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SPIRO-ISOQUINOLINE-PYRROLIDINES AND ANALOGS THEREOF USEFUL AS ALDOSE REDUCTASE INHIBITORS

This invention relates to spiro-isoquinoline-pyrrolidines and their pharmaceutically acceptable salts thereof, to processes for their preparation, to methods for using the compounds, and to pharmaceutical preparations thereof. The compounds have pharmaceutical properties which render them beneficial for the prevention or treatment of complications associated with diabetes mellitus.

The use of insulin and/or oral hypoglycemic agents in the treatment of diabetes mellitus has prolonged the life of many of these patients. However, their use has not had a demonstrable impact on the development of diabetic complications such as neuropathy, nephropathy, retinopathy, cataracts and vascular disease which accompany the underlying metabolic disorder. There is little question that chronic hyperglycemia plays a major role in the genesis of these complications, and that complete normalization of blood glucose would likely prevent most if not all complications. For a number of reasons, though, chronic normalization of blood glucose has not been achieved with the currently available therapies.

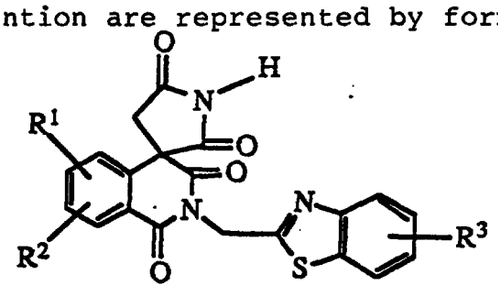
The long-term complications of diabetes develop in tissues where glucose uptake is independent of insulin. In these tissues, which include the lens, retina, kidney and peripheral nerves, the systemic hyperglycemia of diabetes is rapidly transposed into high tissular concentrations of glucose. In all of these tissues this excess glucose is rapidly metabolized by the sorbitol pathway. The intense diabetes-induced flux of glucose through this pathway appears to initiate a cascade of biochemical alterations which slowly progress to cell dysfunction and structural damage. Aldose reductase, the key enzyme in the sorbitol pathway, reduces glucose to sorbitol at the expense of the cofactor NADPH. In animal models of diabetes, compounds which inhibit aldose reductase have been shown to prevent the biochemical, functional and morphological changes induced by hyperglycemia. Early studies by J. H. Kinoshita and collaborators implicated aldose reductase in the etiology of diabetic cataracts. More recent studies have provided compelling evidence that aldose reductase also plays a significant role in the initiation of diabetic nephropathy,

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retinopathy and neuropathy (cf McCaleb et al, J Diab. Comp., 2 16,1989; Robinson et al, Invest. Ophthalmol. Vis. Sci., 30, 2285, 1989; Notvest and Inserra, Diabetes, 36, 500,1987.

The spiro-isoquinoline-pyrrolidine tetrones of the present invention are represented by formula (I):



(I)

and the pharmaceutically acceptable salts thereof, wherein R^1 and R^2 are independently hydrogen, alkyl containing 1 to 6 carbon atoms, halogen, lower alkoxy containing 1 to 6 carbon atoms, trifluoromethyl, nitro, aryl or aryl(lower alkyl)oxy wherein aryl contains 6 to 10 carbon atoms and lower alkyl contains 1 to 6 carbon atoms; R^3 is hydrogen, halogen, alkyl containing 1 to 6 carbon atoms, lower alkoxy containing 1 to 6 carbon atoms, trifluoromethyl, nitro, aryl or aryl(lower alkyl)oxy wherein aryl contains 6 to 10 carbon atoms and lower alkyl contains 1 to 5 carbon atoms.

Examples of 'aryl' are phenyl and naphthyl. Examples of halogen are fluorine, chlorine and bromine. Examples of R^1 and/or R^2 are hydrogen or halogen e.g fluorine such as 6-F. Examples of R^5 are hydrogen, halogen or trifluoromethyl, e.g 5-CF_3 . Examples of alkyl are methyl or ethyl.

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A more preferred group of compounds of the present invention are the compounds of formula (I) wherein R^1 and R^2 are independently hydrogen or halogen; R^3 is hydrogen, halogen or trifluoromethyl.

