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(54) Title: GLYCOGEN PHOSPHORYLASE INHIBITOR COMPOUND AND PHARMACEUTICAL COMPOSITION THEREOF

(57) Abstract: This invention relates to a novel compound which is a glycogen phosphorylase inhibitor and its use in the treatment of diabetes and other conditions associated therewith. The invention further relates to a pharmaceutical composition containing the compound and to processes for preparing the compound and pharmaceutical composition.

WO 2009/045831 A1

GLYCOGEN PHOSPHORYLASE INHIBITOR COMPOUND AND PHARMACEUTICAL COMPOSITION THEREOF

FIELD OF THE INVENTION

The present invention relates to a glycogen phosphorylase inhibitor compound, a pharmaceutical composition of the compound, the use of the compound or pharmaceutical composition containing it in the treatment of diabetes, conditions associated with diabetes, and/or tissue ischemia, including myocardial ischemia, and a process for making the compound.

BACKGROUND OF THE INVENTION

Treatment of diabetes remains a health concern in much of the world. Orally ingested drugs having minimal undesirable side effects are desired over the self-injection of insulin. There is a continuing need for drugs that are better, having fewer side effects, longer acting, or act via different mechanisms.

A number of drugs are available for the treatment of diabetes. These include injected insulin and drugs such as sulfonylureas, glipizide, tobutamide, acetohexamide, tolazimide, biguanides, and metformin (glucophage) which are ingested orally. Insulin self-injection is required in diabetic patients in which orally ingested drugs are not effective. Patients having Type 1 diabetes (also referred to as insulin dependent diabetes mellitus) are usually treated by self-injecting insulin. Patients suffering from Type 2 diabetes (also referred to as non-insulin dependent diabetes mellitus) are usually treated with a combination of diet, exercise, and an oral agent. When oral agents fail, insulin may be prescribed. When diabetic drugs are taken orally, usually multiple daily doses are often required.

Determination of the proper dosage of insulin requires frequent testing of the level of sugar in a patient's urine and/or blood. The administration of an excess dose of insulin generally causes hypoglycemia which has symptoms ranging from mild abnormalities in blood glucose to coma, or even death. Orally ingested drugs are, likewise, not without undesirable side effects. For example, such drugs can be ineffective in some patients and cause gastrointestinal disturbances or impair proper liver function in other individuals. There is always a need for improved drugs having fewer side effects and/or ones that succeed where others fail.

In Type 2 or non-insulin dependent diabetes mellitus, hepatic glucose production is an important target. The liver is the major regulator of plasma glucose levels in the fasting state. The rate of hepatic glucose production in Type 2 patients is typically significantly elevated when compared to non-diabetic individuals. For Type 2 diabetics, in the fed or postprandial state, the liver has a proportionately smaller role in the total plasma glucose supply, and hepatic glucose production is abnormally high.

The liver produces glucose by glycogenolysis (breakdown of the glucose polymer glycogen) and gluconeogenesis (synthesis of glucose from 2- and 3-carbon precursors). Glycogenolysis, therefore, is an important target for interruption of hepatic glucose production. There is some evidence to suggest that glycogenoloysis may contribute to the inappropriate hepatic glucose output in Type 2 diabetic patients. Individuals having liver glycogen storage diseases such as Hers' disease or glycogen phosphorylase deficiency often display episodic hypoglycemia. Further, in normal post-absorptive humans up to about 75% of hepatic glucose production is estimated to result from glycogenolysis.

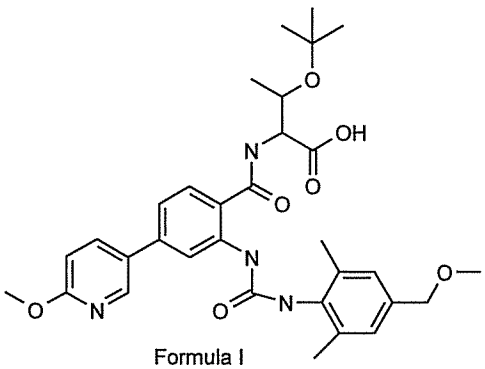
Glycogenolysis is carried out in liver, muscle, and brain by tissue-specific isoforms of the enzyme glycogen phosphorylase. This enzyme cleaves the glycogen macromolecule to release glucose-1-phosphate and a shortened glycogen macromolecule.

Glycogen phosphorylase inhibitors include glucose and its analogs, caffeine and other purine analogs, cyclic amines with various substitutents, acyl ureas, and indole-like compounds. These compounds and glycogen phosphorylase inhibitors, in general, have been postulated to be of potential use in the treatment of Type 2 diabetes by decreasing hepatic glucose production and lowering glycemia. Furthermore, it is believed desirable that a glycogen phosphorylase inhibitor be sensitive to glucose concentrations in blood.

Accordingly, what is desired is a new compound and pharmaceutical composition containing it for the treatment of diabetes and/or conditions associated with diabetes.

SUMMARY OF THE INVENTION

The present invention provides a compound of Formula I,



Formula I

salt, solvate, or physiological functional derivative thereof.

There is also provided a pharmaceutical composition comprising a compound of Formula I, salt, solvate, or physiologically functional derivative thereof.

Further, there is provided a pharmaceutical composition comprising a compound of Formula I, salt, solvate, or physiologically functional derivative thereof and one or more excipients.

There is still further provided a method of treatment comprising administering to a mammal, particularly a human, a pharmaceutical composition comprising a compound of Formula I, pharmaceutically acceptable salt, solvate, or physiologically functional derivative thereof and at least one excipient, wherein said treatment is for a disease or condition selected from the group consisting of diabetes, conditions associated with diabetes, and tissue ischemia, including myocardial ischemia.

Additionally, there is provided a compound of Formula I, salt, solvate, or physiologically functional derivative thereof for use as an active therapeutic substance (in therapy). And, there is also provided a compound of Formula I, salt, solvate, or physiologically functional derivative thereof for use in the treatment of diabetes, conditions associated with diabetes, and/or tissue ischemia, including myocardial ischemia in a mammal, especially a human.

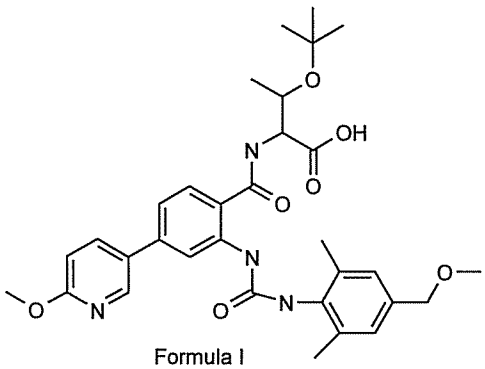
A process for preparing a compound of Formula I, salt, solvate, or physiologically functional derivative thereof is also provided.

DETAILED DESCRIPTION OF THE INVENTION

The activity of glycogen phosphorylase in muscle tissue is important for the generation of glucose and subsequently energy demand. Inhibition of muscle glycogen phosphorylase at the time of exercise may lead to muscle weakness and muscle tissue damage. Therefore, it may be desirable to have the compound of the present invention

which shows a greater effect on glycogen phosphorylase in the liver as compared to the muscle when given orally to mammals. The compound of the present invention shows a strong effect on liver glycogen content with little effect on muscle glycogen content and function after an oral dose. Consequently, the compound of the present invention could exhibit potent in vivo activity, have acceptable solubility and bioavailability properties, as well as having an improved safety/toxicity profile in view of its selectivity for liver tissue.

