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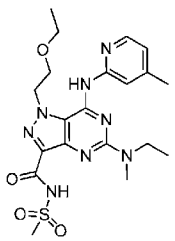
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[Continued on next page]

(54) Title: USE OF A TETRASUBSTITUTED PYRAZOLO[4,3-D]PYRIMIDINE COMPOUND FOR TREATING DIABETIC NEPHROPATHY



1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide

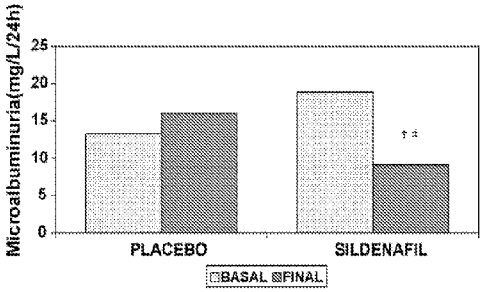


FIG. 1

(57) Abstract: The present invention relates to methods of delaying progression to end stage renal disease (ESRD) in patients comprising administration of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide. The present invention also includes administration of pharmaceutical compositions for delaying progression to ESRD. 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide.

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— *as to the identity of the inventor (Rule 4.17(i))*

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USE OF A TETRASUBSTITUTED PYRAZOLO[4,3-D]PYRIMIDINE COMPOUND FOR TREATING DIABETIC NEPHROPATHY

FIELD OF THE INVENTION

The present invention relates to methods of treating and/or preventing progression of diabetic nephropathy and/or chronic kidney disease using a tetrasubstituted pyrazolo[4,3-d] pyrimidine compound or pharmaceutical compositions containing the tetrasubstituted pyrazolo[4,3-d] pyrimidine compound.

BACKGROUND OF THE INVENTION

Diabetic nephropathy (DN) is a progressive kidney disease caused by diabetes that affects up to 40% of Type I or Type II diabetic patients. DN is characterized by albuminuria (protein in the urine), progressive decline of renal function leading to end stage renal disease (ESRD), hypertension, and increased cardiovascular morbidity and mortality. ESRD is a life-threatening condition of complete or almost complete loss of kidney function requiring dialysis or kidney transplantation.

The current standard-of-care therapy for patients with diabetic nephropathy targets the Renin Angiotensin System (RAS) using angiotensin converting enzyme (ACE) inhibitors and/or angiotensin II receptor blockers (ARBs) but the use of optimal doses of these drugs is limited by the risk for hyperkalaemia (high serum potassium level), which is a serious side-effect. Hyperkalamia can lead to abnormal heart rhythms and, in extreme cases, death. In view of the modest efficacy of currently available treatments to delay the progression of diabetic nephropathy there is an unmet medical need for compounds, alone or in combination with the current standard of care therapies that delay patients progressing to end-stage renal disease.

The present invention relates to a new therapeutic approach for treating DN and CKD that targets the NO signalling pathway, distinct from the RAS approach, using the phosphodiesterase type 5 (PDE5) inhibitor, 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide.

SUMMARY OF THE INVENTION

The present invention relates to methods of delaying progression of CKD, specifically diabetic nephropathy, and/or preventing ESRD in patients comprising the

step of administering to the patient, in need of such treatment, a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide (Example 1) or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to methods of delaying progression of CKD, specifically diabetic nephropathy, and/or preventing ESRD in patients comprising the step of administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, diluent, or excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that microalbuminuria is significantly decreased in Type II diabetic male patients after treatment with sildenafil citrate administered at 50mg daily for 30 days (n=20) relative to baseline or Placebo (n=20)).

DETAILED DESCRIPTION OF THE INVENTION

Nitric oxide (NO) contributes to the maintenance of normal kidney function. NO production and/or availability are decreased in patients with advanced DN. Reduced NO signaling contributes to the development of albuminuria and progression of DN in humans. The more albumin in urine, the faster the progression to ESRD. Microalbuminuria is defined as a urinary albumin to creatinine ratio (UACR) in men between 30mg/g and 300mg/g and macroalbuminuria is defined as a UACR of >300mg/g. The presence of macroalbuminuria in diabetic nephroapthy best correlates with progression of renal disease.

Chronic kidney disease, also known as chronic renal disease, is a progressive loss of renal function over a period of months or years with a declining glomerular filtration rate (GFR) representing reduced renal function and progression of CKD. The GFR in milliters per minute (mL/min) defines the five stages of CKD: $\geq$90 mL/min is stage 1; 60-89 mL/min is stage 2; 30-59 mL/min is stage 3; 15-29 mL/min is stage 4; and <15 mL/min is stage 5. Impaired NO signalling has been associated with CKD. This is caused by a combination of reduced NO production, depletion/inactivation of NO by reactive oxygen species and by a dysfunction of soluble guanylate cyclase (sGC).

The associated NO deficiency and dysfunctional sGC promotes hypertension and accelerates progression of renal disease.

Inhibiting PDE5 may restore the integrity of the NO signalling pathway, resulting in a number of beneficial effects, including lowering albuminuria and decreasing blood pressure. NO is released from nerve endings and vascular endothelial cells in areas of the cardiovascular system, by the action of shear-stress and local vasoactive agents, such as bradykinin. NO causes smooth muscle relaxation through activation of guanylate cyclase and consequent increase in cyclic guanosine 5' monophosphate (cGMP). PDE5 specifically degrades cGMP, and an inhibitor of human PDE5 may therefore cause an increase in the levels of cGMP. Elevated cGMP reduces levels of intracellular calcium causing relaxation of smooth muscle cells and ultimately reductions in arterial pressure, vascular resistance, and increased blood flow.

1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide (Example 1) is a selective and competitive inhibitor (IC_{50} = 0.71 nM) of human PDE5. Example 1 shows comparable enzyme inhbitory potency against rat PDE5 (IC_{50} = 0.93 nM) and functionally potentiates the vasorelaxant action of NO in isolated aorta studies in rats (EC_{50} = 3.1 nM). Example 1 exhibits low clearance in dogs and rats, leading to long half-life values, and oral bioavailability is high in both dogs and rats. Example 1 shows no significant inhibition of the major human cytochrome P450 enzymes and is therefore unlikely to significantly alter the metabolism of coadministered drugs that are substrated for these enzymes.

In another embodiment, the present invention relates to methods of treating or preventing progression of diabetic nephropathy in a patient comprising the step of administering to the patient, in need of such treatment, a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to methods of treating or preventing progression of diabetic nephropathy in a patient comprising the step of administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-

pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one carrier, diluent or excipient.

In another embodiment, the present invention relates to methods of treating or preventing progression of chronic kidney disease in a patient comprising the step of administering to the patient, in need of such treatment, a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide or a pharmaceutically acceptable salt thereof. In particular, the methods of the present invention may be used to treat or prevent CKD stage 3 or 4.

In another embodiment, the present invention relates to methods of treating or preventing progression of chronic kidney disease in a patient comprising the step of administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one carrier, diluent or excipient. In particular, the methods of the present invention may be used to treat or prevent CKD stage 3 or 4.

In another embodiment, the present invention relates to methods of reducing albumin in urine in a patient comprising the step of administering to the patient, in need of such treatment, a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to methods of reducing albumin in urine in a patient comprising the step of administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one carrier, diluent or excipient.

In another embodiment, the present invention relates to methods of treating or preventing macroalbuminuria in a patient comprising the step of administering to the patient, in need of such treatment, a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to methods of treating or preventing macroalbuminuria in a patient comprising the step of administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one carrier, diluent or excipient.

In another embodiment, 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, may be employed in combination with another phosphodiesterase type 5 (PDE5) inhibitor including, but not limited to, avanafil, lodenafil, mirodenafil, sildenafil, tadalafil, vardenafil, and udenafil. The combination may be administered separately or within the same pharmaceutical composition.

In another embodiment, 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, may be employed in combination with an angiotensin-converting-enzyme (ACE) inhibitor including, but not limited to, captopril, enalapril, lisinopril, perindopril, and ramipril. The combination may be administered separately or within the same pharmaceutical composition.

In another embodiment, 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, may be employed in combination with an angiotensin II receptor blocker (ARB) including, but not limited to, losartan, candesartan, valsartan, irbesartan, telmisartan, eprosartan, olmesartan, and azilsartan. The combination may be administered separately or within the same pharmaceutical composition.