The most preferred compounds of the present invention are set forth below:

2-(2-benzothiazolylmethyl)spiro[isoquinoline-4(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrone;

2-(2-benzothiazolylmethyl)-6-fluorospiro[isoquinoline-4(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrone;

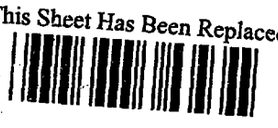
2-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]spiro[isoquinoline-4-(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrone;

6-fluoro-2-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]spiro-[isoquinoline-4(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrone.

The compounds of formula (I) all possess at least one asymmetric carbon atom, namely the spiro carbon atom at position 3' of the pyrrolidine ring. The compounds of formula (I) therefore exist, and may be isolated, in two or more stereoisomeric forms. This invention encompasses the compounds of formula (I) in racemic form or in any optically active form.

The spiro-isoquinoline-pyrrolidines can be prepared by the processes described hereinafter.

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A method is provided for preventing or relieving diabetes mellitus associated complications in a diabetic mammal by administering to said mammal a prophylactic or alleviating amount of the compounds of formula (I). Such complications include neuropathy, nephropathy, retinopathy, keratopathy, diabetic uveitis, and cataracts.

The compounds of formula (I), when admixed with a pharmaceutically acceptable carrier, form a pharmaceutical composition which can be used according to the preceding method.

The spiro-isoquinoline-pyrrolidines of this invention may be administered to mammals, for example, man, cattle, or rabbits, either alone or in dosage forms, i.e., capsules or tablets, combined with pharmacologically acceptable excipients.

The compounds of this invention may be given orally. However, the method of administering the present active ingredients of this invention is not to be construed as limited to a particular mode of administration. For example, the compounds may be administered topically directly to the eye in the form of drops of sterile, buffered ophthalmic solutions, preferably of pH 7.2-7.6. Also, they may be administered orally in solid form containing such excipients as starch, milk sugar, certain types of clay and so forth. They may also be administered orally in the form of solutions or they may be injected parenterally. For parenteral administration, they may be used in the form of a sterile solution, preferaby of pH 7.2-7.6, containing a pharmaceutically acceptable buffer.

The dosage of the spiro-isoquinoline-pyrrolidines will vary with the form of administration and the particular compound chosen. Furthermore, it will vary with the particular host under treatment. Generally, treatment is initiated with small dosages substantially less than the optimal dose of the compound. Thereafter, the dosage is increased by small increments until efficacy is obtained. In general, the compounds of this invention are most desirably administered at a concentration level that will generally afford effective results without causing any harmful or deleterious side effects. For topical administration, a 0.05-1.0% solution may be administered dropwise in the eye. The frequency of instillation varies with the subject under treatment from a drop every two or three days to once daily. For oral or parenteral administration a preferred level of dosage ranges from about 0.1 mg to about 1.0 mg per kilo of body weight per day, although aforementioned variations will occur. However, a dosage level that is in the

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range of from about 0.1 mg to about 1.0 mg per kilo of body weight per day is most satisfactory.

Unit dosage forms such as capsules, tablets, pills and the like may contain from about 5.0 mg to about 25.0 mg of the active ingredients of this invention with a pharmaceutical carrier. Thus, for oral administration, capsules can contain from between about 5.0 mg to about 25.0 mg of the active ingredients of this invention with or without a pharmaceutical diluent. Tablets, either effervescent or noneffervescent, can contain between about 5.0 to 25.0 mg of the active ingredients of this invention together with conventional pharmaceutical carriers. Thus tablets, which may be coated and either effervescent or noneffervescent, may be prepared according to the known art. Inert diluents or carriers, for example, magnesium carbonate or lactose, can be used together with conventional disintegrating agents for example, magnesium stearate.