The present invention provides a compound of Formula I



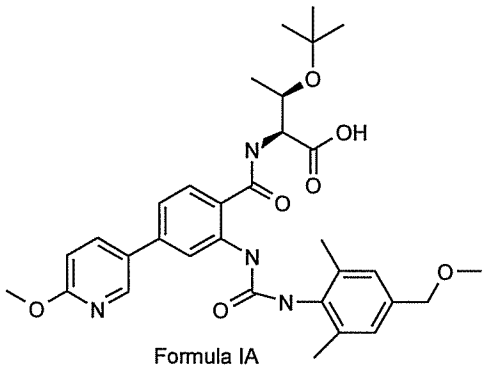
Formula I

salt, solvate, or physiological functional derivative thereof. The chemical name for a compound of Formula I is *O*-(1,1-dimethylethyl)-*N*-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)threonine.

The compound of Formula I or a salt, solvate, or physiologically functional derivative thereof may exist in stereoisomeric forms (e.g., it contains one or more asymmetric carbon atoms). The individual stereoisomers (enantiomers and diastereomers) and mixtures of these are included within the scope of the present invention. The invention also covers the individual isomers of the compound (salt, solvate or physiologically functional derivative) represented by Formula I as mixtures with isomers thereof in which one or more chiral centers are inverted. Likewise, it is understood that a compound (salt, solvate, or physiologically functional derivative) of Formula I may exist in tautomeric forms other than that shown in the formula and these are also included within the scope of the present invention. It is to be understood that the present invention includes all combinations and subsets of the particular groups defined hereinabove. The scope of the present invention includes mixtures of stereoisomers as well as purified enantiomers or enantiomerically/diastereomerically enriched mixtures. Also included within the scope of the invention are individual isomers of the compound represented by Formula I, as well as any wholly or partially equilibrated

mixtures thereof. The present invention also includes the individual isomers of the compound, salt, solvate, or derivative represented by the formula as well as mixtures with isomers thereof in which one or more chiral centers are inverted. It is to be understood that the present invention includes all combinations and subsets of the particular groups defined hereinabove.

The preferred stereochemistry of the compound is shown in Formula IA below:



Formula IA

It will be appreciated by those skilled in the art that the compound of the present invention may also be utilized in the form of a pharmaceutically acceptable salt, solvate, or physiologically functional derivative thereof.

Typically, but not absolutely, the salts of the present invention are pharmaceutically acceptable salts. Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compound of the invention. Salts of the compound of the present invention may include conventional salts formed from pharmaceutically acceptable inorganic or organic acids or bases as well as quaternary ammonium salts. These salts may comprise acid addition salts. In general, the salts are formed from pharmaceutically acceptable inorganic and organic acids. More specific examples of suitable acid salts include hydrochloric, hydrobromic, sulphuric, phosphoric, nitric, perchloric, fumaric, acetic, propionic, succinic, glycolic, formic, lactic, aleic, tartaric, citric, palmoic, malonic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, fumic, toluenesulfonic, methansulfonic (mesylate), naphthalene-2-sulfonic, benzenesulfonic, hydroxynaphthoic, hydroiodic, malic, teroic, tannic, steroic, and the like.

Other acids such as oxalic and trifluoroacetate, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining the compound of the invention and its pharmaceutically acceptable salts. More specific examples of suitable basic salts include sodium, lithium,

potassium, magnesium, aluminium, calcium, zinc, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, and procaine salts.

Other representative salts include acetate, benzenesulfonate, benzoate, bitartrate, borate, calcium edetate, camsylate, carbonate, clavulanate, citrate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylsulfate, monopotassium maleate, mucate, napsylate, nitrate, oxalate, pamoate (embonate), palmitate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate.

As used herein, the term "solvate" refers to a complex of stoichiometry formed by a solute (in this invention, a compound of Formula I, salt, or physiologically functional derivative thereof) and a solvent. Such solvents, for the purpose of the invention, may 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. Most preferably the solvent used is water and the solvate is a hydrate.

As used herein, the term "physiologically functional derivative" refers to any pharmaceutically acceptable derivative of a compound of the present invention that, 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, for example, esters and amides, will be clear to those skilled in the art, without undue experimentation. Reference may be made to the teaching of *Burger's Medicinal Chemistry and Drug Discovery*, 5th Edition, Volume 1: Principles and Practice, which is incorporated herein by reference to the extent that it teaches physiologically functional derivatives.

Processes for preparing pharmaceutically acceptable salts, solvates, and physiologically functional derivatives of the compound of Formula I are generally known in the art. See, for example, *Burger's Medicinal Chemistry and Drug Discovery*, 5th Edition, Volume 1: Principles and Practice.

The compound (salt, solvate, or physiologically functional derivative) of Formula I may be conveniently prepared by the process outlined below. The order of the foregoing

steps is not critical to the practice of the invention and the process may be practiced by performing the steps in any suitable order based on the knowledge of those skilled in the art. In addition some of the steps described may be combined without the isolation all intermediate compounds.

One general method of the synthesis of the compound of Formula I is outlined in Scheme 1 below. The commercially available starting materials methyl 4-chloro-2-nitrobenzoate (2) and [6-(methyloxy)-3-pyridinyl]boronic acid (3) can be coupled under standard conditions using a catalyst such as, but not limited to dichlorobis(tricyclohexylphosphine)palladium(II) or dichlorobis(triphenylphosphine)palladium(II) or tetrakis(triphenylphosphine)palladium in a solvent such as acetonitrile or DME and water in the presence of a base such as cesium fluoride or sodium carbonate to give intermediate 4. Hydrolysis of the ester of intermediate 4 under basic conditions such as lithium hydroxide or sodium hydroxide in solvents which include tetrahydrofuran (THF) and/or methanol (MeOH) and/or water and/or 1,4-dioxane gives the corresponding carboxylic acid (5).