In another embodiment, 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, may be employed in combination with both an angiotensin-converting-enzyme (ACE) inhibitor and an angiotensin II receptor blocker (ARB). The ACE inhibitor includes, but is not limited to, captopril, enalapril, lisinopril, perindopril, and ramipril. The angiotensin II receptor blocker (ARB) includes, but is not limited to, losartan, candesartan, valsartan,

irbesartan, telmisartan, eprosartan, olmesartan, and azilsartan. The combination may be administered separately or within the same pharmaceutical composition.

It is to be understood that the methods of the present invention may use 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide in solution, as a suspension, as the amorphous solid or as a crystalline solid wherein the crystalline solid includes polymorphs, hydrates, solvates or combinations thereof. In particular, the present invention contemplates the use of polymorph Forms A, B and C as disclosed in US 2008/0194591. Preferred polymorphs are Forms B and C, or a combination thereof. The most preferred polymorph is Form C.

Definitions

The term “Example 1” as used herein means 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide or a pharmaceutically acceptable salt thereof.

The term “chronic kidney disease or CKD” includes stages 1-5 unless otherwise noted herein. It is to be understood that the present invention contemplates treating or preventing the progression of all five stages of CKD.

The term “patient” as used herein means a human.

The term “pharmaceutically acceptable salt” as used herein means those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of patients and lower animals without undue toxicity, irritation, allergic response and the like and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well-known in the art. For example, S. M. Berge et al. describe pharmaceutically acceptable salts in detail in Berge et al., J. Pharmaceutical Sciences, 1977, 66: 1-19. The salts can be prepared in situ during the final isolation and purification of Example 1 of the present invention or separately by reacting the free base of Example 1 with a suitable organic or inorganic acid. Representative acid addition salts include, but are not limited to acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bicarbonate, bisulfate, butyrate, camphorate, camphorsufonate, citrate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isethionate), lactate, maleate, methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-

phenylpropionate, phosphate, picrate, pivalate, propionate, succinate, sulphate, tartrate, thiocyanate, and p-toluenesulfonate.

The present invention also provides pharmaceutical compositions which comprise Example 1 formulated together with one or more non-toxic pharmaceutically acceptable carriers. The pharmaceutical compositions may be specially formulated for oral administration in solid or liquid form, for parenteral injection, or for rectal administration.

The term "pharmaceutically acceptable carrier" as used herein means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator. The present invention provides pharmaceutical compositions which comprise Example 1 formulated together with one or more non-toxic pharmaceutically acceptable carriers. The pharmaceutical compositions can be formulated for oral administration in solid or liquid form, for parenteral injection or for rectal administration.

The pharmaceutical compositions of this invention can be administered to patients orally, parenterally, intraperitoneally, topically (as by powders, ointments or drops), bucally or as an oral or nasal spray. The term "parenterally," as used herein, refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous, intraarticular injection and infusion.

Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectable

solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propylene glycol, polyethylene glycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservative agents, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Suspensions, in addition to Example 1, may contain suspending agents, as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, tragacanth, and mixtures thereof.

If desired, and for more effective distribution, Example 1 can be incorporated into slow-release or targeted-delivery systems such as polymer matrices, liposomes, and microspheres. They may be sterilized, for example, by filtration through a bacteria-retaining filter or by incorporation of sterilizing agents in the form of sterile solid compositions, which may be dissolved in sterile water or some other sterile injectable medium immediately before use.

Example 1 can also be in micro-encapsulated form, if appropriate, with one or more pharmaceutically acceptable carriers as noted above. The solid dosage forms of

tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms Example 1 can be admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of such composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Injectable depot forms are made by forming microencapsulated matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides) Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic, parenterally acceptable diluent or solvent such as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms Example 1 is mixed with at least one inert pharmaceutically acceptable carrier such as sodium citrate or calcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and salicylic acid; b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay; and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

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

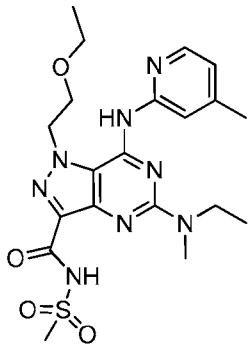
Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Actual dosage levels of Example 1 in the pharmaceutical compositions of this invention can be varied so as to obtain an amount of Example 1 which is effective to achieve the desired therapeutic response for a particular patient, compositions, and mode of administration. The selected dosage level will depend upon the activity of Example 1, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated.

The total daily dose of Example 1 administered to a patient is 0.3 to 400 mgs. If desired, the effective daily dose can be divided into multiple doses for purposes of administration, e.g. two to four separate doses per day.

Synthetic Preparation

Example 1



1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide

The title compound was prepared as described in U.S. Patent No. 7,572,799 (see Example 115). Polymorph Forms A, B, and C of the title compound were prepared as described in U.S. Published Patent Application No. 2008/0194591 (US Patent Application No. 11/913,091). US 7,572,799 and US 2008/0194591 are hereby incorporated by reference.

Biology/Pharmacology

In single dose toxicity studies in mice and rats, no deaths were observed; the maximum non-lethal dose was 2000 mg/kg. In dogs, doses up to 1000 mg/kg were given and no adverse effects were noted.

In humans, Example 1 was evaluated in single dose and multiple dose clinical studies in 46 healthy male volunteers aged 21 to 49 years. Both single and multiple dose clinical studies were conducted with an oral solution or suspension ranging from single doses of 0.3 to 400 mg and multiple doses of 30 to 200 mg. Example 1 was rapidly absorbed in humans following single doses of solution with time of occurrence of C_{max} (T_{max}) of 1.1 to 1.5 hours. The terminal half-life did not alter significantly with dose and ranged from 11.9 to 15.7 hours. Following multiple doses, Example 1 was rapidly absorbed (T_{max} of 1.1 to 3.5 hours) and t½ on days 1 and 13 ranged from 11.6 to 14.5 hours. Single doses of Example 1 were well tolerated, no serious adverse events occurred. In the multiple dose study, Example 1 was well tolerated over the dose range of 30 to 200 mg. No significant changes in vital signs, ECGs, laboratory safety test results, or physical examination results were reported at any dose.

TABLE 1

Assay/Model	Example 1 Potency/Efficacy
PDE5 enzyme (human platelet)	IC_{50} = 0.71 nM
PDE5 enzyme (rat platelet)	IC_{50} = 0.93 nM
PDE5 enzyme (dog platelet)	IC_{50} = 0.65 nM
Aortic ring relaxation (rat)	EC_{50} = 3.1 nM
In vivo SHR (oral dosing)	EC_{max} = 6.5 nM (unbound plasma concentration)
PDE6 enzyme (human retinal cone)	IC_{50} = 28.9 nM
PDE6 enzyme (human retinal rod)	IC_{50} = 63.6 nM
PDE11 enzyme (human)	IC_{50} = 26.3 nM

IC50 = 50% inhibitory concentration; EC50 = efficacious concentration; ECmax = maximally efficacious concentration; SHR = spontaneously hypertensive rat.

Example 1 is a competitive inhibitor of human PDE5, with a mean IC_{50} value of 0.71 nM (0.34 ng/mL) using platelet-derived enzyme. Potencies against rat and dog platelet-derived PDE5 are similar at 0.93 and 0.65 nM, respectively. Example 1 has PDE6 IC_{50} potencies against human retinal cone and rod of 28.9 nM (41-fold selectivity) and 63.6 nM (90-fold selectivity), respectively. The IC_{50} of Example 1 against human

PDE11 is 26.3 nM (37-fold selectivity). Greater than 1000-fold selectivity exists relative to PDE enzymes 1, 2, 3, 4A, 4B, 4D, 7B, 8A, 9, and 10.

The direct functional effects of Example 1 were demonstrated in isolated rat aortic rings. Example 1 induced aortic ring vasorelaxation with a mean EC_{50} value of 3.1 nM.

The antihypertensive efficacy of Example 1 was assessed following daily oral dosing in conscious, spontaneously hypertensive rats (SHR) monitored continuously by radiotelemetry. A significant reduction in mean arterial pressure (MAP) was achieved at free plasma concentrations corresponding to approximately 7-fold the rat PDE5 IC_{50}. Treatment of SHR with Example 1 induced a significant, sustained reduction in MAP over a 14-day dosing period. Similarly, treatment with the ACE inhibitor enalapril induced a significant, sustained reduction in MAP over a 14-day dosing period. When combined with an ACE inhibitor, Example 1 afforded greater MAP lowering effects than the ACE inhibitor alone.

