NOVEL METHOD

This invention relates to a novel method for the treatment of renal diseases.

European Patent Applications, Publication Numbers: 0306228, 0008203, 0139421, 0032128, 0428312, 0489663, 0155845, 0257781, 0208420, 0177353, 0319189, 0332331, 0332332, 0528734, 0508740; International Patent Application, Publication Numbers 92/18501, 93/02079, 93/22445 and United States Patent Number 5104888, disclose certain thiazolidinedione derivatives which are disclosed as having hypoglycaemic and hypolipidaemic activity. The thiazolidinedione derivatives disclosed in these patent applications are examples of a class of hypoglycaemic agent generally referred to as 'insulin sensitisers' and hence these compounds are referred to herein as 'thiazolidinedione insulin sensitisers'.

Another series of compounds generally recognised as having insulin sensitiser activity are those typified by the compounds disclosed in International Patent Applications, Publication Numbers WO93/21166 and WO94/01420. These compounds are herein referred to as 'acyclic insulin sensitisers'. Other examples of acyclic insulin sensitisers are those disclosed in United States Patent Number 5232945 and International Patent Applications, Publication Numbers WO92/03425 and WO91/19702.

Examples of other insulin sensitisers are those disclosed in European Patent Application, Publication Number 0533933, Japanese Patent Application Publication Number 05271204 and United States Patent Number 5264451.

We have now discovered that compounds having insulin sensitiser activity can prevent hydronephrosis and proteinuria, such as albuminuria, and that they are therefore of potential use in the treatment and/or prophylaxis of renal disease, especially renal disease associated with the development of Type II diabetes including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease. The prophylactic action of an insulin sensitiser upon nephropathy is also indicative that an insulin sensitising agent can be expected to prevent, reverse, stabilise or retard the progression of microalbuminuria to albuminuria. This is because microalbuminuria is considered to be a predictor of future nephropathy, especially in patients with clinical evidence of pre-diabetic insulin resistance



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☑ Description : paged ...1.... to paged ...17.....
☑ Claim(s) : paged ...18... to paged ...21.....
☐ Drawing(s) : paged ..... to paged .....
☑ Abstract : paged ...22... to paged .....
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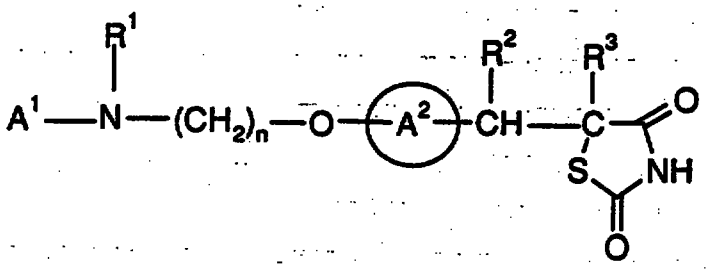
syndrome, alternatively referred to as Syndrome X.

Accordingly, the present invention provides a method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.

Particular insulin sensitisers include thiazolidinedione insulin sensitisers.

Particular insulin sensitisers include acyclic insulin sensitisers.

One favoured group of insulin sensitisers are the thiazolidinedione insulin sensitisers disclosed in EP 0306228 and WO94/05659. Thus in a favoured aspect the present invention provides a method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which method comprises the administration of an effective non-toxic amount of a compound of formula (I):



(I)

or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable solvate thereof, wherein:

A^1 represents a substituted or unsubstituted aromatic heterocyclyl group;

R^1 represents a hydrogen atom, an alkyl group, an acyl group, an aralkyl group, wherein the aryl moiety may be substituted or unsubstituted, or a substituted or unsubstituted aryl group;

R^2 and R^3 each represent hydrogen, or R^2 and R^3 together represent a bond;

A^2 represents a benzene ring having in total up to five substituents; and

n represents an integer in the range of from 2 to 6; to a human or non-human mammal in need thereof.

Suitable aromatic heterocyclyl groups of the compounds of formula (I) include substituted or unsubstituted, single or fused ring aromatic heterocyclyl groups comprising up to 4 hetero atoms in each ring selected from oxygen, sulphur or nitrogen.

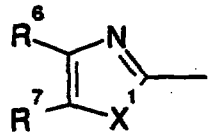
Favoured aromatic heterocyclyl groups of the compounds of formula (I) include substituted or unsubstituted single ring aromatic heterocyclyl groups having 5 to 7 ring atoms, preferably 5 or 6 ring atoms.

In particular, the aromatic heterocyclyl groups of the compounds of formula (I) comprise 1, 2 or 3 heteroatoms, especially 1 or 2, selected from oxygen, sulphur or nitrogen.

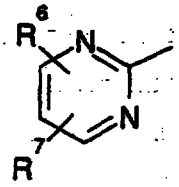
Suitable values for A^1 when it represents a 5- membered aromatic heterocyclyl group include thiazolyl and oxazolyl, especially oxazolyl.

Suitable values for A^1 when it represents a 6- membered aromatic heterocyclyl group include pyridyl or pyrimidinyl, especially pyridyl.

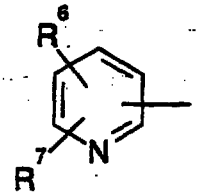
Preferably, A^1 represents a moiety of formula (a), (b) or (c):



(a)



(b)



(c)

wherein:

R^6 and R^7 each independently represents a hydrogen or halogen atom, an alkyl or alkoxy group or a substituted or unsubstituted aryl group or when R^6 and R^7 are each attached to adjacent carbon atoms, then R^6 and R^7 together with the carbon atoms to which they are attached form a benzene ring wherein each carbon atom represented by R^6 and R^7 together is substituted or unsubstituted; and in the moiety of formula (a) X^1 represents oxygen or sulphur.

Aptly, A^1 represents a moiety of the abovedefined formula (a).

Aptly, A^1 represents a moiety of the abovedefined formula (b).

Aptly, A^1 represents a moiety of the abovedefined formula (c).

A particular form of moiety (c) is a moiety (c'):

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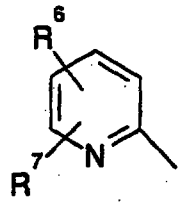
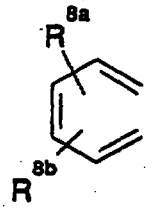


(c')

wherein R^6 and R^7 are as defined in relation to formula (c).

In one favoured aspect R^6 and R^7 together represent a moiety of formula (d):



(d)

wherein R^{8a} and R^{8b} each independently represent hydrogen, halogen, substituted or unsubstituted alkyl or alkoxy.

Suitably, R^{8a} and R^{8b} each independently represent hydrogen, halogen, alkyl or alkoxy. Favourably, R^{8a} represents hydrogen. Favourably, R^{8b} represents hydrogen. Preferably, R^{8a} and R^{8b} both represent hydrogen.

In a further favoured aspect R^6 and R^7 each independently represent hydrogen, alkyl or a substituted or unsubstituted phenyl group and more favourably, R^6 and R^7 each independently represent hydrogen, alkyl or phenyl.

Preferably, for the moiety of formula (a), R^6 and R^7 together represent the moiety of formula (d).

Preferably, for the moieties of formula (b), (c) or (c'), R^6 and R^7 both represent hydrogen.

It will be appreciated that the five substituents of A^2 include three optional substituents. Suitable optional substituents for the moiety A^2 include halogen, substituted or unsubstituted alkyl or alkoxy.