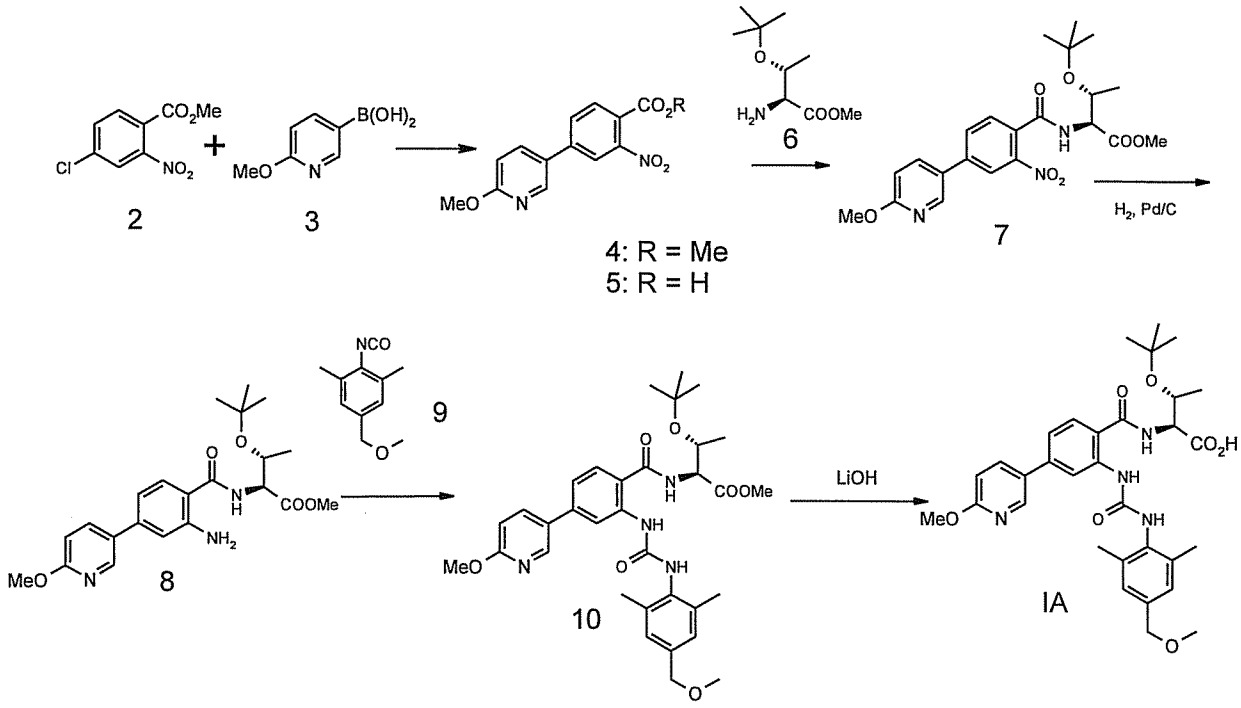
Intermediate 7 is formed by mixing the carboxylic acid (5) with methyl *O*-(1,1-dimethylethyl)-L-threoninate (6) or its hydrochloride salt under standard coupling conditions. These conditions include, but are not limited to, the use of EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride), PyBop (Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate), PyBrOP (Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate), HOBT (N-hydroxybenzotriaole), HOAT (N-hydroxy-9-azabenzotriaole), or DIC (N, N'-diisopropylcarbodiimide), or HATU (2-(1H-9-Azabenzotriazxole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and DIEA (N,N-diisopropylethylamine) or triethylamine at room temperature. Solvents that can be used include DMSO, NMP or preferably DMF. In a preferred method, intermediates 5 and 6 are combined in ethyl acetate in the presence of 1-propanephosphonic acid cyclic anhydride and an organic base such as DIEA or triethylamine to yield intermediate 7.

Reduction of the nitro group of 7 under standard conditions such as, but not limited to, treatment with palladium on carbon under a hydrogen atmosphere in a solvent such as ethyl acetate or methanol yields intermediate 8.

Intermediate 10 is formed by mixing intermediate 8 with the isocyanate, intermediate 9 (method of synthesis outlined below, see Scheme 2) and diisopropylethylamine (DIEA) or triethylamine, in a solvent such as DMF. Preferably intermediates 8 and 9 are combined in pyridine to give intermediate 10.

The final product is formed by cleavage of the ester of intermediate 10 under basic conditions such as lithium hydroxide or sodium hydroxide in solvents which include tetrahydrofuran (THF) and/or methanol (MeOH) and/or water and/or 1,4-dioxane.

Scheme 1: Synthesis of a Compound of Formula I.

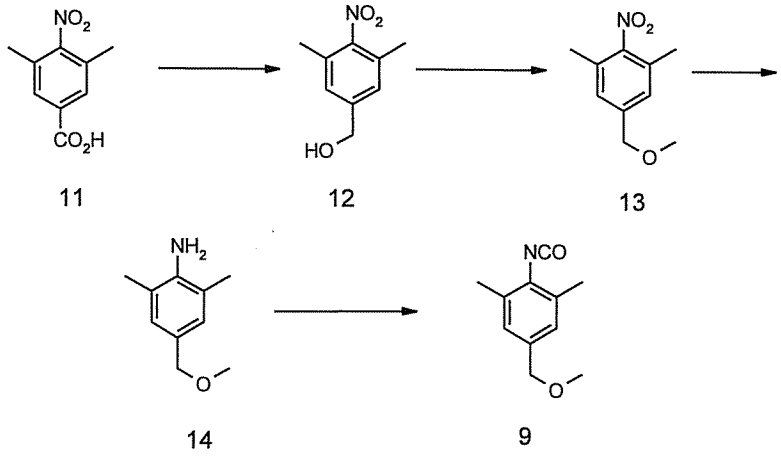


Synthesis of the other isomers of Formula I can be accomplished by utilizing the corresponding isomers, including racemates, of Formula 6.

One general method of the synthesis of intermediate 9 is outlined in Scheme 2 below. Reduction of 11 with sodium borohydride in the presence of boron trifluoride diethyl etherate will give intermediate 12. Methylation of intermediate 12 can be carried out by treatment with a base such as sodium hydride followed by treatment with a methylating agent such as iodomethane or dimethyl sulfate in a solvent such as DMF or NMP to give intermediate 13. Likewise 12 can be reacted with dimethyl sulfate in the presence of aqueous sodium hydroxide and benzyl triethylammonium chloride in toluene (biphasic system) to give intermediate 13. In another method, intermediate 12 can be converted to the corresponding bromide using standard conditions such as treatment with phosphorus tribromide in dichloromethane. The resulting bromide can be converted to intermediate 13 by treatment with sodium methoxide in methanol. Reduction of intermediate 13 can be carried out by treating with zinc and a base such as sodium

hydroxide in a solvent such as ethanol and/or water to give intermediate 14. In an alternative method the reduction of intermediate 13 can be carried out by treating with PtO_2 and hydrogen in a solvent such as ethanol. Intermediate 9 is then obtained by treatment of intermediate 14 with phosgene or triphosgene and a base such as DIEA in a solvent such as dichloromethane.

Scheme 2: Synthesis of Intermediate 9.



The invention further provides a pharmaceutical composition (also referred to as pharmaceutical formulation) comprising a compound of Formula I, salt, solvate, or physiologically functional derivative thereof and one or more excipients (also referred to as carriers and/or diluents in the pharmaceutical arts). The excipients are acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof (i.e., the patient).

In accordance with another aspect of the invention there is provided a process for the preparation of a pharmaceutical composition comprising mixing (or admixing) a compound of Formula I, salt, solvate, or physiologically functional derivative thereof with at least one excipient.

Pharmaceutical compositions may be in unit dose form containing a predetermined amount of active ingredient per unit dose. Such a unit may contain a therapeutically effective dose of the compound of Formula I, salt, solvate, or physiologically functional derivative thereof or a fraction of a therapeutically effective dose such that multiple unit dosage forms might be administered at a given time to achieve the desired therapeutically effective dose. 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. Furthermore, such pharmaceutical compositions may be prepared by any of the methods well-known in the pharmacy art.