Example 1 was assessed in a series of safety pharmacology studies outlined in Table 2. For in vivo studies, the oral route of exposure was used. Rats given diazepam or frusemide served as positive controls in the locomotor activity and fluid/electrolyte excretion studies, respectively. Dofetilide was used for assay validity or a positive control in the dofetilide and hERG assays, respectively. Positive controls or animals given positive controls were not assessed concurrently in the remaining studies as these studies utilized well-characterized models to evaluate safety parameters.

TABLE 2

Study	Concentration or Dose
[^{3}H]dofetilide binding assay	0.003-100 μM
hERG assay	3 and 10 μM
Purkinje fiber assay	0.01, 0.1, 1, 10 μM
In vivo toleration – rat Appearance/behavior - single dose	100, 300, 1000 mg/kg
Central nervous system - rat Locomotor activity	3, 30, 300 mg/kg
Renal – rat Fluid and electrolyte excretion	3, 30, 300 mg/kg
Pulmonary function – rat Respiratory parameters	3, 30, 300 mg/kg
Cardiovascular – anesthetized dog PK/PD evaluation with monitoring	1.47-387 μg/kg/min
Cardiovascular – conscious dogs Hemodynamic and ECG parameters	0.5, 1.5, 5 mg/kg

No relevant effects were noted in the hERG patch clamp assay, the dog Purkinje fiber assay, or the [^{3}H]dofetilide-binding assay up to 10 μM (4.77 μg/mL). Example 1 competitively displaced the [^{3}H]dofetilide by 5.5% at 30 μM (14.3 μg/mL and 32.5% at 100 μM (47.7 μg/mL). The no-effect concentration of 4.77 μg/mL is approximately 426 times the C_{max} (~0.011 μg/mL as free fraction) based on human pharmacokinetics (PK) obtained after a 30 mg dose, which was well tolerated, suggesting a low potential for QT prolongation.

Results in rats given up to 300 mg/kg of Example 1 demonstrated minor, nondose-related effects consisting of decreased rearing and center rearing, but no adverse effects on pulmonary function. Example 1 demonstrated a dose-related decrease in urine volume and electrolyte excretion consistent with results from other PDE5 inhibitors.

In dogs, Example 1 at 0.5, 1.5, and 5 mg/kg induced small, but significant reductions in blood pressure and left ventricular end-diastolic pressure. These cardiovascular effects are consistent with cGMP elevation in vascular smooth muscle, resulting from inhibition of cGMP-specific PDE5. Example 1 was not associated with any relevant effects on ECG parameters in dogs. No clinically-relevant effects on ECG parameters were noted in humans given single and multiple doses of Example 1. C_{max}

in dogs at 5 mg/kg was 12.8 mg/mL or ~8 times higher than the C_{max} (1.57-1.63 μg/mL) determined in humans after a 30 mg dose, which was well tolerated.

Liguid chromatography/mass spectrometry was used to determine Example 1 concentrations in plasma samples from PK and toxicokinetic studies in rats, dogs, and humans. For toxicokinetic studies, this method was validated over a concentration range of 10 to 1000 ng/mL for a 50 μL plasma sample. Radiometric methods were used to measure [^{14}C] Example 1 derived radioactivity in biological samples from the in vitro and in vivo metabolism studies.

Absolute oral bioavailability of Example 1 following a single dose was 82% in rats and 77% in dogs. Following intravenous (IV) administration of Example 1, plasma clearance in rats and dogs was lower that the liver-blood flow in the corresponding species, indicating that Example 1 is a low-clearance compound. The steady-state distribution volume was lower than total body water in the rat and dog.

In a tissue distribution study in rats given radio labeled Example 1, as substantial portion of the dose remained within the gastrointestinal tract and systemic distribution was generally in proportion to tissue-blood flow. Plasma protein binding values for Example 1 were determined to be 99.3% in rat, dog, and human and 98.8% in rabbit plasma.

In rats and dogs, greater than 80% of the administered doses (total radioactivity) were eliminated in feces. The majority of the radioactivity was recovered within 48 hours. Total radioactivity recovered in rats and dogs was greater than 92%.

The potential for Example 1 to inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 (midazolam, testosterone and felodipine) was determined in human liver microsomes. No inhibition was observed with IC_{50} values >30 μM.

There were no deaths, clinical signs, or effects on body weight, and no pathologic findings in mice or rats given single oral doses of Example 1 at 20, 200, or 2000 mg/kg and observed for 14 days. No toxicity was observed in rats given single oral doses of 100, 300, or 1000 mg/kg, whereas mild gastrointestinal effects occurred in dogs given single-oral doses of 100, 500, o 1000 mg/kg.

Micronized Example 1 (polymorph form C) was assessed in single-dose toxicity/toxicokinetic studies in rats and dogs to compare exposure with polymorph B. The micronized Example 1 polymorph form C enabled the administration of higher doses in rats and dogs than was achievable with polymorph form B. At equivalent

doses of polymorph forms B and C (100 mg/kg in dogs and 100 and 300 mg/kg in rats), there were no differences in systemic exposure between the polymorph forms.

Example 1 was assessed in a series of genetic toxicology assays consisting of the microbial reverse mutation, in vitro cytogenetic (human lymphocyte), and in vivo rat micronucleus assays. Study designs and dose selection were consistent with International Conference on Harmonization and Organization for Economic Cooperation and Development guidelines for mutagenicity and clastogenicity assays. All in vitro tests were conducted with and without exogenous metabolic activation using concentrations up to those limited by cytotoxicity or insolubility. Example 1 was not genotoxic in either in vitro or in vivo assays (Table 3).

TABLE 3

Test System	Dose	Result
Mutagenicity: Salmonella typhimurium (Strains TA-1535, TA-1537) and Escherichia coli (Strain WP2uvrA)	50-5000 mg/plate (±S9)	Negative
Clastogenicity In Vitro: Structural Chromosome Aberration in Human Peripheral Lymphocytes	100, 350, 500, μg/mL (3h-S9) 100, 200, 350, μg/mL (3h+S9) 12.5, 50, 100, μg/mL (24h-S9)	Negative
Clastogenicity In Vitro Micronucleus Assay in Rat Bone Marrow	VC, 100, 300, 600 mg/kg/day	Negative

S9 = Postmitochondrial supernatant from livers of rats treated with Aroclor 1254; VC = Vehicle control.

The PDE5 inhibitor sildenafil reduces albuminuria in Type II diabetic patients with early-stage diabetic nephropathy (Figure 1). This data supports the use of PDE5 inhibitors for treating DN and/or CKD that have a suitable pharmacokinetic (PK) and safety profile in humans. Example 1 is a potent and selective PDE5 inhibitor that was well tolerated in humans following oral administration. When compared to sildenafil, Example 1 appears to be a more potent PDE5 inhibitor with greater selectivity for PDE5 over PDE6. Further, Example 1 has a 3-4 fold longer half-life in humans (Table 4).

These advantages make Example 1 superior to sildenafil for treating or delaying the progression of albuminuria, DN and/or CKD in humans, particularly diabetic patients.

TABLE 4

Criteria	Example 1	Sildenafil
PDE5 IC_{50} (nM)	0.71	4*
PDE5/PDE6 selectivity	41 fold	9 fold*
Human Half-life (hr)	11.9 to 15.7	3.7

* As reported in Ballard et al., *The Journal of Urology*, 159:2164-2171, Jun 1998

5

We Claim:

1. 1-(2-Ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, for use in the treatment of end stage renal disease.

2. 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one carrier, diluent or excipient for use in the treatment of end stage renal disease.

3. 1-(2-Ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, for use in the treatment of diabetic nephropathy.

4. 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one carrier, diluent or excipient for use in the treatment of diabetic nephropathy.

5. 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide or a pharmaceutically acceptable salt thereof , for use in the treatment of chronic kidney disease.

6. The use according to claim 5 wherein the chronic kidney disease is stage 3 or 4.

7. 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one carrier, diluent or excipient for use in the treatment of chronic kidney disease.