Further suitable, favoured and preferred values for variables A^2, R^1, R^2, $R^3$and n are as defined in EP 0306228 and WO94/05659.

A preferred compound of formula (I) is 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof

and/or a pharmaceutically acceptable salt thereof, especially a maleic acid salt thereof, and/or a pharmaceutically acceptable solvate thereof.

As indicated above, a compound of formula (I) may exist in one of several tautomeric forms, all of which are encompassed in the method of the present invention. It will be appreciated that the present invention encompasses the administration of all of the isomeric forms of the compounds of formula (I) and the pharmaceutically acceptable salts thereof, including any stereoisomeric forms thereof, whether as individual isomers or as mixtures of isomers.

Suitable substituents for any heterocyclyl group of the compounds of formula (I) include up to 4 substituents selected from the group consisting of: alkyl, alkoxy, aryl and halogen or any two substituents on adjacent carbon atoms, together with the carbon atoms to which they are attached, may form an aryl group, preferably a benzene ring, and wherein the carbon atoms of the aryl group represented by the said two substituents may themselves be substituted or unsubstituted.

The suitable, favoured and preferred thiazolidinedione insulin sensitisers disclosed in European Patent Applications, Publication Numbers: 0306228, 0008203, 0139421, 0032128, 0428312, 0489663, 0155845, 0257781, 0208420, 0177353, 0319189, 0332331, 0332332, 0528734, 0508740; International Patent Application, Publication Numbers 92/18501, 93/02079, 93/22445 and United States Patent Number 5104888 are those compounds defined as suitable, favoured and preferred in the respective patent publications.

The suitable, favoured and preferred acyclic insulin sensitisers disclosed in International Patent Applications, Publication Numbers WO91/19702, WO92/03425, WO93/21166 and WO94/01420 and United States Patent Number 5232945 are those compounds defined as suitable, favoured and preferred in the respective patent publications.

Other suitable, favoured and preferred insulin sensitisers are the suitable, favoured and preferred compounds disclosed in European Patent Application, Publication Number 0533933, International Patent Application, Publication Number WO 93/02079 , Japanese Patent Application Publication Number 05271204 and United States Patent Number 5264451.

Also specifically included in the method of the invention are the specific examples disclosed in the above mentioned patent applications.

When used herein the term 'insulin sensitiser' relates to compounds which

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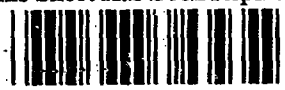

increase the biological response to insulin. In addition, based upon the observed effects in appropriate test animals such as Zucker fatty (fa/fa) rats, insulin sensitisers are indicated to lower elevated fasting plasma insulin concentrations and improve glycaemic control.

When used herein the term 'aryl' includes phenyl and naphthyl optionally substituted with up to five, preferably up to three, groups selected from halogen, alkyl, phenyl, alkoxy, haloalkyl, hydroxy, amino, nitro, carboxy, alkoxycarbonyl, alkoxycarbonylalkyl, alkylcarbonyloxy, or alkylcarbonyl groups.

When used herein the term 'halogen' refers to fluorine, chlorine, bromine and iodine; preferably chlorine.

When used herein the terms 'alkyl' and 'alkoxy' relate to groups having straight or branched carbon chains,containing up to 12 carbon atoms.

When used herein the term 'acyl' includes alkylcarbonyl groups.

Suitable alkyl groups are C_{1-12} alkyl groups, especially C_{1-6} alkyl groups e.g. methyl, ethyl, n-propyl, iso-propyl, n-butyl, isobutyl or tert-butyl groups.

Suitable substituents for any alkyl group include those indicated above in relation to the term "aryl".

Suitable pharmaceutically acceptable salts include salts of carboxy groups and acid addition salts.

Suitable pharmaceutically acceptable salts of carboxy groups include metal salts, such as for example aluminium, alkali metal salts such as lithium, sodium or potassium, alkaline earth metal salts such as calcium or magnesium and ammonium or substituted ammonium salts, for example those with lower alkylamines such as triethylamine, hydroxy alkylamines such as 2-hydroxyethylamine, bis-(2-hydroxyethyl)-amine or tri-(2-hydroxyethyl)-amine, cycloalkylamines such as bicyclohexylamine, or with procaine, dibenzylpiperidine, N-benzyl-b-phenethylamine, dehydroabietylamine, N,N'-bisdehydroabietylamine, glucamine, N-methylglucamine or bases of the pyridine type such as pyridine, collidine, quinine or quinoline.

Suitable acid addition salts include pharmaceutically acceptable inorganic salts such as the sulphate, nitrate, phosphate, borate, hydrochloride and hydrobromide and pharmaceutically acceptable organic acid addition salts such as acetate, tartrate, maleate, citrate, succinate, benzoate, ascorbate, methane-sulphonate, a-keto glutarate and a-glycerophosphate, especially the maleate salt.

Suitable pharmaceutically acceptable solvates include hydrates.

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The active compounds disclosed in the above mentioned patent publications, and referred to herein as insulin sensitisers, including the specific examples disclosed therein, are conveniently prepared according to the methods disclosed in the said patent publications: Thus a compound of formula (I), or the tautomeric form thereof, and/or a pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable solvate thereof, may be prepared using the processes described in EP 0306228 and WO94/05659.

The salts and/or solvates of the compounds of formula (I) may be prepared and isolated according to conventional procedures for example sodium salts may be prepared by using sodium methoxide in methanol.

The present invention also provides an insulin sensitiser,such as a compound of formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, for use in the treatment of and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria.

The present invention also provides an insulin sensitiser,such as a compound of formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, for use in the manufacture of a medicament for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria.

In the above mentioned treatment and or prophylaxis the insulin sensitiser such as a compound of formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, may be administered per se or, preferably, as a pharmaceutical composition also comprising a pharmaceutically acceptable carrier.

Accordingly, the present invention also provides a pharmaceutical composition for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which composition comprises an insulin sensitiser, such as a compound of the formula (I), or a tautomeric form thereof, or a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, and a

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pharmaceutically acceptable carrier therefor.

As used herein the term 'pharmaceutically acceptable' embraces compounds, compositions and ingredients for both human and veterinary use: for example the term 'pharmaceutically acceptable salt' embraces a veterinarily acceptable salt.

The composition may, if desired, be in the form of a pack accompanied by written or printed instructions for use.

Usually the pharmaceutical compositions of the present invention will be adapted for oral administration, although compositions for administration by other routes, such as by injection and percutaneous absorption are also envisaged.

Particularly suitable compositions for oral administration are unit dosage forms such as tablets and capsules. Other fixed unit dosage forms, such as powders presented in sachets, may also be used.

In accordance with conventional pharmaceutical practice the carrier may comprise a diluent, filler, disintegrant, wetting agent, lubricant, colourant, flavourant or other conventional adjuvant.

Typical carriers include, for example, microcrystalline cellulose, starch, sodium starch glycollate, polyvinylpyrrolidone, polyvinylpolypyrrolidone, magnesium stearate, sodium lauryl sulphate or sucrose.

Most suitably the composition will be formulated in unit dose form. Such unit dose will normally contain an amount of the active ingredient in the range of from 0.1 to 1000 mg, more usually 0.1 to 500 mg, and more especially 0.1 to 250 mg.

Conveniently, the active ingredient may be administered as a pharmaceutical composition hereinbefore defined, and this forms a particular aspect of the present invention.