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

When adapted for oral administration, pharmaceutical compositions may be in discrete units such as tablets or capsules; powders or granules; solutions or suspensions in aqueous or non-aqueous liquids; edible foams or whips; oil-in-water liquid emulsions or water-in-oil liquid emulsions. The compound (salt, solvate, or derivative) of the invention or pharmaceutical composition of the invention may also be incorporated into a candy, a wafer, and/or tongue tape formulation for administration as a "quick-dissolve" medicine.

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. Powders or granules are prepared by comminuting the compound to a suitable fine size and mixing with a similarly comminuted pharmaceutical carrier such as an edible carbohydrate, as, for example, starch or mannitol. Flavoring, preservative, dispersing, and coloring agents can also be present.

Capsules are made by preparing a powder mixture, as described above, and filling formed gelatin or non-gelatinous sheaths. Glidants and lubricants such as colloidal silica, talc, magnesium stearate, calcium stearate, solid polyethylene glycol can be added to the powder mixture before the filling operation. A disintegrating or solubilizing agent such as agar-agar, calcium carbonate, or sodium carbonate can also be added to improve the availability of the medicine 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. Suitable binders include starch, gelatin, natural sugars, such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth, sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate,

sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrators include, without limitation, starch, methylcellulose, 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, and aliginate, 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 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 compound (salt, solvate, or derivative) 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 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. Dyestuffs can be added to these coatings to distinguish different 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 active ingredient. Syrups can be prepared by dissolving the compound (salt, solvate, or derivative) of the invention in a suitably flavoured aqueous solution, while elixirs are prepared through the use of a non-toxic alcoholic vehicle. Suspensions can be formulated by dispersing the compound (salt, solvate, or derivative) of the invention in a non-toxic vehicle. Solubilizers and emulsifiers, such as ethoxylated isostearyl alcohols and polyoxyethylene sorbitol ethers, preservatives, flavor additives such as peppermint oil, 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.

In the present invention, tablets and capsules are preferred for delivery of the pharmaceutical composition.

As used herein, the term "treatment" includes prophylaxis and refers to alleviating the specified condition, eliminating or reducing one or more symptoms of the condition, slowing or eliminating the progression of the condition, and preventing or delaying the reoccurrence of the condition in a previously afflicted or diagnosed patient or subject. Prophylaxis (or prevention or delay of disease onset) is typically accomplished by administering a drug in the same or similar manner as one would to a patient with the developed disease or condition.

The present invention provides a method of treatment in a mammal, especially a human, suffering from diabetes or a related condition such as obesity, syndrome X, insulin resistance, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, hyperglycemia, hypercholesterolemia, hyperinsulinemia, hyperlipidemia, cardiovascular disease, stroke, atherosclerosis, lipoprotein disorders, hypertension, tissue ischemia, myocardial ischemia, and depression. Such treatment comprises the step of administering a therapeutically effective amount of a compound of Formula I, salt, solvate, or physiologically functional derivative thereof to said mammal, particularly a human. Treatment can also comprise the step of administering a therapeutically effective amount of a pharmaceutical composition containing a compound of Formula I, salt, solvate, or physiologically functional derivative thereof to said mammal, particularly a human.

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.

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.

For use in therapy, therapeutically effective amounts of a compound of Formula I, as well as salts, solvates, and physiologically functional derivatives thereof, may be administered as the raw chemical. Additionally, the active ingredient may be presented as a pharmaceutical composition.

While it is possible that, for use in therapy, a therapeutically effective amount of a compound of Formula I (salt, solvate, or physiologically functional derivative thereof) may be administered as the raw chemical, it is typically presented as the active ingredient of a pharmaceutical composition or formulation.

The precise therapeutically effective amount of a compound (salt, solvate, or physiologically functional derivative) of the invention will depend on a number of factors, including, but not limited to, the age and weight of the subject (patient) being treated, the precise disorder requiring treatment and its severity, the nature of the pharmaceutical formulation/composition, and route of administration, and will ultimately be at the discretion of the attending physician or veterinarian. Typically, a compound of Formula I (salt, solvate, or physiologically functional derivative thereof) will be given for the treatment in the range of about 0.1 to 100 mg/kg body weight of recipient (patient, mammal) per day and more usually in the range of 0.1 to 10 mg/kg body weight per day. Acceptable daily dosages may be from about 1 to about 1000 mg/day, and preferably from about 1 to about 100 mg/day. 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, solvate, or physiologically functional derivative thereof, may be determined as a proportion of the effective amount of the compound of Formula I per se. Similar dosages should be appropriate for treatment (including prophylaxis) of the other conditions referred herein for treatment. In general, determination of appropriate dosing can be readily arrived at by one skilled in medicine or the pharmacy art.

Additionally, the present invention comprises a compound of Formula I, salt, solvate, or physiological functional derivative thereof, or a pharmaceutical composition thereof with at least one other anti-diabetic drug. Such anti-diabetic drugs can include, for example, injected insulin and drugs such as sulfonylureas, thiazolidinediones, glipizide, glimepiride, tobutamide, acetohexamide, tolazimide, biguanides, rosiglitazone, metformin (glucophage), sitagliptin (Januvia) salts or combinations thereof, and the like, which are ingested orally. When a compound of the invention is employed in combination with another anti-diabetic drug, it is to be appreciated by those skilled in the art that the dose of each compound or drug of the combination may differ from that when the drug or compound is used alone. Appropriate doses will be readily appreciated and determined by those skilled in the art. The appropriate dose of the compound of Formula I (salt, solvate, physiologically functional derivative thereof) and the other

therapeutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect, and are with the expertise and discretion of the attending doctor or clinician.

EXPERIMENTAL

The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way, the invention being defined by the claims. Unless otherwise noted, reagents are commercially available or are prepared according to procedures in the literature.

Example 1: Preparation of the Compound of Formula IA: *O*-(1,1-Dimethylethyl)-*N*-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threonine

Step 1. Methyl 4-[6-(methyloxy)-3-pyridinyl]-2-nitrobenzoate

Two microwave vials were each charged with methyl 4-chloro-2-nitrobenzoate (0.5 g, 2.32 mmol), [6-(methyloxy)-3-pyridinyl]boronic acid (0.51 g, 3.48 mmol), dichlorobis(tricyclohexylphosphine)palladium(II) (0.137 g, 0.186 mmol) and cesium fluoride (1.76 g, 11.6 mmol). To each vial was added acetonitrile (9 mL) and water (1.5 mL). Each vial was heated at 150 °C for 6 min. After cooling to RT the contents of the vials were combined, diluted with ethyl acetate (100 mL), washed with 50 % brine, dried over sodium sulfate and concentrated under reduced pressure. This material was combined with another 0.5 g scale (4-chloro-2-nitrobenzoate) reaction and chromatographed on silica gel with hexane/ethyl acetate gave 2.0 g of the product as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.44 (s, 1H), 8.01 (s, 1H), 7.87-7.80 (m, 3H), 6.88 (d, *J*=8.7 Hz, 1H), 4.01 (s, 3H), 3.94 (s, 3H).

Step 2. 4-[6-(Methyloxy)-3-pyridinyl]-2-nitrobenzoic acid.