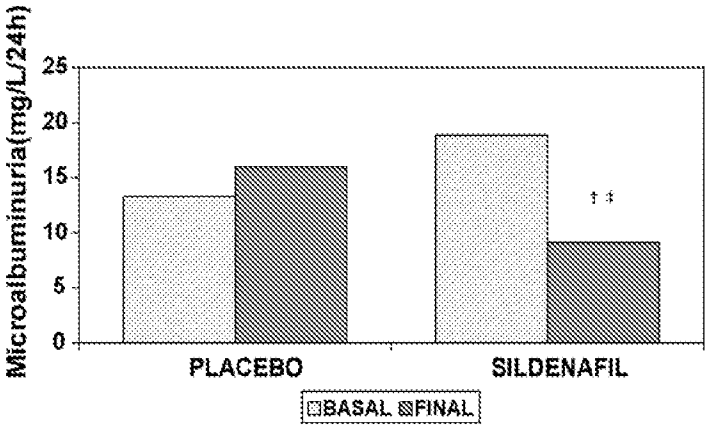
8. The use according to claim 7 wherein the chronic kidney disease is stage 3 or 4.

9. 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide or a pharmaceutically acceptable salt thereof, for use in the reduction of albumin in urine.

10. 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one carrier, diluent or excipient for use in the reduction of albumin in urine .

11. 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, for use in the treatment of macroalbuminuria.

12. 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and at least one carrier, diluent or excipient for use in the treatment of macroalbuminuria.



Grover-Paez et al., Diabetes Research and Clinical Practice, 78:136-140 (2007)

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/059239

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/519 A61P13/12
ADD.

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)
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 practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	paragraphs [0136], [0141]	1-6

Y	WO 2007/054778 A1 (PFIZER PROD INC [US]; TOLLEFSON MICHAEL BRENT [US]) 18 May 2007 (2007-05-18) page 9, lines 24-26 page 8, line 8	1-6

X	WO 2006/016262 A1 (PFIZER LTD [GB]; PFIZER [US]; HUGHES BERNADETTE [GB]) 16 February 2006 (2006-02-16) page 14, line 17	3

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

☒ See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
14 January 2014	24/01/2014

Name and mailing address of the ISA/	Authorized officer
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1

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Information on patent family members

International application No
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(12)发明专利申请

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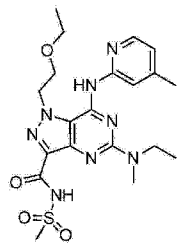
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A61P 13/12(2006.01)

权利要求书1页 说明书11页 附图1页

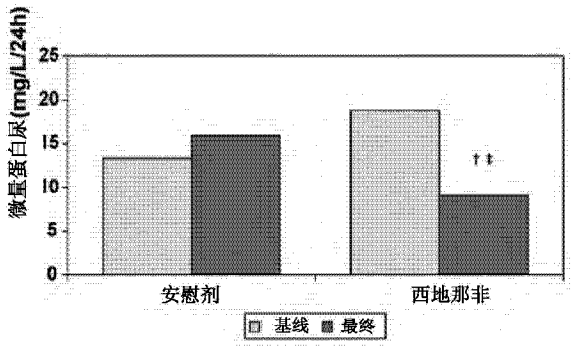
(54)发明名称

四取代的吡唑并[4,3-d]嘧啶化合物用于治疗糖尿病肾病的用途

(57)摘要



1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺本发明涉及在患者中延缓向终末期肾病(ESRD)进展的方法,其包括给药1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺。本发明还包括给药药物组合物以延缓向ESRD的进展。



CN 104755086 A

1. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐,其用于治疗终末期肾病。

2. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐以及至少一种载体、稀释剂或赋形剂,其用于治疗终末期肾病。

3. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐,其用于治疗糖尿病肾病。

4. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐以及至少一种载体、稀释剂或赋形剂,其用于治疗糖尿病肾病。

5. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐,其用于治疗慢性肾病。

6. 权利要求 5 的用途,其中所述慢性肾病是 3 期或 4 期的。

7. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐以及至少一种载体、稀释剂或赋形剂,其用于治疗慢性肾病。

8. 权利要求 7 的用途,其中所述慢性肾病是 3 期或 4 期的。

9. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐,其用于减少尿中的白蛋白。

10. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐以及至少一种载体、稀释剂或赋形剂,其用于减少尿中的白蛋白。

11. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐,其用于治疗巨白蛋白尿。

12. 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐以及至少一种载体、稀释剂或赋形剂,其用于治疗巨白蛋白尿。

四取代的吡唑并 [4,3-d] 嘧啶化合物用于治疗糖尿病肾病的用途

发明领域

[0001] 本发明涉及利用四取代的吡唑并 [4,3-d] 嘧啶化合物或者包含所述四取代的吡唑并 [4,3-d] 嘧啶化合物的药物组合物来治疗和 / 或预防糖尿病肾病和 / 或慢性肾病的进展的方法。

[0002] 发明背景

[0003] 糖尿病肾病 (DN) 是糖尿病引起的进行性肾病，其影响多达 40％的 I 型或 II 型糖尿病患者。DN 的特征在于蛋白尿 (尿中的蛋白)、引起终末期肾病 (ESRD) 的肾功能的进行性下降、高血压以及升高的心血管发病率及死亡率。ESRD 是一种威胁生命的疾病状况，其存在完全或几乎完全的肾功能丧失，从而需要透析或肾脏移植。

[0004] 目前患有糖尿病肾病的患者的标准护理疗法通过利用血管紧张素转化酶 (ACE) 抑制剂和 / 或血管紧张素 II 受体阻断剂 (ARB) 来靶向肾素血管紧张素系统 (RAS)，但是这些药物的最佳剂量的使用受到高钾血症 (高血清钾水平) 的风险的限制，所述高钾血症是一种严重的副作用。高钾血症可以导致心率异常，并且在某些极端情况下引起死亡。鉴于目前可用的延缓糖尿病肾病进展的治疗的效力一般，医药上亟需单独或与目前的护理疗法标准联合使用的化合物，其延缓患者向终末期肾病的进展。

[0005] 本发明涉及一种用于治疗 DN 和 CKD 的新疗法，其靶向 NO 信号传导途径，与 RAS 方法不同，其使用 5 型磷酸二酯酶 (PDE5) 抑制剂 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺。

发明内容

[0006] 本发明涉及延缓患者的 CKD 特别是糖尿病肾病的进展和 / 或预防患者的 ESRD 的方法，其包括向有此需要的患者给药治疗有效量的 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺 (实施例 1 化合物) 或其药学可接受的盐的步骤。

[0007] 在另一实施方案中，本发明涉及延缓患者的 CKD 特别是糖尿病肾病的进展和 / 或预防患者的 ESRD 的方法，其包括向有此需要的患者给药药物组合物的步骤，所述药物组合物包含 ：治疗有效量的 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐以及至少一种药学可接受的载体、稀释剂或赋形剂。

附图说明

[0008] 图 1 示出在 II 型糖尿病男性患者中，用柠檬酸西地那非以每日 50mg 给药 30 天进行治疗后 (n = 20)，相对于基线或安慰剂 (n = 20)，微量蛋白尿明显减少。

[0009] 发明详述

[0010] 一氧化氮 (NO) 有助于维持正常的肾功能。NO 产生和 / 或可用性在患有晚期 DN 的患者中降低。降低的 NO 信号传导促成人中的蛋白尿的产生和 DN 的进展。尿中的白蛋白越多，则向 ESRD 的进展越快。微量蛋白尿被定义为人中的尿白蛋白与肌酐的比例 (UACR) 为 30mg/g-300mg/g，而巨白蛋白尿则被定义为 UACR>300mg/g。糖尿病肾病中巨白蛋白尿的存在与肾病的进展密切相关。

[0011] 慢性肾病又称为慢性肾疾病，是数月或数年内伴随着降低的肾小球滤过率 (GFR) 的进行性肾功能丧失，其表现出降低的肾功能和 CDK 的进展。以毫升 / 分钟 (mL/min) 表示的 GFR 定义 CKD 的 5 个阶段 :>90mL/min 为 1 期 ;60-89mL/min 为 2 期 ;30-59mL/min 为 3 期 ;15-29mL/min 为 4 期 ;并且 <15mL/min 为 5 期。受损的 NO 信号传导与 CKD 有关。这由以下的组合导致 :NO 产生的减少、NO 被活性氧物质耗尽 / 失活以及可溶性鸟苷酸环化酶 (sGC) 功能失调。相关的 NO 缺陷和功能失调的 sGC 促成高血压并且加速肾病的进展。