The spiro-isoquinoline-pyrrolidines also can be used in combination with insulin or oral hypoglycemic agents to produce a beneficial effect in the treatment of diabetes mellitus. In this instance, commercially available insulin preparations or oral hypoglycemic agents, exemplified by acetohexamide, chlorpropamide, tolazamide, tolbutamide and phenformin, are suitable. The compounds hereof can be administered sequentially or simultaneously with insulin or the oral hypoglycemic agent. Suitable methods of administration, compositions and doses of the insulin preparation or oral hypoglycemic agent are described in medical textbooks; for instance, Physicians' Desk Reference, 42 ed., Medical Economics Co., Oradell, N.J., U.S.A., 1988.

The aldose reductase inhibiting property of the compounds of this invention and the utilization of the compounds in preventing, diminishing and alleviating diabetic complications are demonstrable in experiments using galactosemic rats, see Dvornik et al., Science, 182, 1146 (1973). Such experiments are exemplified hereinbelow after the listing of the following general comments pertaining to these experiments:

(a) Four or more groups of six male rats, 50-70 g, Sprague-Dawley strain, were used. The first group, the control group, was fed a mixture of laboratory chow (rodent Laboratory Chow, Purina) and glucose at 20% (w/w %) concentration. An untreated galactosemic group was fed a similar diet in which galactose was substituted for glucose. The third group was fed a diet prepared by mixing a given amount of the

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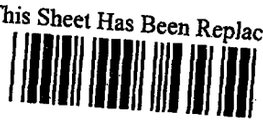
test compound with the galactose containing diet. The concentration of galactose in the diet of the treated groups was the same as that for the untreated galactosemic group.

(b) After four days, the animals were killed by euthanization. Both the lens and sciatic nerve were removed, weighed and stored frozen for polyol determination.

(c) The polyol determination was performed by a modification of the procedure of M. Kraml and L. Cosyns, Clin. Biochem., 2, 373 (1969). Only two minor reagent changes were made: (a) the rinsing mixture was an aqueous 5% (w/v) trichloroacetic acid solution and (b) the stock solution was prepared by dissolving 25 mg of dulcitol in 100 ml of an aqueous trichloroacetic acid solution. [N.B.: For each experiment the average value found in the tissue from rats fed the glucose diet was subtracted from the individual values found in the corresponding tissue in galactose-fed rats to obtain the amount of polyol accumulated.] The aldose reductase inhibiting effects of the compounds of formula (I) were also tested by employing an in vitro testing procedure similar to that described by S. Hayman and J.H. Kinoshita, J. Biol. Chem., 240, 877 (1965). In the present case the procedure of Hayman and Kinoshita was modified in that the final chromatography step was omitted in the preparation of the enzyme from bovine lens.

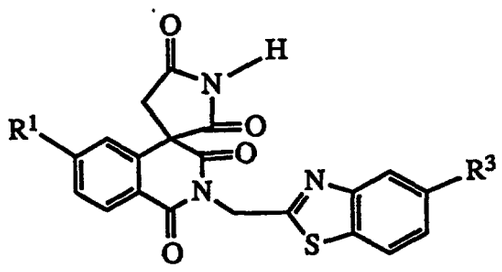
The following tabulated results show that the spiro-isoquinoline-pyrrolidines of this invention show the property that they are active both in vitro and in vivo and diminish the accumulation of dulcitol in the lenses, sciatic nerves and diaphragm of rats fed galactose. The figures under L, N, and D represent the percentage decrease of dulcitol accumulation in the tissues of the lens, sciatic nerve, and diaphragm, respectively, for treated rats as compared to untreated rats.

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ALDOSE REDUCTASE INHIBITORS



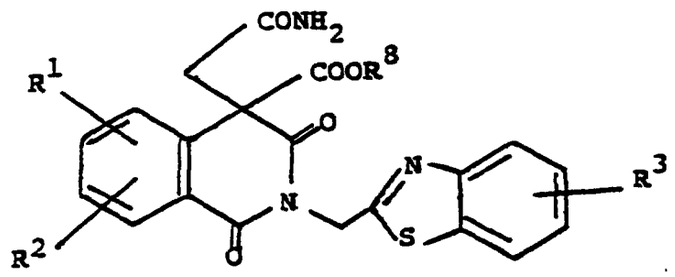
			% inhibition In Vitro				% Lowering Galactitol Accumulation In Vivo		
	R^1	R^3	10^{-6}M	10^{-7} M	4×10^{-8}M	Dose mg/kg/day	(%)L	(%)N	(%)D
	H	H	97	92	81	1.1	NS	48	NS
	H	CF_3	95	93	90	1.1	22	100	89
						0.5	NS	49	94
	F	H	96	94	91	4.6	NS	63	93
	F	CF_3	95	94	92	1.0	NS	53	85