Lithium hydroxide monohydrate (1.748 g, 41.6mmol) in water (15 mL) was added to a solution of methyl 4-[6-(methyloxy)-3-pyridinyl]-2-nitrobenzoate (2.0 g, 6.94 mmol) in THF (50 mL) and Methanol (20 mL). The mixture was stirred at RT for ca. 4.5 h. The

reaction mixture was acidified with 1N aqueous HCl (100 mL) and extracted with ethyl acetate. The organic phase was dried over sodium sulfate, filtered and concentrated under reduced pressure to give 1.9 g (100% yield) of an off white solid. ^{1}H NMR (400 MHz, DMSO-D6) δ ppm: 13.9 (brs, 1H), 8.65 (d, *J* = 2.7 Hz, 1H), 8.27 (d, *J* = 1.6 Hz, 1H), 8.18 (dd, *J* = 2.6, 8.7, 1H), 8.08 (dd, *J* = 1.9. 8.0 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 6.97 (d, *J* = 8.6 Hz, 1H), 3.91 (s, 3H).

Step 3. Methyl *O*-(1,1-dimethylethyl)-*N*-({4-[6-(methyloxy)-3-pyridinyl]-2-nitrophenyl}carbonyl)-L-threoninate.

HATU (3.95 g, 10.4 mmol) was added to a solution of 4-[6-(methyloxy)-3-pyridinyl]-2-nitrobenzoic acid (1.90 g, 6.93 mmol), methyl *O*-(1,1-dimethylethyl)-L-threoninate hydrochloride (1.72 g, 7.6 mmol) and diisopropylethylamine (1.79 g, 13.86 mmol) in DMF (100 mL). The mixture was stirred at RT for ca. 24 h. Most of the DMF was removed under reduced pressure and the residue was dissolved in ethyl acetate (100 mL) and washed with 1 N HCl (50 mL), saturated sodium bicarbonate (50 mL) and brine (50 mL). The ethyl acetate phase was dried over sodium sulfate, filtered and concentrated under reduced pressure. Chromatography on silica gel with hexane/ethyl acetate gave 2.9 g (94% yield) of the product as an amber oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.44 (d, *J* = 2.6 Hz, 1H), 8.21 (d, *J* = 1.9 Hz, 1H), 7.85-7.81 (m, 2H), 7.73 (d, *J* = 7.8 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 1H), 6.66 (d, *J* = 9.5 Hz, 1H), 4.76 (dd, *J* = 1.7, 9.3 Hz, 1H), 4.36 (m, 1H), 4.01 (s, 3H), 3.79 (s, 3H), 1.36 (d, *J* = 6.3 Hz, 3H), 1.13 (s, 9H).

Step 4. Methyl *N*-({2-amino-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-*O*-(1,1-dimethylethyl)-L-threoninate.

Palladium (10% on carbon, 2.0 g) was added to a solution of methyl *O*-(1,1-dimethylethyl)-*N*-({4-[6-(methyloxy)-3-pyridinyl]-2-nitrophenyl}carbonyl)-L-threoninate (2.90 g, 6.51 mmol) in methanol (125 mL) under a nitrogen atmosphere. The reaction was evacuated and flushed with hydrogen. The mixture was then stirred under a hydrogen atmosphere for ca. 18 h. After flushing with nitrogen the mixture was filtered and the solvent evaporated under reduced pressure to give 2.45 g (91% yield) of the product as a foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.39 (d, *J* = 2.7 Hz, 1H), 7.78 (dd,

J = 2.5, 8.6 Hz, 1H), 7.55 (d, J = 8.0 Hz, 1H), 6.88-6.81 (m, 4H), 5.61 (brs, 2H), 4.68 (dd, J = 1.9, 9.3 Hz, 1H), 4.33-4.31 (m, 1H), 3.98 (s, 3H), 3.75 (s, 3H), 1.27 (d, J = 6.4 Hz, 3H), 1.16 (s, 9H).

Step 5. (3,5-Dimethyl-4-nitrophenyl)methanol.

To a suspension of sodium borohydride (2.91 g, 76.7 mmol) in THF (100 mL) was added 3,5-dimethyl-4-nitrobenzoic acid (8.5 g, 43.55 mmol), after stirring ca. 5 min boron trifluoride diethyl etherate (14.53 g, 102.4 mmol) was added dropwise. The reaction was stirred at room temperature for ca. 16 hours. The reaction was poured slowly into water (150 mL) and extracted with ethyl acetate (2 X 300 mL), the organic phase was washed with brine, dried over sodium sulfate and concentrated under reduced pressure to give 8.05 g (102 % yield) of product as an off white solid. ^{1}H NMR (400 MHz, DMSO-D6) δ ppm: 7.19 (s, 2H), 4.48 (s, 2H), 2.23 (s, 6H).

Step 6. 1,3-Dimethyl-5-[(methyloxy)methyl]-2-nitrobenzene

To (3,5-dimethyl-4-nitrophenyl)methanol (7.0 g, 38.68 mmol) in DMF (150 mL) was added sodium hydride (1.85 g of 60 % oil dispersion, 46.36 mmol). After stirring for ca. 40 min, methyl iodide was added and the reaction was stirred at RT for 3 days. The reaction was quenched by the slow addition of water (500 mL) and extracted with ethyl acetate (2 X 500 mL). The organic phase was washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate/hexanes) to give 5.3 g (70%) of the product. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.09 (s, 2H), 4.42 (s, 2H), 3.41 (s, 3H), 2.31 (s, 6H).

Step 7. 2,6-Dimethyl-4-[(methyloxy)methyl]aniline

1,3-Dimethyl-5-[(methyloxy)methyl]-2-nitrobenzene (5.0 g, 25.6 mmol) was dissolved in EtOH (120 mL) and warmed to 80 °C. A solution of NaOH (5.9 g, 128 mmol) in water (10 mL) was added, followed by the addition zinc (15 g, 230 mmol) in 5 g portions. Once addition was complete the solution was refluxed for ca. 4 h and then cooled and stirred at RT for 3 days. The mixture was then filtered and the filtrate was

concentrated and the residue was partitioned between ethyl acetate and brine. The brine layer was extracted with ethyl acetate, and the combined organics were dried over sodium sulfate, filter and concentrated under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate/hexanes) to give 4.5 g (106%) of the product as a oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.93 (s, 2H), 4.31 (s, 2H), 3.58 (brs, 2H), 3.34 (s, 3H), 2.18 (s, 6H).

Step 8. 2-Isocyanato-1,3-dimethyl-5-[(methyloxy)methyl]benzene

To a mixture of 2,6-dimethyl-4-[(methyloxy)methyl]aniline (3.45 g, 20.88 mmol), and N,N-(diisopropy)aminomethylpolystyrene (PS-DIEA, Argonaut, 17.6 g, load of 3.56 mmol/g) in dichloromethane (200 mL) was added phosgene (5.17 g of 25 % toluene solution, 52.2 mmol) over ca. 2-3 min. The mixture was stirred at RT for ca. 24 h and then filtered to remove the PS-DIEA. Concentration under reduce pressure gave 4.0 g (100%) of the product as a tan oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.02 (s, 2H), 4.36 (s, 2H), 3.38 (s, 3H), 2.32 (s, 6H).