[0012] 抑制 PDE5 可以恢复 NO 信号传导途径的完整性，从而获得许多有益效果，包括降低蛋白尿和降低血压。NO 通过剪应力和诸如缓激肽的局部血管活性物质的作用从神经末端和心血管系统中的血管内皮细胞释放。NO 通过活化鸟苷酸环化酶引起平滑肌舒张，从而引起环状 5'- 单磷酸鸟苷 (cGMP) 增加。PDE5 特异性降解 cGMP，因此人 PDE5 的抑制剂可以使得 cGMP 水平升高。升高的 cGMP 降低细胞内钙水平，从而使得平滑肌细胞舒张，并最终导致动脉压降低、血管阻力降低和血流增加。

[0013] 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺 (实施例 1 化合物) 是人 PDE5 的选择性和竞争性抑制剂 (IC_{50}= 0.71nM)。实施例 1 化合物对大鼠 PDE5 表现出相当的酶抑制效力 (IC_{50}= 0.93nM)，并且在大鼠的分离主动脉研究中功能性地增强 NO 的血管舒张作用 (EC_{50}= 3.1nM)。实施例 1 化合物在犬和大鼠中表现出低清除，从而实现长半衰期值，并且在犬和大鼠中都表现出高口服生物利用度。实施例 1 化合物对主要的人细胞色素 P450 酶没有表现出明显的抑制，因此不大可能显著改变作为这些酶的底物的共给药药物的代谢。

[0014] 在另一实施方案中，本发明涉及治疗或预防患者的糖尿病肾病的进展的方法，其包括向需要此类治疗的患者给药治疗有效量的 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐的步骤。

[0015] 在另一实施方案中，本发明涉及治疗或预防患者的糖尿病肾病的进展的方法，其包括向需要此类治疗的患者给药药物组合物的步骤，所述药物组合物包含治疗有效量的 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐以及至少一种载体、稀释剂或赋形剂。

[0016] 在另一实施方案中，本发明涉及治疗或预防患者的慢性肾病的进展的方法，其包括向需要此类治疗的患者给药治疗有效量的 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐的步骤。特别地，本发明的方法可以用于治疗或预防 3 期或 4 期 CKD。

[0017] 在另一实施方案中，本发明涉及治疗或预防患者的慢性肾病的进展的方法，其

包括向需要此类治疗的患者给药药物组合物的步骤，所述药物组合物包含治疗有效量的1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺或其药学可接受的盐以及至少一种载体、稀释剂或赋形剂。特别地，本发明的方法可以用于治疗或预防3期或4期CKD。

[0018] 在另一实施方案中，本发明涉及降低患者的尿中的白蛋白的方法，其包括向需要此类治疗的患者给药治疗有效量的1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺或其药学可接受的盐的步骤。

[0019] 在另一实施方案中，本发明涉及降低患者的尿中的白蛋白的方法，其包括向需要此类治疗的患者给药药物组合物的步骤，所述药物组合物包含治疗有效量的1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺或其药学可接受的盐以及至少一种载体、稀释剂或赋形剂。

[0020] 在另一实施方案中，本发明涉及治疗或预防患者的巨白蛋白尿的方法，其包括向需要此类治疗的患者给药治疗有效量的1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺或其药学可接受的盐的步骤。

[0021] 在另一实施方案中，本发明涉及治疗或预防患者的巨白蛋白尿的方法，其包括向需要此类治疗的患者给药药物组合物的步骤，所述药物组合物包含治疗有效量的1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺或其药学可接受的盐以及至少一种载体、稀释剂或赋形剂。

[0022] 在另一实施方案中，1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺或其药学可接受的盐可以与其它5型磷酸二酯酶(PDE5)抑制剂组合使用，所述其它PDE5抑制剂包括但不限于阿伐那非、罗地那非、米罗那非、西地那非、他达拉非、伐地那非和乌地那非。所述组合可以分别给药或者在同一药物组合物中给药。

[0023] 在另一实施方案中，1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺或其药学可接受的盐可以与血管紧张素转化酶(ACE)抑制剂组合使用，所述ACE抑制剂包括但不限于卡托普利、依那普利、赖诺普利、培哚普利和雷米普利。所述组合可以分别给药或者在同一药物组合物中给药。

[0024] 在另一实施方案中，1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺或其药学可接受的盐可以与血管紧张素II受体阻断剂(ARB)组合使用，所述ARB包括但不限于氯沙坦、坎地沙坦、缬沙坦、厄贝沙坦、替米沙坦、依普罗沙坦、奥美沙坦和阿齐沙坦。所述组合可以分别给药或者在同一药物组合物中给药。

[0025] 在另一实施方案中，1-(2-乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4-甲基吡啶-2-基)氨基)-N-(甲基磺酰基)-1H-吡唑并[4,3-d]嘧啶-3-甲酰胺或其药学可接受的盐可以与血管紧张素转化酶(ACE)抑制剂和血管紧张素II受体阻断剂(ARB)这两者组

合使用。所述 ACE 抑制剂包括但不限于卡托普利、依那普利、赖诺普利、培哚普利和雷米普利。所述血管紧张素 II 受体阻断剂 (ARB) 包括但不限于氯沙坦、坎地沙坦、缬沙坦、厄贝沙坦、替米沙坦、依普罗沙坦、奥美沙坦和阿齐沙坦。所述组合可以分别给药或者在同一药物组合物中给药。

[0026] 应当理解，本发明的方法可以使用溶液、悬浮液、无定形固体或晶体固体形式的 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺，其中所述晶体固体包括多晶型物、水合物、溶剂合物或其组合。特别地，本发明涵盖 US2008/0194591 中公开的多晶型物 A、B 和 C 的用途。优选的多晶型物是形式 B 和 C 或其组合。最优选的多晶型物是形式 C。

[0027] 定义

[0028] 本文所用的术语“实施例 1 化合物”指 1-(2- 乙氧基乙基)-5-(乙基 (甲基) 氨基)-7-((4- 甲基吡啶 -2- 基) 氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺或其药学可接受的盐。

[0029] 除非本文另外指明，术语“慢性肾病或 CKD”包括 1-5 期。应当理解，本发明涵盖治疗或预防 CKD 的所有五个阶段的进展。

[0030] 本文所用的术语“患者”指人。

[0031] 本文所用的术语“药学可接受的盐”表示这样的盐，其在合理的医疗判断的范围内，适合于与患者和低等动物的组织接触而没有不适当的毒性、刺激、过敏应答等，并且具有相称的合理益处 / 风险比例。药学可接受的盐是本领域公知的。例如，S.M.Berge 等人在 Berge 等人，J.Pharmaceutical Sciences,1977,66:1-19 中详细描述了药学可接受的盐。所述盐可以在本发明的实施例 1 化合物的最终分离和纯化期间原位制备，也可以通过使实施例 1 化合物的游离碱与合适的有机或无机酸反应来分别制备。代表性的酸加成盐包括但不限于乙酸盐、己二酸盐、藻酸盐、柠檬酸盐、天冬氨酸盐、苯甲酸盐、苯磺酸盐、碳酸氢盐、硫酸氢盐、丁酸盐、樟脑酸盐、樟脑磺酸盐、柠檬酸盐、二葡糖酸盐 (digluconate)、甘油磷酸盐、半硫酸盐、庚酸盐、己酸盐、富马酸盐、盐酸盐、氢溴酸盐、氢碘酸盐、2- 羟基乙磺酸盐 (羟乙基磺酸盐)、乳酸盐、马来酸盐、甲磺酸盐、烟酸盐、2- 萘磺酸盐、草酸盐、扑酸盐、果胶酸盐 (pectinate)、过硫酸盐、3- 苯基丙酸盐、磷酸盐、苦味酸盐、新戊酸盐、丙酸盐、琥珀酸盐、硫酸盐、酒石酸盐、硫氰酸盐和对甲苯磺酸盐。

[0032] 本发明还提供药物组合物，其包含与一种或多种无毒的药学可接受的载体配制的实施例 1 化合物。所述药物组合物可以特别地配制为用于以固体或液体形式口服给药，用于胃肠外注射，或者用于直肠给药。