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This invention provides processes for preparing compounds of formula I. These processes include:

a) cyclising an amide of formula (A)

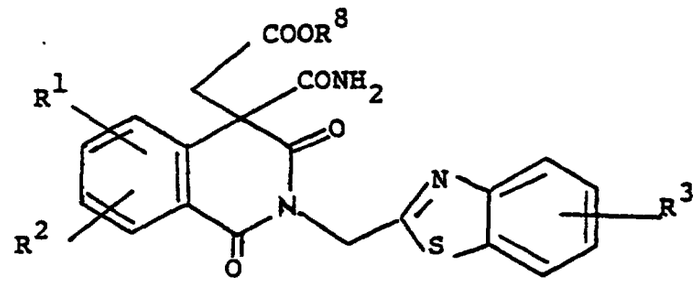


(A)

wherein R^1, R^2 and R^3 are as defined above and $COOR^8$ is ester group, using a suitable base such as sodium hydride to give a compound of formula I;

or

b) cyclising a compound of formula (B)

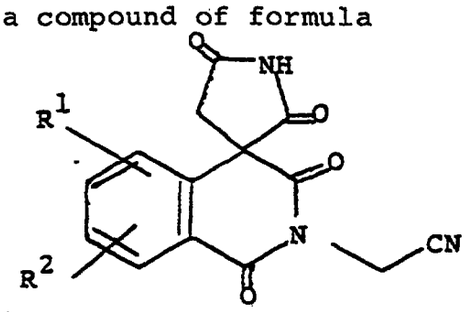


(B)

wherein R^1, R^2, R^3 and $COOR^8$ are as defined above using a suitable base such as sodium hydride or lithium bis-(trimethylsilyl)amide to give a compound of formula I;

or

c) reacting a compound of formula

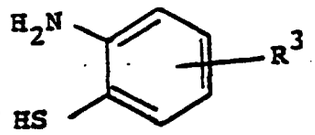


(D)

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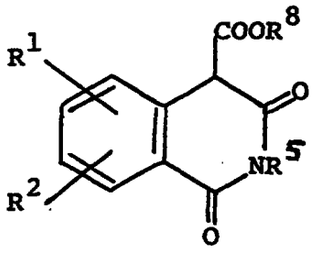
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with a 2-aminothiophenol of formula



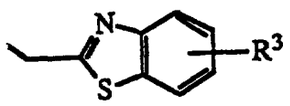
(E)

to give a compound of formula I. Starting materials of formulae (A), (B) and (D) can be prepared via intermediates of formula (G) shown below:

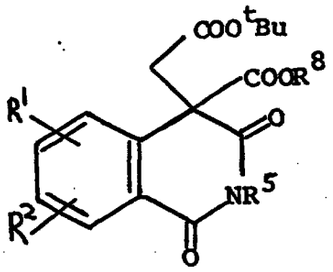


(G)

wherein R^1, R^2, and $COOR^8$ are as defined above and R^5 is CH_2CN or a group of formula



Examples of R^8 are alkyl groups such as C_1-C_4 alkyl especially methyl. R^8 may also be ^tBu. For example compounds of formula (A) and (D) are prepared by reacting a compound of formula (G) with $Br(CH_2)_n CO_2{}^tBu$ in the presence of a suitable base e.g $Li\text{-}N(SiMe_3)_2$ to give a compound of formula



(H)