In the above mentioned treatments an insulin sensitiser, such as a compound of the formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, may be taken in doses, such as those described above, one to six times a day in a manner such that the total daily dose for a 70 kg adult will generally be in the range of from 0.1 to 6000 mg, and more usually about 1 to 1500 mg, generally about 0.5 to 10 mg. That is in the range of from 1.429×10^{-3} to 85.714 mg/kg/day, more usually about 1.429×10^{-2} to 21.429 mg/kg/day, generally about 7.143×10^{-3} to 0.1429 mg/kg/day.

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The following Examples illustrate the invention but do not limit it in any way.

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Example 1: Studies Into The Effects Of The Test Compound Upon Renal Pathology

Obese Zucker rats are known to develop chronic nephropathy in addition to hyperlipidaemia, hyperinsulinaemia and peripheral insulin resistance (Kasiske *et al* 1985).

5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione (herein after referred to as 'test compound') was administered to 2-3 month old obese Zucker fa/fa rats by dietary administration to provide a daily dose over a period of 3 months ranging from 2.0 - 7.0 μmole/kg body weight. A group of age-matched obese Zucker fa/fa rats were given the same diet without the addition of the test compound, as was a group of lean Fa/? rats. At the end of the study, all of the control Zucker fa/fa rats had chronic nephropathy which involved dilated tubules, atrophied or hyperplastic tubular epithelial cells, thickened tubular basement membranes, segmented glomeruli or global glomerulosclerosis.

In the corresponding lean animals, 3/15 animals, a minimal degree of chronic nephropathy was seen.

Treatment with test compound resulted in a reduction in the incidence and degree of chronic nephropathy compared to the control group of Zucker fa/fa rats.

In the kidneys of the control Zucker fa/fa rats, mild-moderate hydronephrosis was seen in 4/9 animals. Characteristically this involved dilation of the kidney pelvis. No hydronephrosis was seen in the rats treated with test compound.

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TABLE OF SIGNIFICANT HISTOPATHOLOGICAL FINDINGS IN THE KIDNEY OF FATTY ZUCKER RATS

Group/Treatment Animal Number	Hydronephrosis	Chronic Nephropathy
Group 1		
Test Compound in diet		
2	0	±
7	0	±
9	0	0
15	0	++
16	0	±
17	0	+
21	0	±
22	0	±
24	0	±
27	0	±
28	0	±
Group 2		
Diet only		
3	++	+
5	+++	+
13	0	+
14	++	++
18	0	+
23	+++	++
26	0	+
29	0	+
30	0	++

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Severity Key

0 None seen

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±	Minimal
+	Mild
++	Moderate
+++	Marked

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Example 2: Studies Into The Effect Of Test CompoundsUpon Systolic Blood Pressure, Urinary Total Protein And Urinary N-Acetyl ß-D-Glucosaminidase (NAG) Activity Measurments.

A second study was performed in Zucker rats over a period of 9 months to investigate the longitudinal effects of the drug on systolic blood pressure and various indices of renal function, two of which are exemplified here. In one arm of the study, the drug was given in the diet (50 µmole/kg of diet) from aged 6-7weeks in Zucker fatty (fa/fa) rats, whilst in a second arm of the study, drug treatment as above was delayed until proteinuria had become established after 4 months, indicative of structural damage already present in the kidneys. A third group of Zucker fatty rats and a further group of lean rats were given diet alone throughout the period of study.

After dosing with the test compound, at monthly intervals measurements were made of systolic blood pressure, urinary total protein and urinary N-acetyl ß-D-glucosaminidase (NAG) activity.

Measurement of Systolic Blood Pressure

Rats were enclosed in custom-built restrainers and placed on a shelf in a warming cabinet, whose temperature was controlled at approximately 30°C. An inflatable cuff with integral pulse sensor was attached to the tail of each rat. After warming for 20-30 min the tail cuff was inflated and deflated automatically and a measurement of systolic blood pressure made using an IITC Non-Invasive Blood Pressure Monitor. This cycle was repeated several times for each rat until stable values of blood pressure were obtained.

Measurement of Urinary Parameters

Twenty-four hour urine collections, made in metabolism cages, were aliquoted and frozen at -70°C until required for assay.

(i) Urinary Protein

Urinary protein concentration was measured using the Bio-Rad protein assay as modified for use in a 96-well microtitre plate. The assay is a dye-binding assay based on the differential colour change of a dye in response to various

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concentrations of protein (Bradford, Anal. Biochem.,72, 248, 1976). After a period of incubation with Dye Reagent, diluted urine samples are read at an optical density of 595 nm using a Molecular Devices multiplate reader.

(ii) Urinary NAG Activity

NAG activity was assayed using a reagent kit on a Hitachi 717 analyser (both suppied by Boehringer Mannheim UK, Lewes). Enzyme activity was measured by monitoring the rate of chlorophenol (570 nm) released from the substrate chlorophenol red-ß-D-glucosaminide.

Results and Statistics

In the tables below, results are given as mean values for the group ± the standard error of the mean. Results have been analysed by one way ANOVA and significant differences from the Zucker fatty rat control group have been indicated by an asterisk.

Conclusion

The results of Example 2 demonstrate that treatment of Zucker fatty (fa/fa) rats from the age of 6-7 weeks, for a period of 9 months, with BRL 49653 given via the diet, prevented the development of hypertension and markedly reduced both the elevation of urinary NAG activity and the rate of development of proteinuria. When drug treatment was commenced after proteinuria had become established, both systolic blood pressure and urinary NAG activity were prevented from rising further and again there was a marked reduction of the rate of increase in the urinary protein concentration.

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Effects of starting treatment with Test Compound prior to the development of renal complications.

SYSTOLIC BLOOD PRESSURE (mm Hg)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
0	102 ± 3	106 ± 3	123 ± 3*
1	110 ± 3 *	120 ± 4	128 ± 3
2	122 ± 4 *	137 ± 4	132 ± 4
3	122 ± 4 *	142 ± 6	134 ± 3
4	126 ± 6 *	146 ± 4	132 ± 4*
5	131 ± 4 *	157 ± 6	138 ± 4*
6	128 ± 3 *	150 ± 6	133 ± 2*
7	125 ± 6*	157 ± 6	128 ± 6*
8	133 ± 5*	155 ± 4	130 ± 3*
9	143 ± 4*	164 ± 4	136 ± 3*

Effects of starting treatment with Test Compound prior to the development of renal complications

URINARY PROTEIN CONCENTRATION (µg/hour)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
0	226 ± 15 *	287 ± 17	198 ± 19*
1	373 ± 22	355 ± 18	321 ± 23*
2	402 ± 22 *	509 ± 28	396 ± 20*
3	221 ± 32 *	874 ± 102	393 ± 25*
4	376 ± 54 *	3965 ± 731	613 ± 52*
5	1573 ± 105 *	5823 ± 809	1395 ± 88*
6	1585 ± 147 *	8946 ± 1171	1192 ± 92*
7	2124 ± 293 *	12052 ± 1535	1176 ± 96*
8	2664 ± 370*	14534 ± 1540	1409 ± 92*
9	3152 ± 515*	16182 ± 1581	1373 ± 113*

Effects of starting treatment with Test Compound prior to the development of renal complications.