[0033] 本文所用的术语“药学可接受的载体”表示任何类型的无毒、惰性的固体、半固体或液体填充剂、稀释剂、封装材料 (encapsulating material) 或配制助剂。可以充当药学可接受的载体的材料的一些实例有糖，如乳糖、葡萄糖和蔗糖；淀粉，如玉米淀粉和马铃薯淀粉；纤维素及其衍生物，如羧甲基纤维素钠、乙基纤维素和醋酸纤维素；粉状黄芪胶；麦芽；明胶；滑石；赋形剂，如可可脂和栓剂蜡；油，如花生油、棉籽油、红花油、芝麻油、橄榄油、玉米油和豆油；多元醇，如丙二醇；酯，如油酸乙酯和月桂酸乙酯；琼脂；缓冲剂，如氢氧化镁和氢氧化铝；藻酸；无热原水；等渗盐水；林格氏溶液；乙醇和磷酸盐缓冲溶液；以及其他无毒的相容润滑剂，如月桂基硫酸钠和硬脂酸镁；并且根据配制人员的判断，着色剂、释放

剂、包衣剂、甜味剂、风味和芳香剂、防腐剂以及抗氧化剂也可以存在于所述组合物中。本发明提供药物组合物，其包含与一种或多种无毒的药学可接受的载体配制的实施例 1 化合物。所述药物组合物可以配制为用于以固体或液体形式口服给药，用于胃肠外注射，或者用于直肠给药。

[0034] 本发明的药物组合物可以以下方式向患者给药：口服、肠胃外、腹膜内、局部（例如通过散剂、软膏剂或滴剂）、含服或者作为口服或鼻喷雾剂。本文所用的术语“肠胃外”指给药方式，其包括静脉内、肌肉内、腹膜内、胸骨内、皮下、关节内注射和输注。

[0035] 用于肠胃外注射的本发明的药物组合物包括药学可接受的无菌水性或非水性溶液剂、分散液、混悬剂或乳剂以及用于复原成无菌注射溶液剂或分散液的无菌粉末。合适的水性或非水性载体、稀释剂、溶剂或媒介物的实例包括水、乙醇、多元醇（丙二醇、聚二乙醇、甘油等）、其合适的混合物、植物油（如橄榄油）和可注射有机酯如油酸乙酯。合适的流动性可以通过例如使用诸如卵磷脂的包衣，通过在分散液的情况下维持所需的粒径以及通过使用表面活性剂来维持。

[0036] 这些组合物还可以包含辅剂，例如防腐剂、润湿剂、乳化剂和分散剂。可以通过各种抗菌剂和抗真菌剂如对羟苯甲酸酯、氯丁醇、酚、山梨酸等来确保防止微生物的作用。还可以期望地包含等渗剂如糖、氯化钠等。可注射药物形式的延长吸收可以通过使用延缓吸收的物质如单硬脂酸铝和明胶来实现。

[0037] 在一些情况下，为了延长药物的效应，通常期望延缓药物从皮下或肌内注射的吸收。这可以通过使用水溶性不好的晶体或无定形材料来实现。药物的吸收速率取决于其溶出速率，这又可以取决于晶体大小和晶形。或者，肠胃外给药的药物形式的延迟吸收是通过将所述药物溶于或悬浮于油媒介物中来实现的。

[0038] 除了实施例 1 化合物外，混悬剂还可以包含助悬剂，例如乙氧基化异硬脂醇、聚氧乙烯山梨醇酯和聚氧乙烯脱水山梨醇酯、微晶纤维素、偏氢氧化铝 (aluminum metahydroxide)、膨润土、琼脂、黄芪胶及其混合物。

[0039] 若期望，并且为了更有效的分布，实施例 1 化合物可以被并入缓释或靶向递送系统中，例如聚合物基质、脂质体和微球。它们可以例如通过经细菌过滤器 (bacteria-retaining filter) 过滤或者通过掺入无菌固体组合物形式的灭菌剂来进行灭菌，所述无菌固体组合物形式的灭菌剂可以在使用前即溶于无菌水或其他无菌可注射介质。

[0040] 若需要，实施例 1 化合物还可以为微囊化形式，其包含一种或多种上文所述的药学可接受的载体。片剂、糖锭剂、胶囊剂、丸剂和颗粒剂的固体剂型可以用包衣和壳来制备，例如肠溶衣、控释包衣和药物配制领域公知的其他包衣。在此类固体剂型中，实施例 1 化合物可以与至少一种惰性稀释剂如蔗糖、乳糖或淀粉混合。在通常的实践中，此类剂型还可以包含除了惰性稀释剂以外的其他物质，例如压片润滑剂和其他压片助剂如硬脂酸镁和微晶纤维素。在胶囊剂、片剂和丸剂的情况下，所述剂型还可以包含缓冲剂。它们可以任选地包含遮光剂，并且还可以是这样的组合物，其仅或者优选地在肠道的某一部分以延迟方式释放一种或多种活性成分。可用的包埋组合物的实例包括聚合物质和蜡。

[0041] 可注射贮库 (depot) 形式可以通过在生物可降解聚合物如聚乳酸 - 聚乙醇酸 (polylactide-polyglycolide) 中形成药物的微囊化基质来制备。取决于药物与聚合物的

比例以及所用的特定聚合物的性质，可以控制药物释放的速率。其他生物可降解聚合物的实例包括聚（原酸酯）和聚（酸酐）。贮库可注射制剂还可以通过将药物包埋（entrap）在与身体组织相容的脂质体或微乳剂中来制备。

[0042] 可注射制剂可以通过经细菌过滤器过滤或者通过掺入无菌固体组合物形式的灭菌剂来进行灭菌，所述无菌固体组合物形式的灭菌剂可以在使用前即溶于或分散于无菌水或其他无菌可注射介质。

[0043] 可注射制剂如无菌可注射水性或油性混悬剂可以根据已知的技术利用合适的分散或润湿剂和助悬剂来配制。无菌可注射制剂还可以是在无毒、肠胃外可接受的稀释剂或溶剂中的无菌可注射溶液剂、混悬剂或乳剂，例如在 1,3- 丁二醇中的溶液剂。可以使用的可接受媒介物和溶剂包括水、林格氏溶液、U.S.P. 和等渗氯化钠溶液。此外，无菌不挥发油通常用作溶剂或悬浮介质。为此目的，可以使用任何温和的不挥发油，包括合成的单甘油酯或二甘油酯。此外，脂肪酸如油酸也用于制备可注射制剂。

[0044] 用于口服给药的固体剂型包括胶囊剂、片剂、丸剂、散剂和颗粒剂。在此类固体剂型中，将实施例 1 化合物与以下混合 ：至少一种惰性药学可接受的载体如柠檬酸钠或磷酸钙，和 / 或 a) 填充剂或填料 (extender)，如淀粉、乳糖、蔗糖、葡萄糖、甘露醇和水杨酸 ；b) 粘合剂，如羧甲基纤维素、藻酸盐、明胶、聚乙烯吡咯烷酮、蔗糖和阿拉伯树胶 ；c) 湿润剂，如甘油 ；d) 崩解剂，如琼脂、碳酸钙、马铃薯或木薯淀粉、藻酸、某些硅酸盐和碳酸钠 ；e) 溶解阻滞剂 (solution retarding agent)，如石蜡 ；f) 吸收促进剂，如季铵化合物 ；g) 润湿剂，如鲸蜡醇和单硬脂酸甘油酯 ；h) 吸附剂，如高岭土和膨润土 ；和 i) 润滑剂，如滑石、硬脂酸钙、硬脂酸镁、固体聚乙二醇、月桂基硫酸钠 ；以及它们的混合物。在胶囊剂、片剂和丸剂的情况下，所述剂型还可以包含缓冲剂。

[0045] 相似类型的固体组合物还可以在软和硬明胶胶囊中利用乳糖 (sucrose) 或乳糖 (milk sugar) 以及高分子量聚乙二醇等作为填充剂。

[0046] 片剂、糖锭剂、胶囊剂、丸剂和颗粒剂的固体剂型可以用包衣和壳来制备，例如肠溶衣和制药领域公知的其他包衣。它们可以任选地包含遮光剂，并且还可以是这样的组合物，其仅或者优选地在肠道的某一部分以延迟方式释放一种或多种活性成分。可用的包埋组合物的实例包括聚合物质和蜡。

[0047] 用于口服给药的液体剂型包括药学可接受的乳剂、微乳剂、溶液剂、混悬剂、糖浆剂和酏剂。除了实施例 1 化合物以外，所述液体剂型还可以包含本领域中常用的惰性稀释剂，例如水或其他溶剂，增溶剂和乳化剂，如乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苯甲醇、苯甲酸苄酯、丙二醇、1,3- 丁二醇、二甲基甲酰胺、油（特别是棉籽油、花生油、玉米油、胚芽油、橄榄油、蓖麻油和芝麻油）、甘油、四氢糠醇、聚乙二醇和脱水山梨醇的脂肪酸酯以及它们的混合物。