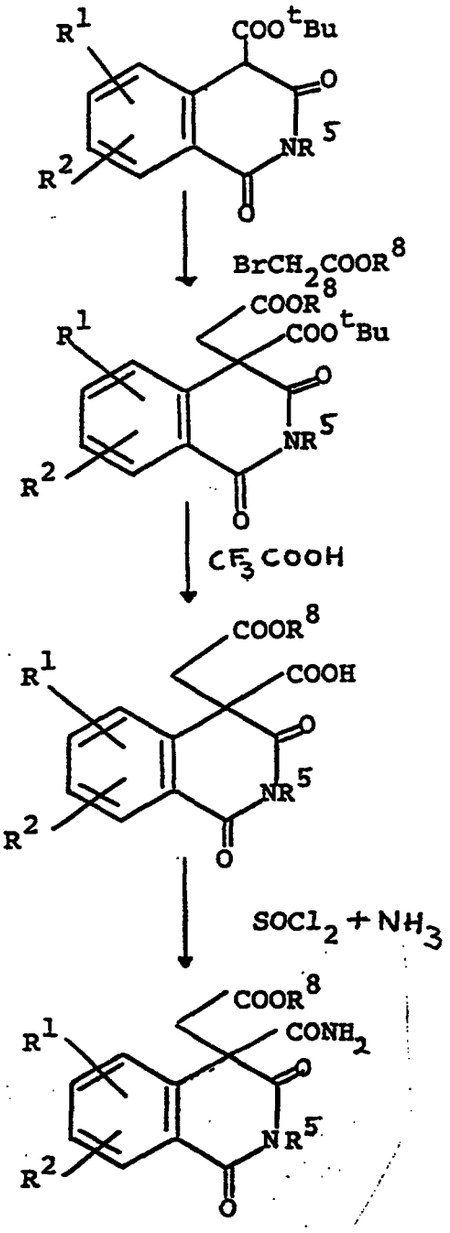
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wherein R^1, R^2 and R^5 are as defined above and $COOR^8$ is an ester group (other than ^tBu ester); treating the compound of formula (H) with trifluoracetic acid to remove the ^tBu group, reacting the resulting acid with $SOCl_2$ and ammonia to give the amide of formula (A) or (D)

Intermediates of formula (B) or (D) may be prepared via the route



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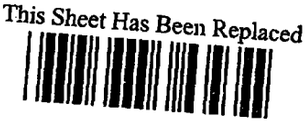
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where $COOR^8$ is an ester group providing R^8 is not ^tBu. Other routes to the various intermediates will be apparent to the skilled chemist.

Starting materials for the processes herein described are known compounds or may be prepared by analogous methods.

The compounds of formula (I) form salts with suitable therapeutically acceptable inorganic and organic bases. These derived salts possess the

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activity as their parent compound and are included within the scope of this invention. The compounds of formula (I) are transformed in excellent yield into the corresponding therapeutically acceptable salts by neutralization with the appropriate inorganic or organic base. The salts are administered usually in the same manner as the parent compounds. Suitable inorganic bases to form these salts include, for example, the hydroxides, carbonates or bicarbonates of the therapeutically acceptable alkali metals or alkaline earth metals, for example, sodium, potassium, magnesium, calcium and the like. Suitable organic bases include the following amines: benzylamine; lower mono-, di-, and trialkylamines, the alkyl radicals of which contain up to three carbon atoms, such as methylamine, dimethylamine, trimethylamine, ethylamine, di- and triethylamine, methylethylamine, and the like; mono-, di-, and trialkanolamines, the alkanol radicals of which contain up to three carbon atoms, for example, mono-, di-, and triethanolamine; alkylene-diamines which contain up to six carbon atoms, such as hexamethylenediamine; cyclic saturated or unsaturated bases containing up to six carbon atoms, such as pyrrolidine, piperidine, morpholine, piperazine and their N-alkyl and N-hydroxyalkyl derivatives, such as N-methyl-morpholine and N-(2-hydroxyethyl)-piperidine, as well as pyridine. Furthermore, there may be mentioned the corresponding quaternary salts, such as the tetraalkyl (for example tetramethyl), alkyl-alkanol (for example methyl-triethanol and trimethyl-monoethanol) and cyclic ammonium salts, for example the N-methyl-pyridinium, N-methyl-N-(2-hydroxyethyl)-morpholinium, N,N-dimethyl-morpholinium, N-methyl-N-(2-hydroxyethyl)-morpholinium, N,N-dimethylpiperidinium salts, which are characterized by having good water-solubility. In principle, however, there can be used all the ammonium salts which are physiologically compatible.