URINARY N-ACETYL -ß- D-GLUCOSAMINIDASE ACTIVITY (mU/hour)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
0	5.7 ± 0.4	5.9 ± 0.3	4.4 ± 0.3*
1	7.5 ± 0.7	9.1 ± 0.8	5.1 ± 0.5*
2	3.8 ± 1.1 *	8.2 ± 1.5	6.5 ± 0.3
3	4.5 ± 0.9 *	8.2 ± 1.0	5.2 ± 0.4*
4	5.1 ± 1.2 *	9.3 ± 0.5	6.9 ± 0.5*
5	6.5 ± 0.7 *	10.7 ± 0.4	7.6 ± 0.7*
6	6.5 ± 1.1 *	10.8 ± 1.7	7.4 ± 0.5
7	8.9 ± 0.8*	11.7 ± 0.7	7.1 ± 0.5*
8	6.2 ± 1.2*	11.8 ± 1.3	7.4 ± 0.5*
9	9.1 ± 1.2*	13.4 ± 0.8	7.7 ± 0.6*

Effects of starting treatment with Test Compound once renal complications have become established.

SYSTOLIC BLOOD PRESSURE (mm Hg)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
-4	106 ± 3	106 ± 3	123 ± 3*
-3	123 ±5	120 ± 4	128 ± 3
-2	138 ± 7	137 ± 4	132 ± 4
-1	141 ± 6	142 ± 6	134 ± 3
0	146 ± 4	146 ± 4	132 ± 4*
1	140 ± 5*	157 ± 6	138 ± 4*
2	134 ± 3*	150 ± 6	133 ± 2*
3	144 ± 3*	157 ± 6	128 ± 6*
4	139 ± 3*	155 ± 4	130 ± 3*
5	146 ± 6*	164 ± 4	136 ± 3*

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Effects of starting treatment with Test Compound once renal complications have become established.

URINARY PROTEIN CONCENTRATION (µg/hour)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
-4	220 ± 13	287 ± 17	198 ± 19*
-3	347 ± 32	355 ± 18	321 ± 23*
-2	457 ± 21	509 ± 28	396 ± 21*
-1	958 ± 194	874 ± 102	393 ± 25*
0	3520 ± 905	3965 ± 731	613 ± 52*
1	3692 ± 386*	5823 ± 809	1395 ± 88*
2	5326 ± 967*	8946 ± 1171	1192 ± 92*
3	6230 ± 835*	12052 ± 1535	1178 ± 96*
4	5379 ± 708*	14534 ± 1540	1409 ± 92*
5	7677 ± 825*	16182 ± 1581	1373 ± 113*

Effects of starting treatment with Test Compound once renal complications have become established.

URINARY N-ACETYL-ß-D-GLUCOSAMINIDASE ACTIVITY (mU/hour)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
-4	5.4 ± 0.2	5.9 ± 0.3	4.4 ± 0.3*
-3	8.3 ± 0.5	9.1 ± 0.8	5.1 ± 0.5*
-2	9.5 ± 1.1	8.2 ± 1.5	6.5 ± 0.3
-1	7.8 ± 0.6	8.2 ± 1.0	5.2 ± 0.4*
0	7.6 ± 0.7*	9.3 ± 0.5	6.9 ± 0.5*
1	7.6 ± 0.7*	10.7 ± 0.4	7.6 ± 0.7*
2	5.7 ± 0.9*	10.8 ± 1.7	7.4 ± 0.5
3	7.4 ± 0.9*	11.7 ± 0.7	7.1 ± 0.5*
4	5.4 ± 1.0*	11.8 ± 1.3	7.4 ± 0.5*
5	8.0 ± 1.1*	13.4 ± 0.8	7.7 ± 0.6*

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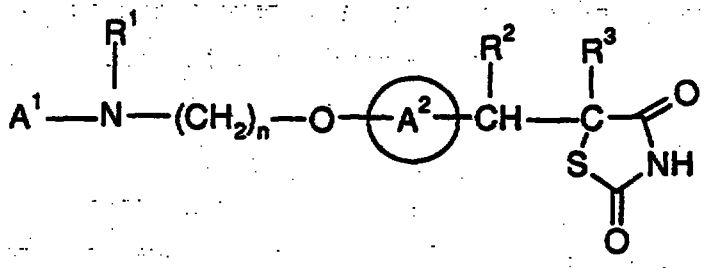
Claims:

1. A method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.

2. A method according to claim 1, wherein the insulin sensitiser is a thiazolidinedione insulin sensitiser.

3. A method according to claim 1, wherein the insulin sensitiser is an acyclic insulin sensitiser.

4. A method according to claim 1, wherein the insulin sensitiser is a compound of formula (I):



(I)

or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable solvate thereof, wherein:
A^1 represents a substituted or unsubstituted aromatic heterocyclyl group;
R^1 represents a hydrogen atom, an alkyl group, an acyl group, an aralkyl group, wherein the aryl moiety may be substituted or unsubstituted, or a substituted or unsubstituted aryl group;
R^2 and R^3 each represent hydrogen, or R^2 and R^3 together represent a bond;
A^2 represents a benzene ring having in total up to five substituents; and
n represents an integer in the range of from 2 to 6; to a human or non-human

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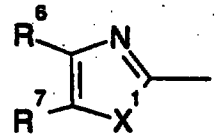
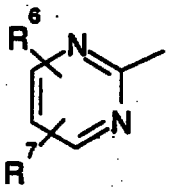
mammal in need thereof.

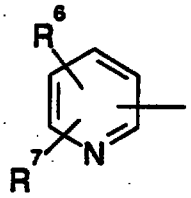
5. A method according to claim 4, wherein in the compound of formula (I) A^1 represents a moiety of formula (a), (b) or (c):



(a)



(b)

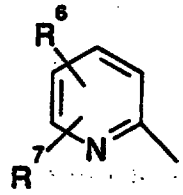


(c)

wherein:

R^6 and R^7 each independently represents a hydrogen or halogen atom, an alkyl or alkoxy group or a substituted or unsubstituted aryl group or when R^6 and R^7 are each attached to adjacent carbon atoms, then R^6 and R^7 together with the carbon atoms to which they are attached form a benzene ring wherein each carbon atom represented by R^6 and R^7 together is substituted or unsubstituted; and in the moiety of formula (a) X^1 represents oxygen or sulphur.

6. A method according to claim 5, wherein in the compound of formula (I) A^1 represents a moiety of the above defined formula (c).



(c')

wherein R^6 and R^7 are as defined in claim 5.

7. A method according to claim 1, wherein the insulin sensitiser is 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof.

8. A method according to claim 7, wherein the insulin sensitiser is a maleic

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* C L M S *

acid salt of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof and/or a pharmaceutically acceptable solvate thereof.

9. The use of an insulin sensitiser for the manufacture of a medicament for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria.

10. A pharmaceutical composition for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria which composition comprises an insulin sensitiser and a pharmaceutically acceptable carrier therefor.

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Abstract

Novel Method

A method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.

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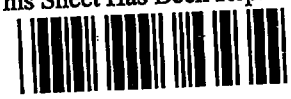


REPLACEMENT SHEETS SG
☑ Description : paged 1... to paged 17...
☑ Claim(s) : paged 18. to paged 30.
☐ Drawing(s) : paged ..... to paged .....
☑ Abstract : paged 31.. to paged ....
DATE OF RECEIPT 26/3/97

Use of insulin sensitisers for treating renal diseases

This invention relates to a novel method for the treatment of renal diseases.

European Patent Applications, Publication Numbers: 0306228, 0008203, 0139421, 0032128, 0428312, 0489663, 0155845, 0257781, 0208420, 0177353, 0319189, 0332331, 0332332, 0528734, 0508740; International Patent Application, Publication Numbers 92/18501, 93/02079, 93/22445 and United States Patent Number 5104888, disclose certain thiazolidinedione derivatives which are disclosed as having hypoglycaemic and hypolipidaemic activity. The thiazolidinedione derivatives disclosed in these patent applications are examples of a class of hypoglycaemic agent generally referred to as 'insulin sensitisers' and hence these compounds are referred to herein as 'thiazolidinedione insulin sensitisers'.

