacological properties. In short, the present invention provides unexpected superior performance characteristics not heretofore appreciated.

The protocol used to demonstrate the pharmacological response of the present invention is based on the ability of the kinase to phosphorylate a biotinylated peptide, the sequence of which is derived from the phosphorylation site of glycogen synthase and its sequence is: Biotin-Ahx-AAAKREILLSRPS(PO$_3$$_2$)YR-amide. The phosphorylated biotinylated peptide is then captured onto streptavidin coated scintillation proximity assay (SPA) beads from Amersham Technology, where the signal from the $^{33}$P is amplified via the scintillant contained in the beads.

GSK-3β is commercially available or may be cloned and expressed in E coli using standard techniques to produce soluble, active protein. The production of active
protein involves purification in two steps using Metal Chelate and Ion Exchange Chromatography. Protein eluting from Ion Exchange provides >90% pure product that may then be concentrated for use in high throughput screening.

The kinase was assayed at a concentration of 20 nM final in 100 mM HEPES, pH 7.2 containing 10 mM magnesium chloride, 0.1 mg/mL bovine serum albumin, and 2.5 uM [γ-35P]-ATP. After 40 minutes incubation at room temperature, the reaction was stopped by addition of 100mM EDTA solution followed by an additional solution of diluted Streptavidin coated SPA beads to give a final concentration of 0.25 mg of beads per assay well in a 96-well microtiter plate.

10 mM stock solutions of the compounds of the invention in 100% DMSO are generated as a first step in the screening process. The second step involves the creation of dose response plates where these compounds are diluted 3-fold in 100% DMSO across the plate such that the final top concentration of inhibitor is 0.033 mM in the 30 uL kinase assay. The third step involves the creation of the assay plates.

This is achieved by transferring 1 uL of the compounds to assay plates by automated liquid handling. The fourth step is to perform the assay as described and count the resulting plates in the Packard TopCount NXT microplate scintillation and luminescence counter. The final step is data acquisition and analysis where IC50 values are generated for each compound by normalizing curve data to the equation

\[ 100^* (U1-C2)/(C1-C2) \] (where U1 is the cpm value, C2 is the background, and C1 is the maximum number of counts), then fitting the normalized data to the equation \[ y = V_{max}^* (1-(x/(K+x))) \].

The compound of the present invention shows an IC50 value in the range of 50 nM.
What is claimed is:

1. A compound of formula (I):

\[
\begin{array}{c}
\text{HN} \\
\text{N} \\
\text{N} \\
\text{Br} \\
\end{array}
\quad
\begin{array}{c}
\text{F} \\
\end{array}
\]

including salts, solvates, and physiologically functional derivatives thereof.

2. A compound of according to claim 1 substantially as hereinbefore defined with reference to any one of the Examples.

3. A pharmaceutical composition comprising:
   a compound of claim 1; and
   a pharmaceutically acceptable carrier.

4. A compound according to claim 1 for use as an active therapeutic substance.

5. A compound according to claim 1 for use in inhibiting GSK3.

6. A compound according to claim 1 for use the treatment or prophylaxis of diabetes.

7. Use of a compound according to claim 1 in the manufacture of a medicament for use in the inhibition of GSK3.

8. Use of a compound according to claim 1 in the manufacture of a medicament for use in the treatment of diabetes.
9. A method for the treatment or prophylaxis of a disease or condition that are mediated by GSK3 activity, comprising:

administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to claim 1.

10. A method for the treatment or prophylaxis of diabetes comprising:

administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to claim 1.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7 A61K31/4164 C07D401/04 A61P3/00

According to International Patent Classification (IPC) or to both national classification and IPC.

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
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<tbody>
<tr>
<td>Y</td>
<td>WO 96 18626 A (HOFFMAN LA ROCHE ;HARMON CHARLES STANFORD (US); KAMBER MARKUS (CH) 20 June 1996 (1996-06-20) page 6</td>
<td>1-8</td>
</tr>
</tbody>
</table>

**X** Further documents are listed in the continuation of box C. **X** Patent family members are listed in annex.

A* document defining the general state of the art which is not considered to be of particular relevance

C* earlier document but published on or after the international filing date

L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date or another citation or other special reason (as specified)

O* document referring to an oral disclosure, use, exhibition or other means

P* document published prior to the international filing date but later than the priority date claimed

*P* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

*X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

**X** document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

*S* document member of the same patent family

**Date of the actual completion of the International search**

25 November 2002

**Date of mailing of the International search report**

09/12/2002

**Name and mailing address of the ISA**

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Authorized officer

Lauro, P
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<thead>
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<td>WO 00 38675 A (HOLDER JULIE CAROLINE; SMITH DAVID GLYNN (GB); COGHLAN MATTHEW PAU) 6 July 2000 (2000-07-06) cited in the application page 1-6</td>
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INTERNATIONAL SEARCH REPORT

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(e) for the following reasons:

1. **X** Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
   
   Although claims 9-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. **☐** Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. **☐** Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. **☐** As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. **☐** As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. **☐** As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. **☐** No required additional search fees were timely paid by the applicant. Consequently, this international Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- **☐** The additional search fees were accompanied by the applicant's protest.
- **☐** No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1996)
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