The transformations to the salts can be carried out by a variety of methods known in the art. For example, in the case of the inorganic salts, it is preferred to dissolve the compound of formula (I) in water containing at least one equivalent amount of a hydroxide, carbonate, or bicarbonate corresponding to the inorganic salt desired. Advantageously, the reaction is performed in a water-miscible, inert organic solvent, for example, methanol, ethanol, dioxane, and the like in the presence of water. For example, such use of sodium hydroxide, sodium carbonate or sodium bicarbonate gives a solution of the sodium salt. Evaporation of the solution or addition of a water-miscible solvent

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of a more moderate polarity, for example, a lower alkanol, for instance, butanol, or a lower alkanone, for instance, ethyl methyl ketone, gives the solid inorganic salt if that form is desired.

To produce an amine salt, the acidic compound of formula (I) is dissolved in a suitable solvent of either moderate or low polarity, for example, ethanol, methanol, ethyl acetate, diethyl ether and benzene. At least an equivalent amount of the amine corresponding to the desired cation is then added to that solution. If the resulting salt does not precipitate, it can usually be obtained in solid form by addition of a miscible diluent of lower polarity, for example, benzene or petroleum ether, or by evaporation. If the amine is relatively volatile, any excess can easily be removed by evaporation. It is preferred to use substantially equivalent amounts of the less volatile amines.

Salts wherein the cation is quaternary ammonium are produced by mixing the compound of formula (I) with an equivalent amount of the corresponding quaternary ammonium hydroxide in water solution, followed by evaporation of the water.

The following processes exemplify preferred embodiments.

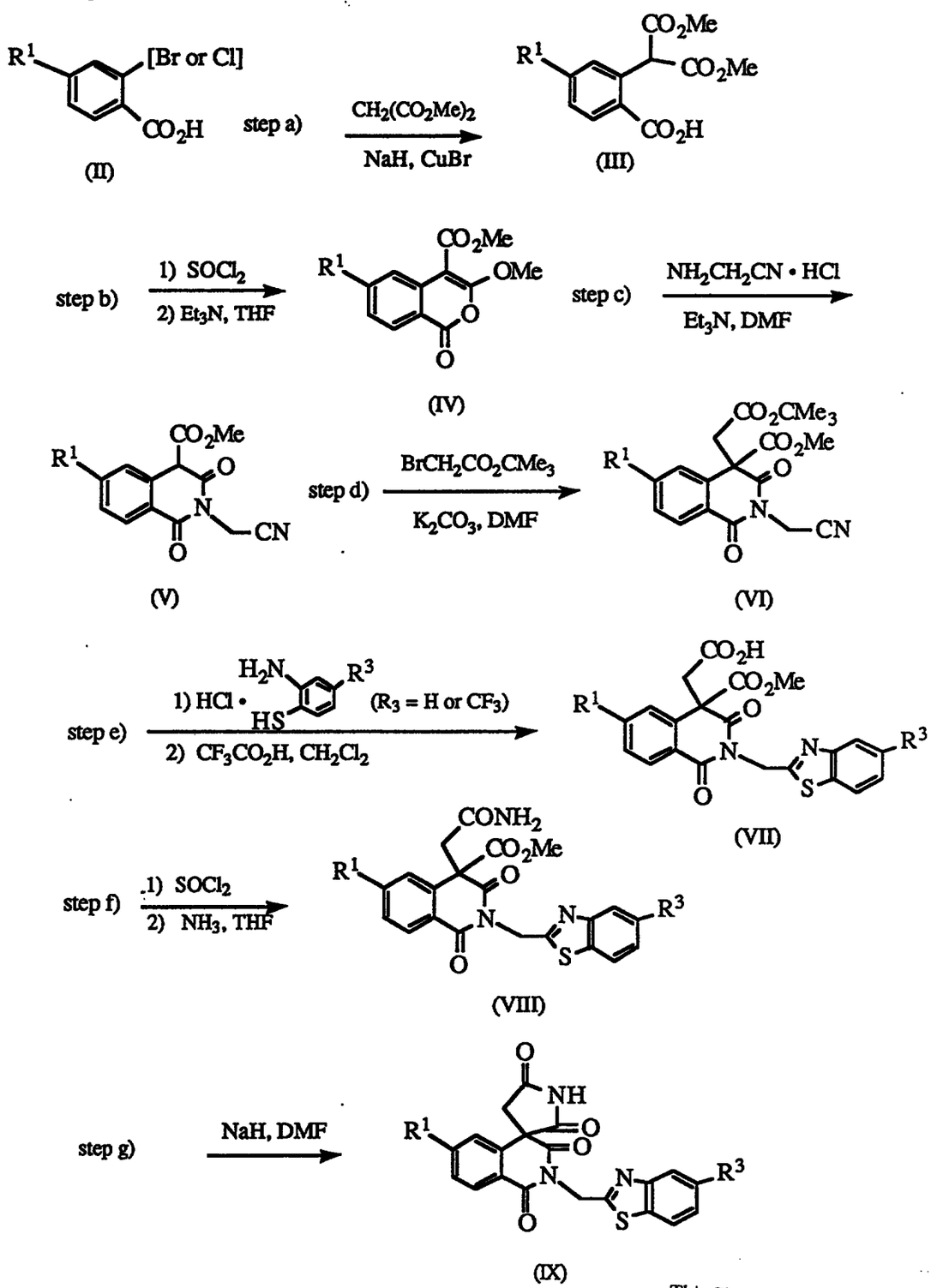
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The Process:

The spiro-isoquinoline-pyrrolidines of the present invention were prepared by the following reaction scheme:



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Step a) Reacting either 2-bromobenzoic acid or 2-chlorobenzoic acid of formula (II) wherein R^1 is as defined above with dimethyl malonate and NaH in the presence of a catalytic amount of CuBr to produce the propanedioic acid dimethyl ester of formula (III) wherein R^1 is as defined above.

The 2-bromobenzoic acids or 2-chlorobenzoic acids of formula (II) required for the present invention are commerically available compounds or can be prepared by known methods.

Step b) The propanedioic acid dimethyl ester of formula (III) can be reacted with thionyl chloride under refluxing conditions to produce the corresponding acid chloride which upon treatment with Et_3N in a conventional solvent which does not adversely influence the reaction, for example, tetrahydrofuran, can produce the compound of formula (IV), wherein R^1 is as defined above.

Step c) The compound of formula (IV), wherein R^1 is as defined above, is reacted with aminoacetonitrile hydrochloride in the presence of Et_3N in a conventional solvent which does not adversely influence the reaction, for example, N,N-dimethylformamide, produces the compound of the formula (V), wherein R^1 is as defined above.

Step d) The compound of formula (V), wherein R^1 is as defined above, is reacted with an inorganic base such as potassium carbonate in a conventional solvent which does not adversely influence the reaction, for example, N,N-dimethylformamide and subsequent addition of the tert-butyl bromoacetate produces the compound of formula (VI), wherein R^1 is as defined above.

Step e) The compound of formula (VI), wherein R^1 is as defined above, can be reacted with 2-aminothiophenols (R^3 is as defined above) under refluxing conditions in a conventional solvent which does not adversely influence the reaction, for example, ethyl alcohol, and subsequent treatment of the crude product with an organic acid such as trifluoroacetic acid in a conventional solvent which does not adversely influence the reaction, for example, methylene chloride, to produce the compound of formula (VII), wherein R^1 and R^3 are as defined above.

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Step f) The compound of formula (VII), wherein R^1 and R^3 are as defined above, can be reacted with thionyl chloride under refluxing conditions to produce the corresponding acid chloride which upon treatment with a saturated tetrahydrofuran-ammonium solution produces the compound of formula (VIII), wherein R^1 and R^3 are as defined above.