Another series of compounds generally recognised as having insulin sensitiser activity are those typified by the compounds disclosed in International Patent Applications, Publication Numbers WO93/21166 and WO94/01420. These compounds are herein referred to as 'acyclic insulin sensitisers'. Other examples of acyclic insulin sensitisers are those disclosed in United States Patent Number 5232945 and International Patent Applications, Publication Numbers WO92/03425 and WO91/19702.

Examples of other insulin sensitisers are those disclosed in European Patent Application, Publication Number 0533933, Japanese Patent Application Publication Number 05271204 and United States Patent Number 5264451.

We have now discovered that compounds having insulin sensitiser activity can prevent hydronephrosis and proteinuria, such as albuminuria, and that they are therefore of potential use in the treatment and/or prophylaxis of renal disease, especially renal disease associated with the development of Type II diabetes including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease. The prophylactic action of an insulin sensitiser upon nephropathy is also indicative that an insulin sensitising agent can be expected to prevent, reverse, stabilise or retard the progression of microalbuminuria to albuminuria. This is because microalbuminuria is considered to be a predictor of future nephropathy, especially in patients with clinical evidence of pre-diabetic insulin resistance

REPLACEMENT SHEET
26 MAR 1997

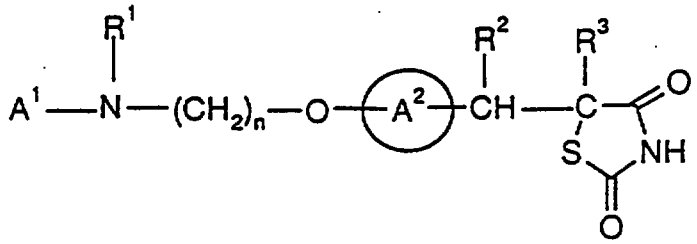
syndrome, alternatively referred to as Syndrome X.

Accordingly, the present invention provides a method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.

Particular insulin sensitisers include thiazolidinedione insulin sensitisers.

Particular insulin sensitisers include acyclic insulin sensitisers.

One favoured group of insulin sensitisers are the thiazolidinedione insulin sensitisers disclosed in EP 0306228 and WO94/05659. Thus in a favoured aspect the present invention provides a method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which method comprises the administration of an effective non-toxic amount of a compound of formula (I):



(I)

or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable solvate thereof, wherein:

A^1 represents a substituted or unsubstituted aromatic heterocyclyl group;

R^1 represents a hydrogen atom, an alkyl group, an acyl group, an aralkyl group, wherein the aryl moiety may be substituted or unsubstituted, or a substituted or unsubstituted aryl group;

R^2 and R^3 each represent hydrogen, or R^2 and R^3 together represent a bond;

A^2 represents a benzene ring having in total up to five substituents; and

n represents an integer in the range of from 2 to 6; to a human or non-human mammal in need thereof.

REPLACEMENT SHEET
26 MAR 1997

Suitable aromatic heterocyclyl groups of the compounds of formula (I) include substituted or unsubstituted, single or fused ring aromatic heterocyclyl groups comprising up to 4 hetero atoms in each ring selected from oxygen, sulphur or nitrogen.

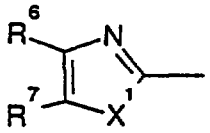
Favoured aromatic heterocyclyl groups of the compounds of formula (I) include substituted or unsubstituted single ring aromatic heterocyclyl groups having 5 to 7 ring atoms, preferably 5 or 6 ring atoms.

In particular, the aromatic heterocyclyl groups of the compounds of formula (I) comprise 1, 2 or 3 heteroatoms, especially 1 or 2, selected from oxygen, sulphur or nitrogen.

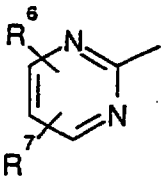
Suitable values for A^1 when it represents a 5- membered aromatic heterocyclyl group include thiazolyl and oxazolyl, especially oxazolyl.

Suitable values for A^1 when it represents a 6- membered aromatic heterocyclyl group include pyridyl or pyrimidinyl, especially pyridyl.

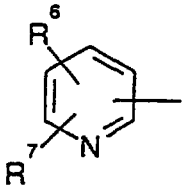
Preferably, A^1 represents a moiety of formula (a), (b) or (c):



(a)



(b)



(c)

wherein:

R^6 and R^7 each independently represents a hydrogen or halogen atom, an alkyl or alkoxy group or a substituted or unsubstituted aryl group or when R^6 and R^7 are each attached to adjacent carbon atoms, then R^6 and R^7 together with the carbon atoms to which they are attached form a benzene ring wherein each carbon atom represented by R^6 and R^7 together is substituted or unsubstituted; and in the moiety of formula (a) X^1 represents oxygen or sulphur.

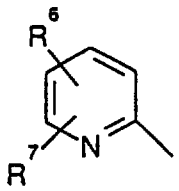
Aptly, A^1 represents a moiety of the abovedefined formula (a).

Aptly, A^1 represents a moiety of the abovedefined formula (b).

Aptly, A^1 represents a moiety of the abovedefined formula (c).

A particular form of moiety (c) is a moiety (c'):

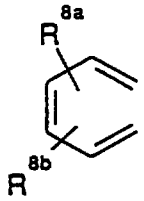
REPLACEMENT SHEET
26 MAR 1997



(c')

wherein R^6 and R^7 are as defined in relation to formula (c).

In one favoured aspect R^6 and R^7 together represent a moiety of formula (d):



(d)

wherein R^{8a} and R^{8b} each independently represent hydrogen, halogen, substituted or unsubstituted alkyl or alkoxy.

Suitably, R^{8a} and R^{8b} each independently represent hydrogen, halogen, alkyl or alkoxy. Favourably, R^{8a} represents hydrogen. Favourably, R^{8b} represents hydrogen. Preferably, R^{8a} and R^{8b} both represent hydrogen.

In a further favoured aspect R^6 and R^7 each independently represent hydrogen, alkyl or a substituted or unsubstituted phenyl group and more favourably, R^6 and R^7 each independently represent hydrogen, alkyl or phenyl.

Preferably, for the moiety of formula (a), R^6 and R^7 together represent the moiety of formula (d).

Preferably, for the moieties of formula (b), (c) or (c'), R^6 and R^7 both represent hydrogen.

It will be appreciated that the five substituents of A^2 include three optional substituents. Suitable optional substituents for the moiety A^2 include halogen, substituted or unsubstituted alkyl or alkoxy.

Further suitable, favoured and preferred values for variables A^2, R^1, R^2, $R^3$and n are as defined in EP 0306228 and WO94/05659.

A preferred compound of formula (I) is 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof

REPLACEMENT SHEET
26 MAR 1997

REPLACEMENT SHEET
26 MAR 1997

and/or a pharmaceutically acceptable salt thereof, especially a maleic acid salt thereof, and/or a pharmaceutically acceptable solvate thereof.

As indicated above, a compound of formula (I) may exist in one of several tautomeric forms, all of which are encompassed in the method of the present invention. It will be appreciated that the present invention encompasses the administration of all of the isomeric forms of the compounds of formula (I) and the pharmaceutically acceptable salts thereof, including any stereoisomeric forms thereof, whether as individual isomers or as mixtures of isomers.