[0048] 除了惰性稀释剂以外，口服组合物还可以包含辅剂，如润湿剂、乳化剂和助悬剂、甜味剂、风味剂和芳香剂。

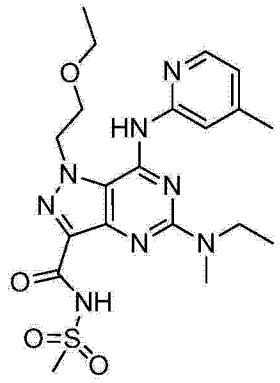
[0049] 在本发明的药物组合物中，可以改变实施例 1 化合物的实际剂量水平以获得实现对于特定患者、组合物和给药方式期望的治疗应答的实施例 1 化合物的有效量。所选的剂量水平会取决于实施例 1 化合物的活性、给药途径、治疗的疾病状况的严重性以及治疗的患者的健康状况和之前的治疗历史。

[0050] 向患者给药的实施例 1 化合物的总每日剂量为 0.3-400mg。若期望，可以为了给药目的将有效的每日剂量分为多个剂量，例如每天 2-4 次单独的剂量。

[0051] 合成制备

[0052] 实施例 1 化合物

[0053]



[0054] 1-(2- 乙氧基乙基)-5-(乙基(甲基)氨基)-7-((4- 甲基吡啶 -2- 基)氨基)-N-(甲基磺酰基)-1H- 吡唑并 [4,3-d] 嘧啶 -3- 甲酰胺

[0055] 标题化合物如美国专利 No. 7,572,799(见实施例 115) 所述制备。标题化合物的多晶型形式 A、B 和 C 如美国公开专利申请 No. 2008/0194591(US 专利申请 No. 11/913,091) 所述制备。US 7,572,799 和 US 2008/0194591 援引加入本文。

[0056] 生物学 / 药理学

[0057] 在小鼠和大鼠的单剂量毒性研究中，没有观察到死亡；最大非致死剂量为 2000mg/kg。在犬中，给药高达 1000mg/kg 的剂量，没有观察到副作用。

[0058] 在人中，在 46 名 21-49 岁的健康男性志愿者中，以单剂量和多剂量临床研究评价实施例 1 化合物。单剂量和多剂量临床研究都以口服溶液剂或混悬剂进行，单剂量为 0.3-400mg，多剂量为 30-200mg。在给药单剂量溶液剂后，实施例 1 化合物在人中快速吸收，C_{max}的发生时间 (T_{max}) 为 1.1-1.5 小时。终末半衰期没有随剂量明显改变，并且为 11.9-15.7 小时。给药多剂量后，实施例 1 化合物快速吸收 (T_{max}为 1.1-3.5 小时)，并且第 1 天和第 13 天的 t1/2 为 11.6-14.5 小时。实施例 1 化合物的单剂量良好耐受，没有发生严重的副作用。在多剂量研究中，实施例 1 化合物在 30-200mg 的剂量范围中良好耐受。生命体征没有明显改变，报导任何剂量的 ECG、实验室安全性测试结果或者身体检查结果。

[0059] 表 1

[0060]

测定 / 模型	实施例 1 化合物效力 / 效果
PDE5 酶 (人血小板)	IC_{50} = 0.71nM
PDE5 酶 (大鼠血小板)	IC_{50} = 0.93nM
PDE5 酶 (犬血小板)	IC_{50} = 0.65nM

主动脉环舒张(aortic ring relaxation,大鼠)	EC_{50}= 3.1nM
体内 SHR(口服给药)	EC_{max}= 6.5nM(未结合的血浆浓度)
PDE6 酶(人视锥)	IC_{50}= 28.9nM
PDE6 酶(人视杆)	IC_{50}= 63.6nM
PDE11 酶(人)	IC_{50}= 26.3nM

[0061] IC_{50}= 50%抑制浓度;EC_{50}=有效浓度;EC_{max}=最大有效浓度;SHR=自发性高血压大鼠。

[0062] 实施例 1 化合物是人 PDE5 的竞争性抑制剂,利用源自血小板的酶测定的平均 IC_{50} 为 0.71nM(0.34ng/mL)。针对大鼠和犬的源自血小板的 PDE5 的效力类似,分别为 0.93 和 0.65nM。实施例 1 化合物的针对人视锥和视杆的 PDE6IC_{50}效力分别为 28.9nM(41 倍选择性)和 63.6nM(90 倍选择性)。实施例 1 化合物针对人 PDE11 的 IC_{50}为 26.3nM(37 倍选择性)。相对于 PDE 酶 1、2、3、4A、4B、4D、7B、8A、9 和 1,存在大于 1000 倍选择性。

[0063] 在分离的大鼠主动脉环中证实了实施例 1 化合物的直接功能性效应。实施例 1 化合物诱导主动脉环血管舒张,平均 EC_{50}值为 3.1nM。

[0064] 每日口服给药后,在通过无线电遥测技术连续监测的有意识的自发性高血压大鼠(SHR)中评价实施例 1 化合物的抗高血压效力。在对应于约 7 倍大鼠 PDE5IC_{50}的游离血浆浓度下实现平均动脉压(MAP)的显著降低。用实施例 1 化合物治疗 SHR 在 14 天的给药期内诱导 MAP 明显且持续的降低。类似地,用 ACE 抑制剂依那普利治疗在 14 天的给药期内诱导 MAP 明显且持续的降低。当与 ACE 抑制剂组合时,实施例 1 化合物提供了比单独的 ACE 抑制剂更大的 MAP 降低效果。

[0065] 在如表 2 所示的一系列安全性药理学研究中评价实施例 1 化合物。对于体内研究,使用口服暴露途径。给药地西泮或呋塞米的大鼠分别在运动行为和流体/电解质分泌研究中充当阳性对照。多非利特在多非利特和 hERG 测定中分别用于测定可靠性或用作阳性对照。在剩余的研究中没有同时评价阳性对照或给药阳性对照的动物,因为这些研究使用良好表征的模型来评价安全性参数。

[0066] 表 2

[0067]

研究	浓度或剂量
[^{3}H]多非利特结合测定	0.003-100 μM
hERG 测定	3 μM 和 10 μM
浦肯野纤维测定	0.01 μM, 0.1 μM, 1 μM, 10 μM
体内耐受 - 大鼠 外观/行为 - 单剂量	100 mg/kg, 300 mg/kg, 1000 mg/kg
中枢神经系统 - 大鼠 运动行为	3 mg/kg, 30 mg/kg, 300 mg/kg
肾 - 大鼠 流体和电解质分泌	3 mg/kg, 30 mg/kg, 300 mg/kg
肺功能 - 大鼠 呼吸参数	3 mg/kg, 30 mg/kg, 300 mg/kg
心血管 - 麻醉的犬 PK/PD 评价，进行监测	1.47-387 μg/kg/min
心血管 - 有意识的犬 血液动力学和 ECG 参数	0.5 mg/kg, 1.5 mg/kg, 5 mg/kg

[0068] 在 hERG 膜片钳测定、犬浦肯野纤维测定或高达 10 μM(4.77 μg/mL) 的 [^{3}H] 多非利特结合测定中没有观察到相关效果。在 30 μM(14.3 μg/mL) 和 100 μM(47.7 μg/mL) 下，实施例 1 化合物分别竞争性地代替 5.5％和 32.5％的 [^{3}H] 多非利特。4.77 μg/mL 的无效果浓度约为 30mg 剂量后获得的基于人药物动力学 (PK) 的 C_{max}（约 0.011 μg/mL 作为游离部分）的 426 倍，这是良好耐受的，表明 QT 延长的低可能性。

[0069] 给药高达 300mg/kg 的实施例 1 化合物的大鼠的结果证实了由降低的抬升 (rearing) 和中心抬升 (center rearing) 组成的小的非剂量相关的效果，但是对肺功能没有副作用。实施例 1 化合物在尿量和电解质分泌中证实了剂量相关的减少，这与其他 PDE5 抑制剂的结果一致。