Step 9. Methyl *O*-(1,1-dimethylethyl)-*N*-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threoninate

Methyl *N*-({2-amino-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-*O*-(1,1-dimethylethyl)-L-threoninate (2.45 g, 5.89 mmol) and 2-isocyanato-1,3-dimethyl-5-[(methyloxy)methyl]benzene (2.25 g, 11.79 mmol) were dissolved in pyridine (80 mL) and stirred for ca. 24 h. The reaction was concentrated under reduce pressure and the residue was dissolved in ethyl acetate, and filtered. The ethyl acetate phase was washed with sodium bicarbonate and brine. After drying over sodium sulfate, filtering and concentrating under reduced pressure the residue was chromatographed on silica gel (ethyl acetate/hexanes) to give 3.2 g (89 %) of the product. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.20 (brs, 1H), 8.80 (s, 1H), 8.46 (d, *J* = 2.5 Hz, 1H), 7.89 (dd, *J* = 2.7, 8.5 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 7.21 (d, *J* = 8.3 Hz, 1H), 7.10 (s, 2H), 6.82 (m, 2H), 5.97 (brs, 1H), 4.51 (m, 1H), 4.43 (s, 2H), 4.29 (m, 1H), 3.98 (s, 3H), 3.78 (s, 3H), 3.39 (s, 3H), 2.31 (s, 6H), 1.20 (d, *J* = 5.6, 3H), 1.14 (s, 9H).

Step 10. *O*-(1,1-Dimethylethyl)-*N*-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threonine

Methyl O-(1,1-dimethylethyl)-N-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threoninate (3.2 g, 5.27 mmol) was dissolved in THF (150 mL) and methanol (50 mL). To this was added lithium hydroxide monohydrate (1.328 g, 31.6 mmol) in water (50 mL). The mixture was stirred at RT for ca. 24 h. To the mixture was added 1N HCl (200 mL) and it was extracted with ethyl acetate (2 X 300 mL). The organic phase was dried over sodium sulfate, filtered, and concentrate under reduced pressure to give 3.16 g (100 %) of the product as a white foam. ^{1}H NMR (400 MHz, DMSO-D6) δ ppm: 12.84 (brs, 1H), 10.12 (brs, 1H), 8.77 (brs, 1H), 8.56 (s, 1H), 8.46 (d, J = 2.5 Hz, 1H), 8.10 (brs, 1H), 7.95 (dd, J = 2.4, 8.5 Hz, 1H), 7.76 (brs, 1H), 7.33 (dd, J = 1.7, 8.3 Hz, 1H), 7.00 (s, 2H), 6.92 (d, J = 8.8 Hz, 1H), 4.44 (m, 1H), 4.32 (s, 2H), 4.18 (m, 1H), 3.89 (s, 3H), 3.26 (s, 3H), 2.17 (s, 6H), 1.18-1.13 (m, 12H). ES MS m/z 593 (M+H).

Example 2: Preparation of the Potassium Salt of the Compound of Formula IA.

Potassium O-(1,1-dimethylethyl)-N-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threoninate

To *O*-(1,1-Dimethylethyl)-*N*-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threonine (1.0 g, 1.69 mmol) in acetonitrile (100 mL) is added potassium t-butoxide (1.0 M in THF, 1.69 mL). The mixture is stirred for ca. 15 min and the solvent is removed under reduced pressure to give the product.

Biological Protocols

The utility of the compounds of Formula I, a salt, solvate, or physiologically functional derivative thereof, in the treatment or prevention of diseases (such as detailed herein) in animals, particularly mammals (e.g., humans) may be demonstrated by the

activity in conventional assays known to one of ordinary skill in the relevant art, including the in vitro and in vivo assays described below.

The purified glycogen phosphorylase (GP) enzyme, wherein glycogen phosphorylase is in the activated "a" state, referred to as human liver glycogen phosphorylase a (HLGPa), can be obtained according to the following procedures.

Appropriate Cloning and Expression of Human Liver Glycogen Phosphorylase:

Human liver glycogen phosphorylase cDNA was amplified by polymerase chain reaction (PCR) from a commercially available human liver cDNA library (BD Biosciences). The cDNA was amplified as 2 overlapping fragments using the primers 5'GGCGAAGCCCCTGACAGACCAGGAGAAG3' with 5'CGATGTCTGAGTGGATTTTAGCCACGCC3' and 5'GGATATAGAAGAGTTAGAAGAAATTG3' with 5'GGAAGCTTATCAATTTCCATTGACTTTGTTAGATTCATTGG3'. PCR conditions were 94°C 1 min., 55°C 1 min., 72°C 2 min. for 40 cycles using the enzyme Pfu Turbo (Stratagene), 0.5% DMSO, 250uM each nucleotide triphosphate, and 0.4uM each primer plus the buffer recommended by the polymerase manufacturer. Each PCR fragment was molecularly cloned and the DNA sequence of each insert was determined. The 2 DNA fragments of the glycogen phosphorylase cDNA were then joined together in a bacterial expression plasmid, pTXK1007LTev (GlaxoSmithKline), creating a full-length cDNA fused at the 5' end to codons for methionine-glycine-alanine-histidine-histidine-histidine-histidine-histidine-histidine-glycine-glycine-glutamate-asparagine-leucine-tyrosine-phenylalanine-glutamine-glycine-glycine-. The protein product would have a 6Xhistidine tag followed by a Tev protease cleavage site. The DNA sequence of both strands of the cDNA in pTXK1007LTev was determined.

Purification of Human Liver Glycogen Phosphorylase:

The frozen cell paste (100g) was thawed and suspended in 1200ml of 50mM Tris, 100mM NaCl, 15 mM imidazole, pH 8.0. The cells were disrupted gently with a Polytron (Brinkman, PT10-35), and passed twice through an AVP homogenizer. The E. coli cell lysates were clarified by centrifugation at 27,500 x g for 45 minutes and filtered through a 0.8 micron filter. The solution was applied to a 21ml Ni-NTA Superflow (Qiagen) column (ID 26mm X H 4.0 cm) pre-equilibrated with 50mM Tris, 100mM NaCl,

and 15 mM imidazole, pH 8.0. The column was washed with equilibration buffer until the A280 returned to baseline. The weakly bound proteins were eluted from the column with 10 bed column volumes of 50mM imidazole in the same buffer. The glycogen phosphorylase was eluted with steps of 100 mM and 250 mM imidazole. Both the100mM and 250 mM fractions were pooled and then diluted 5 fold with 50mM Tris, pH 8.0 buffer. This solution was loaded on a 21ml Q fast flow column (Amersham Pharmacia Biotech AB, ID 2.6cm X H 4.0 cm) pre-equilibrated with 50 mM Tris, pH 8.0. Glycogen phosphorylase was eluted with a continuous gradient from 0- 30% of 1M NaCl in 50 mM Tris, pH 8.0 (buffer B). Fractions of purified glycogen phosphorylase between 15% and 20% buffer B were pooled, aliquoted into microfuge tubes, and stored at -80°C. The purified fraction formed a single ~100kd band on a SDS-PAGE gel.

Activation of Human Liver Glycogen Phosphorylase:

The activation of human liver glycogen phosphorylase (i.e., conversion of the inactive HLGPb form to the activated HLGPa form) was achieved by phosphorylating HLGPb with immobilized phosphorylase kinase.