Suitable substituents for any heterocyclyl group of the compounds of formula (I) include up to 4 substituents selected from the group consisting of: alkyl, alkoxy, aryl and halogen or any two substituents on adjacent carbon atoms, together with the carbon atoms to which they are attached, may form an aryl group, preferably a benzene ring, and wherein the carbon atoms of the aryl group represented by the said two substituents may themselves be substituted or unsubstituted.

The suitable, favoured and preferred thiazolidinedione insulin sensitisers disclosed in European Patent Applications, Publication Numbers: 0306228, 0008203, 0139421, 0032128, 0428312, 0489663, 0155845, 0257781, 0208420, 0177353, 0319189, 0332331, 0332332, 0528734, 0508740; International Patent Application, Publication Numbers 92/18501, 93/02079, 93/22445 and United States Patent Number 5104888 are those compounds defined as suitable, favoured and preferred in the respective patent publications.

The suitable, favoured and preferred acyclic insulin sensitisers disclosed in International Patent Applications, Publication Numbers WO91/19702, WO92/03425, WO93/21166 and WO94/01420 and United States Patent Number 5232945 are those compounds defined as suitable, favoured and preferred in the respective patent publications.

Other suitable, favoured and preferred insulin sensitisers are the suitable, favoured and preferred compounds disclosed in European Patent Application, Publication Number 0533933, International Patent Application, Publication Number WO 93/02079 , Japanese Patent Application Publication Number 05271204 and United States Patent Number 5264451.

Also specifically included in the method of the invention are the specific examples disclosed in the above mentioned patent applications.

When used herein the term 'insulin sensitiser' relates to compounds which

REPLACEMENT SHEET
26 MAR 1997

increase the biological response to insulin. In addition, based upon the observed effects in appropriate test animals such as Zucker fatty (fa/fa) rats, insulin sensitisers are indicated to lower elevated fasting plasma insulin concentrations and improve glycaemic control.

When used herein the term 'aryl' includes phenyl and naphthyl optionally substituted with up to five, preferably up to three, groups selected from halogen, alkyl, phenyl, alkoxy, haloalkyl, hydroxy, amino, nitro, carboxy, alkoxycarbonyl, alkoxycarbonylalkyl, alkylcarbonyloxy, or alkylcarbonyl groups.

When used herein the term 'halogen' refers to fluorine, chlorine, bromine and iodine; preferably chlorine.

When used herein the terms 'alkyl' and 'alkoxy' relate to groups having straight or branched carbon chains,containing up to 12 carbon atoms.

When used herein the term 'acyl' includes alkylcarbonyl groups.

Suitable alkyl groups are C_{1-12} alkyl groups, especially C_{1-6} alkyl groups e.g. methyl, ethyl, n-propyl, iso-propyl, n-butyl, isobutyl or tert-butyl groups.

Suitable substituents for any alkyl group include those indicated above in relation to the term "aryl".

Suitable pharmaceutically acceptable salts include salts of carboxy groups and acid addition salts.

Suitable pharmaceutically acceptable salts of carboxy groups include metal salts, such as for example aluminium, alkali metal salts such as lithium, sodium or potassium, alkaline earth metal salts such as calcium or magnesium and ammonium or substituted ammonium salts, for example those with lower alkylamines such as triethylamine, hydroxy alkylamines such as 2-hydroxyethylamine, bis-(2-hydroxyethyl)-amine or tri-(2-hydroxyethyl)-amine, cycloalkylamines such as bicyclohexylamine, or with procaine, dibenzylpiperidine, N-benzyl-b-phenethylamine, dehydroabietylamine, N,N'-bisdehydroabietylamine, glucamine, N-methylglucamine or bases of the pyridine type such as pyridine, collidine, quinine or quinoline.

Suitable acid addition salts include pharmaceutically acceptable inorganic salts such as the sulphate, nitrate, phosphate, borate, hydrochloride and hydrobromide and pharmaceutically acceptable organic acid addition salts such as acetate, tartrate, maleate, citrate, succinate, benzoate, ascorbate, methane-sulphonate, a-keto glutarate and a-glycerophosphate, especially the maleate salt.

Suitable pharmaceutically acceptable solvates include hydrates.

REPLACEMENT SHEET
26 MAR 1997

The active compounds disclosed in the above mentioned patent publications, and referred to herein as insulin sensitisers, including the specific examples disclosed therein, are conveniently prepared according to the methods disclosed in the said patent publications: Thus a compound of formula (I), or the tautomeric form thereof, and/or a pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable solvate thereof, may be prepared using the processes described in EP 0306228 and WO94/05659.

The salts and/or solvates of the compounds of formula (I) may be prepared and isolated according to conventional procedures for example sodium salts may be prepared by using sodium methoxide in methanol.

The present invention also provides an insulin sensitiser,such as a compound of formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, for use in the treatment of and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria.

The present invention also provides an insulin sensitiser,such as a compound of formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, for use in the manufacture of a medicament for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria.

In the above mentioned treatment and or prophylaxis the insulin sensitiser such as a compound of formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, may be administered per se or, preferably, as a pharmaceutical composition also comprising a pharmaceutically acceptable carrier.

Accordingly, the present invention also provides a pharmaceutical composition for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which composition comprises an insulin sensitiser, such as a compound of the formula (I), or a tautomeric form thereof, or a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, and a

REPLACEMENT SHEET
26 MAR 1997

pharmaceutically acceptable carrier therefor.

As used herein the term 'pharmaceutically acceptable' embraces compounds, compositions and ingredients for both human and veterinary use: for example the term 'pharmaceutically acceptable salt' embraces a veterinarily acceptable salt.

The composition may, if desired, be in the form of a pack accompanied by written or printed instructions for use.

Usually the pharmaceutical compositions of the present invention will be adapted for oral administration, although compositions for administration by other routes, such as by injection and percutaneous absorption are also envisaged.

Particularly suitable compositions for oral administration are unit dosage forms such as tablets and capsules. Other fixed unit dosage forms, such as powders presented in sachets, may also be used.

In accordance with conventional pharmaceutical practice the carrier may comprise a diluent, filler, disintegrant, wetting agent, lubricant, colourant, flavourant or other conventional adjuvant.

Typical carriers include, for example, microcrystalline cellulose, starch, sodium starch glycollate, polyvinylpyrrolidone, polyvinylpolypyrrolidone, magnesium stearate, sodium lauryl sulphate or sucrose.

Most suitably the composition will be formulated in unit dose form. Such unit dose will normally contain an amount of the active ingredient in the range of from 0.1 to 1000 mg, more usually 0.1 to 500 mg, and more especially 0.1 to 250 mg.

Conveniently, the active ingredient may be administered as a pharmaceutical composition hereinbefore defined, and this forms a particular aspect of the present invention.

In the above mentioned treatments an insulin sensitiser, such as a compound of the formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, may be taken in doses, such as those described above, one to six times a day in a manner such that the total daily dose for a 70 kg adult will generally be in the range of from 0.1 to 6000 mg, and more usually about 1 to 1500 mg, generally about 0.5 to 10 mg. That is in the range of from 1.429×10^{-3} to 85.714 mg/kg/day, more usually about 1.429×10^{-2} to 21.429 mg/kg/day, generally about 7.143×10^{-3} to 0.1429 mg/kg/day.

The following Examples illustrate the invention but do not limit it in any way.