[0070] 在犬中，0.5mg/kg、1.5mg/kg 和 5mg/kg 的实施例 1 化合物诱导小的但显著的血压和左心室舒张末期压的降低。这些心血管效果与得自 cGMP 特异性 PDE5 的抑制的血管平滑肌中的 cGMP 升高一致。在犬中，实施例 1 化合物与 ECG 参数的任何相关效果无关。在给药单剂量和多剂量的实施例 1 化合物的人中没有观察到对 ECG 参数的临床相关效果。在 5mg/kg 下犬中的 C_{max} 为 12.8mg/mL，或者是 30mg 剂量后在人中测定的 C_{max} (1.57-1.63 μg/mL) 的 8 倍，这是良好耐受的。

[0071] 液相色谱 / 质谱法用于测定来自大鼠、犬和人的 PK 和毒动学研究的血浆样品中的实施例 1 化合物浓度。对于毒动学研究，对于 50 μL 血浆样品，该方法在 10-1000ng/mL 的浓度范围内得到了验证。放射测量法用于测定来自体外和体内代谢研究的生物学样品中的来自 [^{14}C] 实施例 1 化合物的放射性。

[0072] 给药单剂量后的实施例 1 化合物的绝对口服生物利用度在大鼠中为 82％并且在犬中为 77％。静脉内 (IV) 给药实施例 1 化合物后，大鼠和犬中的血浆清除低于对应物种中的肝脏血流的清除，表明实施例 1 化合物是低清除化合物。在大鼠和犬中，稳态分布体积低于全身含水量。

[0073] 在给药放射性标记的实施例 1 化合物的大鼠的组织分布研究中，由于大部分的剂量保留在胃肠道中，全身分布通常与组织血流成比例。实施例 1 化合物的血浆蛋白结合值

在大鼠、犬和人中测定为 99.3%，并且在兔血浆中测定为 98.8%。

[0074] 在大鼠和犬中，大于 80%的给药剂量（总放射性）在粪便中消除。大部分的放射性在 48 小时内恢复。大鼠和犬中恢复的总放射性大于 92%。

[0075] 在人肝微粒体中测定实施例 1 化合物抑制 CYP1A2、CYP2C9、CYP2C19、CYP2D6 和 CYP3A4（咪达唑仑、睾酮和非洛地平）的潜力。没有观察到 IC_{50}值 >30 μM 的抑制。

[0076] 在口服单剂量给药 20mg/kg、200mg/kg 或 2000mg/kg 的实施例 1 化合物并且观察 14 天的小鼠或大鼠中，没有死亡、临床体征或对体重的影响，并且没有病理发现。在口服单剂量给药 100mg/kg、300mg/kg 或 1000mg/kg 的大鼠中没有观察到毒性，而在口服单剂量给药 100mg/kg、500mg/kg 或 1000mg/kg 的犬中发生轻度的胃肠效应。

[0077] 在大鼠和犬中的单剂量毒性 / 毒动学研究中评价微粉化的实施例 1 化合物（多晶型 C），以与多晶型 B 的暴露进行比较。微粉化的实施例 1 化合物的多晶型 C 使得能在大鼠和犬中比多晶型 B 给药更高的剂量。在等同剂量的多晶型 B 和 C 下（犬中 100mg/kg，并且大鼠中 100mg/kg 和 300mg/kg），这些多晶型的全身暴露之间没有差异。

[0078] 在由微生物回复突变测定、体外细胞遗传测定（人淋巴细胞）和体内大鼠微核测定组成的一系列遗传毒理学测定中评价实施例 1 化合物。研究设计和剂量选择与经济合作与发展的协调和组织的国际会议 (International Conference on Harmonization and Organization for Economic Cooperation and Development) 对诱变性和诱裂性测定的指导一致。有或无外源代谢激活的所有体外测试均利用高达细胞毒性或不溶性所限制的浓度进行。实施例 1 化合物在体外或体内测定中没有遗传毒性（表 3）。

[0079] 表 3

[0080]

测试系统	剂量	结果
诱变性: 鼠伤寒沙门氏杆菌(Salmonella typhimurium)(菌株 TA-1535, TA-1537)和大肠杆菌(Escherichia coli) (菌株 WP2uvrA)	50-5000 mg/平板 (±S9)	阴性
体外诱裂性: 人外周淋巴细胞中的结构染色体畸变	100, 350, 500, μg/mL (3h-S9) 100, 200, 350, μg/mL (3h+S9) 12.5, 50, 100, μg/mL (24h-S9)	阴性
体外诱裂性 **大鼠骨髓中的微核测定**	VC, 100, 300, μ600 mg/kg/day	阴性

[0081] S9 = 来自用 Aroclor 1254 处理的大鼠的肝的线粒体后上清液；VC = 媒介物对照。

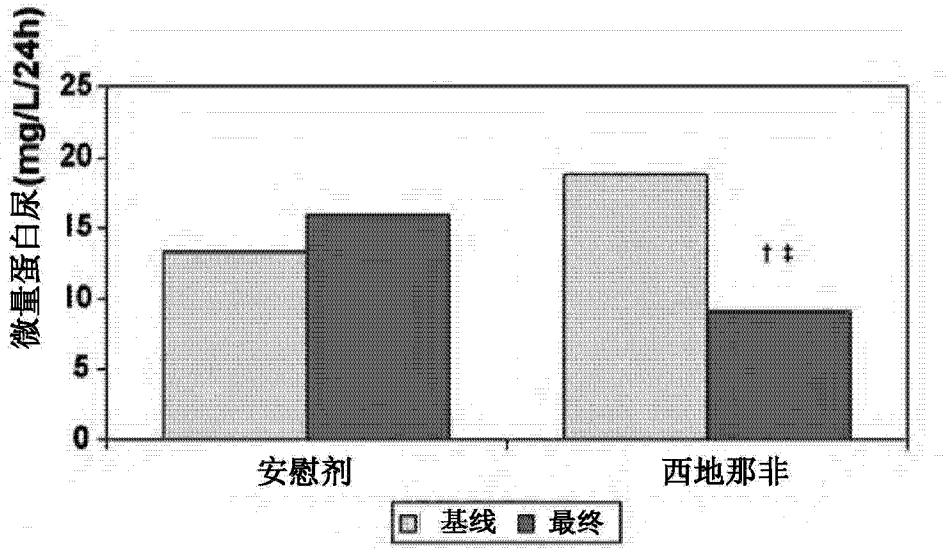
[0082] PDE5 抑制剂西地那非在患有早期糖尿病肾病的 II 型糖尿病患者中减少蛋白尿（图 1）。该数据支持 PDE5 抑制剂用于治疗在人中具有合适的药物动力学 (PK) 和安全性特性的 DN 和 / 或 CKD 的用途。实施例 1 化合物是强效且选择性的 PDE5 抑制剂，其在人中口服给药后良好耐受。与西地那非相比，实施例 1 化合物看起来是更为强效的 PDE5 抑制剂，并且具有更大的 PDE5/PDE6 选择性。此外，实施例 1 化合物在人中具有 3-4 倍长的半衰期（表 4）。这些优势使得实施例 1 化合物对于治疗或延缓人特别是糖尿病患者中的蛋白尿、DN 和 / 或 CKD 的进展优于西地那非。

[0083] 表 4

[0084]

标准	实施例 1 化合物	西地那非
PDE5IC_{50}(nM)	0.71	4*
PDE5/PDE6 选择性	41 倍	9 倍 *
人半衰期 (hr)	11.9-15.7	3.7

[0085] * 如 Ballard 等人 , The Journal of Urology, 159:2164-2171, Jun 1998 报导。

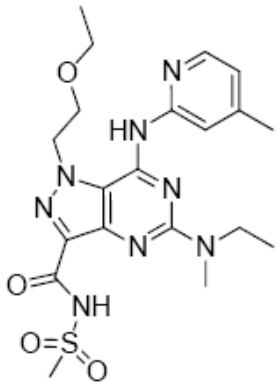


Grover-Paez 等人, Diabetes Research and Clinical Practice, 78: 136-140 (2007)

图 1

Abstract

The present invention relates to methods of delaying progression to end stage renal disease (ESRD) in patients comprising administration of 1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide. The present invention also includes administration of pharmaceutical compositions for delaying progression to ESRD.



1-(2-ethoxyethyl)-5-(ethyl(methyl)amino)-7-((4-methylpyridin-2-yl)amino)-N-(methylsulfonyl)-1H-pyrazolo[4,3-d]pyrimidine-3-carboxamide