10mg of phosphorylase kinase (Sigma, P-2014) was dissolved in 2.5 ml of 100mM HEPES, 80mM CaCl2 (pH 7.4) and gently mixed with 1ml of Affi-Gel (Active Ester Agarose, BioRad # 153-6099) beads previously equilibrated in the same buffer. The mixture was rocked 4 hours at 4°C. The beads were washed once with the same buffer and blocked for 1 hour at room temperature with a solution of 50mM HEPES, 1M glycine methyl ester, pH 8.0. The beads were then washed with 50mM HEPES, 1 mM β-mercaptoethanol, pH 7.4 and stored at 4°C.

Frozen purified glycogen phosphorylase (HLGPb) was thawed in at 4°C then dialyzed overnight into 50 mM HEPES, 100mM NaCl, pH 7.4. 15 mg of the dialyzed HLGPb, 3mM ATP and 5mM MgCl2 was incubated with 500ul of the prepared Affi-Gel immobilized phosphorylase kinase beads equilibrated with 50mM HEPES, 100mM NaCl, pH 7.4. The degree of phosphorylation was monitored by following the increase in activity at 10 minute intervals using the assay system outlined below. Briefly, the assay contained 0.1 uM human liver glycogen phosphorylase, 50mM HEPES, 100mM KCl, 2.5 mM EGTA, $MgCl_2$, 3.5 mM KH_2PO_4, 0.5mM DTT, 0.4mg/mL glycogen, 7.5 mM Glucose, 0.50 mM β-nicotinamide adenine dinucleotide (β-NAD), 3 U/mL phosphoglucomutase, and 5 U/mL glucose-6-phosphate dehydrogenase, Activity was monitored by following the reduction of NAD^+ at 340 nm. The reaction was stopped by removal of the beads

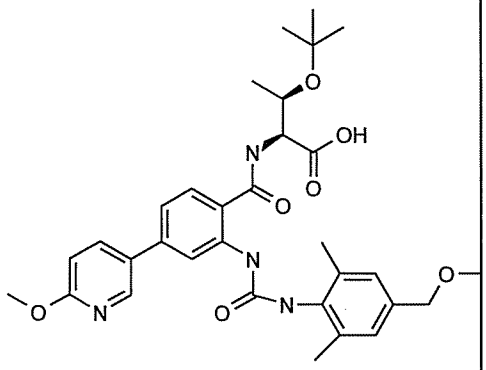
from the mixture when no further increase in activity was observed (30-60 minutes). Phosphorylation was further confirmed by analysis of the sample by mass spectroscopy. The supernatant containing the activated sample was dialyzed in 50mM HEPES, 100mM NaCl, pH 7.4 overnight. The final sample was mixed with an equal volume of glycerol, aliquoted into microfuge tubes and stored at -20°C.

Human Liver Glycogen Phosphorylase a Enzymatic Activity Assay:

An enzymatic assay was developed to measure the response of the activated form of glycogen phosphorylase (HLGPa) to small molecule (<1000 Da.) compounds. The assay was configured to monitor the pharmacologically relevant glycogenolytic reaction by coupling the production of glucose-1-phosphate from glycogen and inorganic phosphate to phosphoglucomutase, glucose-6-phosphate dehydrogenase, NADH oxidase and horseradish peroxidase to produce the fluorescent product resorufin. The concentrations of the reagent components were as follows: 15 nM human liver glycogen phosphorylase a, 1mg/mL glycogen, 5 mM K_2HPO_4, 40 U/mL phosphoglucomutase (Sigma), 20 U/mL glucose-6-phosphate dehydrogenase (Sigma), 200 nM Thermus thermophilus NADH oxidase (prepared as described in Park, H.J.; Kreutzer, R.; Reiser, C.O.A.; Sprinzl, M. *Eur. J. Biochem.* 1992, 205, 875-879.), 2 U/mL horseradish peroxidase (Sigma), 30 uM FAD, 250 uM NAD^+, 50 uM amplex red, +/- 10 mM glucose. The base assay buffer used was 50 mM HEPES, 100 mM NaCl, pH 7.6. To aid in the identification of glucose-sensitive inhibitors of glycogen phosphorylase, the assay was performed with and without 10 mM glucose. In order to scrub the assay of contaminating components that may contribute to non-HLGPa specific resorufin production, the reagents were prepared as two 2x concentrated cocktails. A solution of catalase-coated agarose beads was prepared in the base assay buffer. The first cocktail (cocktail #1) consisted of Thermus thermophilus NADH oxidase, NAD^+, glycogen, phosphoglucomutase, glucose-6-phosphate dehydrogenase, K_2HPO_4, FAD, and 50U/mL catalase-coated agarose beads +/- 10 mM glucose. Amplex red was added to this solution after incubation at 25°C for 30 minutes and the catalase-coated agarose beads were removed by centrifugation and retention of supernatant. The second cocktail (cocktail #2) contained human liver glycogen phosphorylase-a and horseradish peroxidase +/- 10 mM glucose. The assays were performed with preincubation of compounds of this invention with cocktail #2 for 15 minutes, followed by the addition of cocktail #1 to initiate the reaction. The assays were performed in 96 (black ½ volume

Costar #3694) or 384-well microtiter plates (small volume black Greiner). The change in fluorescence due to product formation was measured on a fluorescence plate reader (Molecular Devices SpectraMax M2) with excitation at 560 nm and emission at 590 nm. Activity of example compound 1 is shown in Table 1 below.

Table 1: Activity of the compound in human liver glycogen phosphorylase a enzymatic assay.

Ex #	Structure	Chemical Name	ESMS +m/z	IC50 (uM)
1		*O*-(1,1-Dimethylethyl)-*N*-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threonine	593 (M+H)	0.005

In Vivo Glucagon Challenge Model:

Jugular vein cannulated male CD rats (220-260g) (Charles Rivers, Raleigh, NC) were received 1-2 days after cannulation, housed individually on Alpha-dri™ bedding (Shepherd Specialty Papers, Inc., Kalamazoo, MI) with free access to food (Lab Diet 5001, PMI Nutrition International, Brentwood, MO) and water and maintained on a 12h light/dark cycle at 21°C and 50% relative humidity for 3-4 days prior to the glucagon challenge studies. On the day of the study, the rats were sorted by body weight into treatment groups (N=4-5) and housed individually in shoe box cages with clean Alpha-dri bedding. The cannula lines were opened by removal of 0.2 ml blood and flushed with 0.2 ml sterile saline. After a one hour acclimation, blood samples were collected to determine basal glucose and the rats were orally dosed with vehicle (5% DMSO: 30% Solutol HS15: 20% PEG400: 45% 25 mM N-methylglucamine) or drug (5 ml/kg). Two hr after drug dosing, a time zero blood sample (0.4 ml) was collected for determination of glucose and the rats were dosed through the jugular vein with Sandostatin, 0.5 mg/kg, (Novartis Pharmaceuticals Corp., East Hanover, NJ) and glucagon, 10 ug/kg (Bedford Laboratories, Bedford, OH). Blood samples were collected after 10 and 20 min for

glucose determination. Whole blood was placed in a Terumo Capiject blood collection tube (Terumo Medical Corp., Elkton, MD), allowed to sit at room temperature for 20-30 minutes and then centrifuged (3,000 X G) to obtain serum. Serum levels of glucose were determined using an Olympus AU640™ clinical chemistry immuno-analyzer (Olympus America Inc., Melville, NY). The % reduction (% R) of the vehicle glucose AUC was calculated for each drug treatment using the formula % reduction = 100 * 1-(AUC drug/AUC vehicle), where AUC was calculated from serum glucose values using the equation AUC = (T0+T10)/2*10 + (T10+T20)/2*10 – (T0*20). Activity of example compound 1 is shown in table 2 below.