REPLACEMENT SHEET
26 MAR 1997

REPLACEMENT SHEET
26 MAR 1997

Example 1: Studies Into The Effects Of The Test Compound Upon Renal Pathology

Obese Zucker rats are known to develop chronic nephropathy in addition to hyperlipidaemia, hyperinsulinaemia and peripheral insulin resistance (Kasiske *et al* 1985).

5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione (herein after referred to as 'test compound') was administered to 2-3 month old obese Zucker fa/fa rats by dietary administration to provide a daily dose over a period of 3 months ranging from 2.0 - 7.0 μmole/kg body weight. A group of age-matched obese Zucker fa/fa rats were given the same diet without the addition of the test compound, as was a group of lean Fa/? rats. At the end of the study, all of the control Zucker fa/fa rats had chronic nephropathy which involved dilated tubules, atrophied or hyperplastic tubular epithelial cells, thickened tubular basement membranes, segmented glomeruli or global glomerulosclerosis.

In the corresponding lean animals, 3/15 animals, a minimal degree of chronic nephropathy was seen.

Treatment with test compound resulted in a reduction in the incidence and degree of chronic nephropathy compared to the control group of Zucker fa/fa rats.

In the kidneys of the control Zucker fa/fa rats, mild-moderate hydronephrosis was seen in 4/9 animals. Characteristically this involved dilation of the kidney pelvis. No hydronephrosis was seen in the rats treated with test compound.

REPLACEMENT SHEET
26 MAR 1997

TABLE OF SIGNIFICANT HISTOPATHOLOGICAL FINDINGS IN THE KIDNEY OF FATTY ZUCKER RATS

Group/Treatment Animal Number	Hydronephrosis	Chronic Nephropathy
Group 1		
Test Compound in diet		
2	0	±
7	0	±
9	0	0
15	0	++
16	0	±
17	0	+
21	0	±
22	0	±
24	0	±
27	0	±
28	0	±
Group 2		
Diet only		
3	++	+
5	+++	+
13	0	+
14	++	++
18	0	+
23	+++	++
26	0	+
29	0	+
30	0	++

Severity Key

0 None seen

±	Minimal
+	Mild
++	Moderate
+++	Marked

REPLACEMENT SHEET
26 MAR 1997

REPLACEMENT SHEET
26 MAR 1997

Example 2: Studies Into The Effect Of Test CompoundsUpon Systolic Blood Pressure, Urinary Total Protein And Urinary N-Acetyl ß-D-Glucosaminidase (NAG) Activity Measurments.

A second study was performed in Zucker rats over a period of 9 months to investigate the longitudinal effects of the drug on systolic blood pressure and various indices of renal function, two of which are exemplified here. In one arm of the study, the drug was given in the diet (50 µmole/kg of diet) from aged 6-7weeks in Zucker fatty (fa/fa) rats, whilst in a second arm of the study, drug treatment as above was delayed until proteinuria had become established after 4 months, indicative of structural damage already present in the kidneys. A third group of Zucker fatty rats and a further group of lean rats were given diet alone throughout the period of study.

After dosing with the test compound, at monthly intervals measurements were made of systolic blood pressure, urinary total protein and urinary N-acetyl ß-D-glucosaminidase (NAG) activity.

Measurement of Systolic Blood Pressure

Rats were enclosed in custom-built restrainers and placed on a shelf in a warming cabinet, whose temperature was controlled at approximately 30°C. An inflatable cuff with integral pulse sensor was attached to the tail of each rat. After warming for 20-30 min the tail cuff was inflated and deflated automatically and a measurement of systolic blood pressure made using an IITC Non-Invasive Blood Pressure Monitor. This cycle was repeated several times for each rat until stable values of blood pressure were obtained.

Measurement of Urinary Parameters

Twenty-four hour urine collections, made in metabolism cages, were aliquoted and frozen at -70°C until required for assay.

(i) Urinary Protein

Urinary protein concentration was measured using the Bio-Rad protein assay as modified for use in a 96-well microtitre plate. The assay is a dye-binding assay based on the differential colour change of a dye in response to various

REPLACEMENT SHEET
26 MAR 1997

concentrations of protein (Bradford, Anal. Biochem.,72, 248, 1976). After a period of incubation with Dye Reagent, diluted urine samples are read at an optical density of 595 nm using a Molecular Devices multiplate reader.

(ii) Urinary NAG Activity

NAG activity was assayed using a reagent kit on a Hitachi 717 analyser (both suppied by Boehringer Mannheim UK, Lewes). Enzyme activity was measured by monitoring the rate of chlorophenol (570 nm) released from the substrate chlorophenol red-ß-D-glucosaminide.

Results and Statistics

In the tables below, results are given as mean values for the group ± the standard error of the mean. Results have been analysed by one way ANOVA and significant differences from the Zucker fatty rat control group have been indicated by an asterisk.

Conclusion

The results of Example 2 demonstrate that treatment of Zucker fatty (fa/fa) rats from the age of 6-7 weeks, for a period of 9 months, with BRL 49653 given via the diet, prevented the development of hypertension and markedly reduced both the elevation of urinary NAG activity and the rate of development of proteinuria. When drug treatment was commenced after proteinuria had become established, both systolic blood pressure and urinary NAG activity were prevented from rising further and again there was a marked reduction of the rate of increase in the urinary protein concentration.

REPLACEMENT SHEET
26 MAR 1997

Effects of starting treatment with Test Compound prior to the development of renal complications.

SYSTOLIC BLOOD PRESSURE (mm Hg)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
0	102 ± 3	106 ± 3	123 ± 3*
1	110 ± 3 *	120 ± 4	128 ± 3
2	122 ± 4 *	137 ± 4	132 ± 4
3	122 ± 4 *	142 ± 6	134 ± 3
4	126 ± 6 *	146 ± 4	132 ± 4*
5	131 ± 4 *	157 ± 6	138 ± 4*
6	128 ± 3 *	150 ± 6	133 ± 2*
7	125 ± 6*	157 ± 6	128 ± 6*
8	133 ± 5*	155 ± 4	130 ± 3*
9	143 ± 4*	164 ± 4	136 ± 3*

Effects of starting treatment with Test Compound prior to the development of renal complications

URINARY PROTEIN CONCENTRATION (µg/hour)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
0	226 ± 15 *	287 ± 17	198 ± 19*
1	373 ± 22	355 ± 18	321 ± 23*
2	402 ± 22 *	509 ± 28	396 ± 20*
3	221 ± 32 *	874 ± 102	393 ± 25*
4	376 ± 54 *	3965 ± 731	613 ± 52*
5	1573 ± 105 *	5823 ± 809	1395 ± 88*
6	1585 ± 147 *	8946 ± 1171	1192 ± 92*
7	2124 ± 293 *	12052 ± 1535	1176 ± 96*
8	2664 ± 370*	14534 ± 1540	1409 ± 92*
9	3152 ± 515*	16182 ± 1581	1373 ± 113*

REPLACEMENT SHEET
26 MAR 1997

Effects of starting treatment with Test Compound prior to the development of renal complications.

URINARY N-ACETYL -ß- D-GLUCOSAMINIDASE ACTIVITY (mU/hour)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
0	5.7 ± 0.4	5.9 ± 0.3	4.4 ± 0.3*
1	7.5 ± 0.7	9.1 ± 0.8	5.1 ± 0.5*
2	3.8 ± 1.1 *	8.2 ± 1.5	6.5 ± 0.3
3	4.5 ± 0.9 *	8.2 ± 1.0	5.2 ± 0.4*
4	5.1 ± 1.2 *	9.3 ± 0.5	6.9 ± 0.5*
5	6.5 ± 0.7 *	10.7 ± 0.4	7.6 ± 0.7*
6	6.5 ± 1.1 *	10.8 ± 1.7	7.4 ± 0.5
7	8.9 ± 0.8*	11.7 ± 0.7	7.1 ± 0.5*
8	6.2 ± 1.2*	11.8 ± 1.3	7.4 ± 0.5*
9	9.1 ± 1.2*	13.4 ± 0.8	7.7 ± 0.6*

Effects of starting treatment with Test Compound once renal complications have become established.

SYSTOLIC BLOOD PRESSURE (mm Hg)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
-4	106 ± 3	106 ± 3	123 ± 3*
-3	123 ±5	120 ± 4	128 ± 3
-2	138 ± 7	137 ± 4	132 ± 4
-1	141 ± 6	142 ± 6	134 ± 3
0	146 ± 4	146 ± 4	132 ± 4*
1	140 ± 5*	157 ± 6	138 ± 4*
2	134 ± 3*	150 ± 6	133 ± 2*
3	144 ± 3*	157 ± 6	128 ± 6*
4	139 ± 3*	155 ± 4	130 ± 3*
5	146 ± 6*	164 ± 4	136 ± 3*

REPLACEMENT SHEET
26 MAR 1997

Effects of starting treatment with Test Compound once renal complications have become established.

URINARY PROTEIN CONCENTRATION (µg/hour)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
-4	220 ± 13	287 ± 17	198 ± 19*
-3	347 ± 32	355 ± 18	321 ± 23*
-2	457 ± 21	509 ± 28	396 ± 21*
-1	958 ± 194	874 ± 102	393 ± 25*
0	3520 ± 905	3965 ± 731	613 ± 52*
1	3692 ± 386*	5823 ± 809	1395 ± 88*
2	5326 ± 967*	8946 ± 1171	1192 ± 92*
3	6230 ± 835*	12052 ± 1535	1178 ± 96*
4	5379 ± 708*	14534 ± 1540	1409 ± 92*
5	7677 ± 825*	16182 ± 1581	1373 ± 113*

Effects of starting treatment with Test Compound once renal complications have become established.

URINARY N-ACETYL-ß-D-GLUCOSAMINIDASE ACTIVITY (mU/hour)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
-4	5.4 ± 0.2	5.9 ± 0.3	4.4 ± 0.3*
-3	8.3 ± 0.5	9.1 ± 0.8	5.1 ± 0.5*
-2	9.5 ± 1.1	8.2 ± 1.5	6.5 ± 0.3
-1	7.8 ± 0.6	8.2 ± 1.0	5.2 ± 0.4*
0	7.6 ± 0.7*	9.3 ± 0.5	6.9 ± 0.5*
1	7.6 ± 0.7*	10.7 ± 0.4	7.6 ± 0.7*
2	5.7 ± 0.9*	10.8 ± 1.7	7.4 ± 0.5
3	7.4 ± 0.9*	11.7 ± 0.7	7.1 ± 0.5*
4	5.4 ± 1.0*	11.8 ± 1.3	7.4 ± 0.5*
5	8.0 ± 1.1*	13.4 ± 0.8	7.7 ± 0.6*

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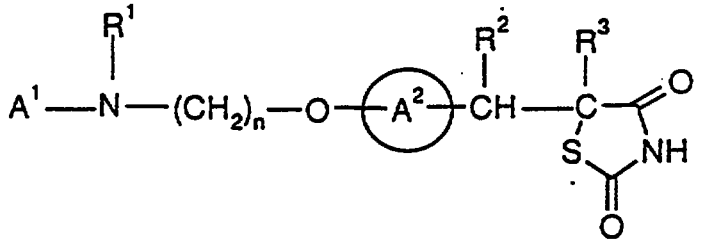
Claims:

1. A method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.

2. A method according to claim 1, wherein the insulin sensitiser is a thiazolidinedione insulin sensitiser.

3. A method according to claim 1, wherein the insulin sensitiser is an acyclic insulin sensitiser.

4. A method according to claim 1, wherein the insulin sensitiser is a compound of formula (I):



(I)

or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable solvate thereof, wherein:
A^1 represents a substituted or unsubstituted aromatic heterocyclyl group;
R^1 represents a hydrogen atom, an alkyl group, an acyl group, an aralkyl group, wherein the aryl moiety may be substituted or unsubstituted, or a substituted or unsubstituted aryl group;
R^2 and R^3 each represent hydrogen, or R^2 and R^3 together represent a bond;
A^2 represents a benzene ring having in total up to five substituents; and
n represents an integer in the range of from 2 to 6; to a human or non-human

REPLACEMENT SHEET
26 MAR 1997

19
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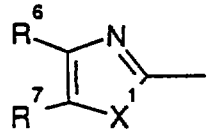
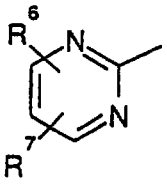
mammal in need thereof.

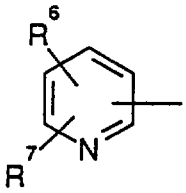
5. A method according to claim 4, wherein in the compound of formula (I) A^1 represents a moiety of formula (a), (b) or (c):



(a)



(b)



(c)

REPLACEMENT SHEET
26 MAR 1997

wherein:
R^6 and R^7 each independently represents a hydrogen or halogen atom, an alkyl or alkoxy group or a substituted or unsubstituted aryl group or when R^6 and R^7 are each attached to adjacent carbon atoms, then R^6 and R^7 together with the carbon atoms to which they are attached form a benzene ring wherein each carbon atom represented by R^6 and R^7 together is substituted or unsubstituted; and in the moiety of formula (a) X^1 represents oxygen or sulphur.

6. A method according to claim 5, wherein in the compound of formula (I) A^1 represents a moiety of the above defined formula (c).

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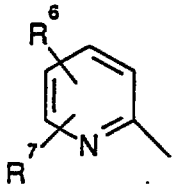

(c')

wherein R^6 and R^7 are as defined in claim 5.

7. A method according to claim 1, wherein the insulin sensitiser is 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof.

8. A method according to claim 7, wherein the insulin sensitiser is a maleic

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acid salt of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof and/or a pharmaceutically acceptable solvate thereof.

9. The use of an insulin sensitiser for the manufacture of a medicament for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria.

10. A pharmaceutical composition for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria which composition comprises an insulin sensitiser and a pharmaceutically acceptable carrier therefor.

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REPLACEMENT SHEET
26 MAR 1997

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Use of insulin sensitisers for treating renal diseases

Abstract

A method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.

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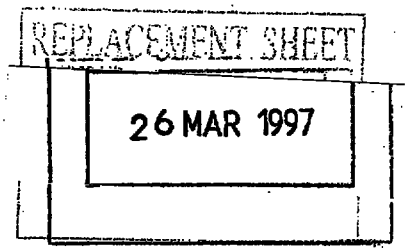

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Claims:

1. The use of a thiazolidinedione insulin sensitiser for the manufacture of a medicament for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria.

2. A use according to claim 1, wherein the insulin sensitiser is 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof.

3. A use according to claim 2, wherein the insulin sensitiser is a maleic acid salt of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof and/or a pharmaceutically acceptable solvate thereof.

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04 JAN 2002

Use of insulin sensitisers for treating renal diseases

Abstract

A method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.

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