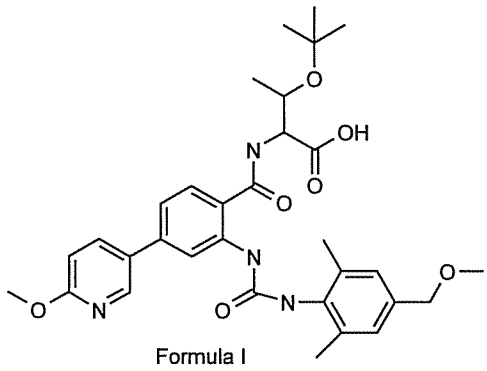
Table 2: Activity of the compound in the in vivo glucagon challenge model.

Dose of *O*-(1,1-Dimethylethyl)-*N*-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threonine (compound 1) (mg/kg)	% R
0.5	32
2	53
5	63
15	70

CLAIMS

What is claimed is:

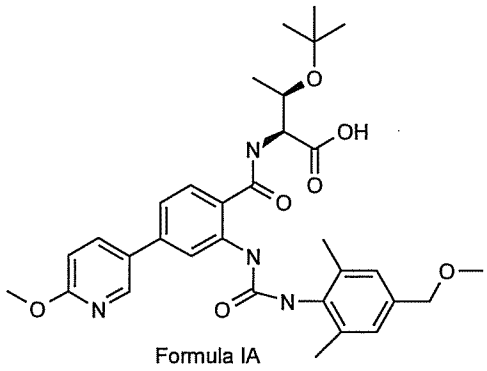
1. A compound of Formula I



Formula I

salt, solvate, or physiological functional derivative thereof.

2. The compound of Claim 1 wherein the stereochemistry is that shown in Formula IA



Formula IA

3. A pharmaceutical composition comprising a compound of Claim 1 or 2, salt, solvate, or physiologically functional derivative thereof.

4. A pharmaceutical composition comprising a compound of Claim 1 or 2, a salt, a solvate, or physiologically functional derivative thereof and one or more excipients.

5. The pharmaceutical composition of Claim 1 or 2 in the form of a tablet or capsule.

6. A method of treatment comprising the administering to a mammal a pharmaceutical composition comprising a compound of Claim 1 or 2, a pharmaceutically acceptable salt, solvate, or physiologically functional derivative thereof and at least one excipient, wherein said treatment is for a disease or condition selected from the group consisting of diabetes and conditions associated with diabetes.

7. The method of Claim 5 wherein said conditions associated with diabetes are selected from the group consisting of obesity, syndrome X, insulin resistance, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, hyperglycemia, hypercholesterolemia, hyperinsulinemia, hyperlipidemia, cardiovascular disease, stroke, atherosclerosis, lipoprotein disorders, hypertension, tissue ischemia, myocardial ischemia, and depression.

8. The method of Claim 6 said treatment is for diabetes.

9. The method of Claim 6 wherein said mammal is a human.

10. A compound of Claim 1 or 2, salt, solvate, or physiologically functional derivative thereof for use as an active therapeutic substance.

11. A compound of Claim 1 or 2, salt, solvate, or physiologically functional derivative thereof for use in therapy.

12. A compound of Claim 1 or 2, salt, solvate, or physiologically functional derivative thereof for use in the treatment of a disease or condition selected from the group consisting of diabetes, obesity, syndrome X, insulin resistance, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, hyperglycemia, hypercholesterolemia, hyperinsulinemia, hyperlipidemia, cardiovascular disease, stroke, atherosclerosis, lipoprotein disorders, hypertension, tissue ischemia, myocardial ischemia, and depression in a mammal.

13. The compound of Claim 12 for use in the treatment of diabetes.

14. The compound of Claim 12 wherein said mammal is a human.

15. A process for preparing a compound of Claim 1 or 2, salt, solvate, or physiologically functional derivative thereof comprising the steps of:

a. conversion of 4-chloro-2-nitrobenzoate and [6-(methyloxy)-3-pyridinyl]boronic acid to methyl 4-[6-(methyloxy)-3-pyridinyl]-2-nitrobenzoate;
b. conversion of methyl 4-[6-(methyloxy)-3-pyridinyl]-2-nitrobenzoate to 4-[6-(methyloxy)-3-pyridinyl]-2-nitrobenzoic acid;
c. conversion of 4-[6-(methyloxy)-3-pyridinyl]-2-nitrobenzoic acid to methyl *O*-(1,1-dimethylethyl)-*N*-({4-[6-(methyloxy)-3-pyridinyl]-2-nitrophenyl}carbonyl)-L-threoninate;
d. conversion of methyl *O*-(1,1-dimethylethyl)-*N*-({4-[6-(methyloxy)-3-pyridinyl]-2-nitrophenyl}carbonyl)-L-threoninate to methyl *N*-({2-amino-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-*O*-(1,1-dimethylethyl)-L-threoninate;
e. conversion of 3,5-dimethyl-4-nitrobenzoic acid to (3,5-dimethyl-4-nitrophenyl)methanol;
f. conversion of (3,5-dimethyl-4-nitrophenyl)methanol to 1,3-Dimethyl-5-[(methyloxy)methyl]-2-nitrobenzene;
g. conversion of 1,3-Dimethyl-5-[(methyloxy)methyl]-2-nitrobenzene to 2,6-dimethyl-4-[(methyloxy)methyl]aniline;
h. conversion of 2,6-dimethyl-4-[(methyloxy)methyl]aniline to 2-isocyanato-1,3-dimethyl-5-[(methyloxy)methyl]benzene;
i. conversion of methyl *N*-({2-amino-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-*O*-(1,1-dimethylethyl)-L-threoninate and 2-isocyanato-1,3-dimethyl-5-[(methyloxy)methyl]benzene to methyl O-(1,1-dimethylethyl)-N-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threoninate; and
j. conversion of methyl O-(1,1-dimethylethyl)-N-({2-{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threoninate to *O*-(1,1-Dimethylethyl)-*N*-({2-

{[({2,6-dimethyl-4-[(methyloxy)methyl]phenyl}amino)carbonyl]amino}-4-[6-(methyloxy)-3-pyridinyl]phenyl}carbonyl)-L-threonine.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/077626

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D213/64 A61K31/4418 A61P3/10

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)
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, WPI Data, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006/052722 A (SMITHKLINE BEECHAM CORP [US]; EVANS KAREN [US]; CICHY-KNIGHT MARIA [US) 18 May 2006 (2006-05-18) claims; examples 225,236,323	1-15

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

"E" 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 of 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

"T" 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

"Y" 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.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2008	29/12/2008

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/077626

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