Step g) The compound of formula (VIII), wherein R^1 and R^3 are as defined above is reacted with a base such as sodium hydride in a conventional solvent which does not adversely influence the reaction, for example, N,N-dimethylformamide, to produce the compound of the formula (IX), wherein R^1 and R^3 are as defined above.

The following examples further illustrate this invention:

EXAMPLE 1

2-[[5-(Trifluoromethyl)-2-benzothiazolyl]methyl]spiro[isoquinoline-4-(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrone

Step a)

(2-Carboxyphenyl)propanedioic Acid Dimethyl Ester

To a rapidly stirred cold suspension (O°C) of 2-bromobenzoic acid (30.0 g, 149.32 mmol), cuprous bromide (2.14 g, 14.93 mmol) and dimethyl malonate (300 mL) was added NaH (80% in mineral oil, 10.75 g, 358.37 mmol) over a 30 minute period, while a stream of dry N_2 was passed over the mixture. After the additon of the NaH had been completed, the mixture was stirred for 10 minutes at room temperature and 30 minutes at 70°C (external oil bath temperature). At this point, the suspension had turned to a solid mass, which was dissolved in H_2O (1000 mL). The aqueous layer was extracted with diethyl ether (3x500 mL) and was acidified with HCl (2N). The mixture was extracted with EtOAc and dried over $MgSO_4$. Evaporation gave an off-white solid which was recrystallized from Et_2O/hexane (-20°C) to give a white solid (34.2 g, 90.9%).

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^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.67 [s, 6H, -CH($CO_2\underline{CH_3}$)$_2$], 5.72 [s, 1H, -$\underline{CH}$(CO_2CH_3)$_2$], 7.3 (d, J=7.76 Hz, 1H, Ar-$\underline{H}$), 7.45 (dt, J=7.66 Hz, 1.12 Hz, 1H, Ar-$\underline{H}$), 7.6 (dt, J=7.66 Hz, 1.45 Hz, 1H, Ar-$\underline{H}$), 7.94 (dd, J=7.8 Hz, 1.33 Hz, 1H, Ar-$\underline{H}$), 13.2 (s, 1H, -$CO_2\underline{H}$)

IR (KBr, cm^{-1}):	3300-2700 (CO_2 H), 1750 (C=0), 1730 (C=0), 1680 (C=0)
MS (m/e):	252 (M^+), 220 (M^+-CH_3OH), 188 (M^+-2xCH_3OH)
Anal. Calcd.:	C, 57.14; H, 4.80
Found:	C, 57.05; H, 4.78
M.P. 119-120°C.	

The following compound was prepared in substantially the same manner as that of Example 1, Step a):

(2-Carboxy-6-fluorophenyl)propanedioic Acid Dimethyl Ester

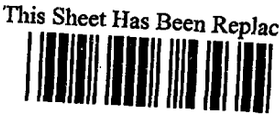
^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.68 [s, 6H, (–$CO_2\underline{Me}$)$_2$], 5.79 (s, 1H, Ar-$\underline{H}$), 7.12 (dd, J=10.06 Hz, 2.61 Hz, 1H, Ar-$\underline{H}$), 7.33 (dt, J=8.48 Hz, 2.64 Hz, 1H, Ar-$\underline{H}$), 8.03 (dd, 8.77 Hz, 6.17 Hz, 1H, Ar-$\underline{H}$)

IR (KBr, cm^{-1}): 3400-2700 (CO_2H), 1730 (C=0), 1680 (C=0)

MS (m/e): 270 (M^+), 238 (M^+-CH_3OH), 210 (M^+-CH_3OH, -CO), 151 (M^+ -CH_3OH, -CO, -CO_2CH_3)

Anal. Calcd.:	C. 53.34; H, 4.10
Found:	C, 53.36; H, 3.93
M.P. 121.5-123.0°C.	

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Step b)

3-Methoxy-1-oxo-1H-2-benzopyran-4-carboxylic Acid Methyl Ester

A mixture of (2-carboxyphenyl)propanedioic acid dimethyl ester (10.09 g, 39.68 mmol) and $SOCl_2$ (100 g) was refluxed for 2 hours. The volatiles were removed in vacuo and the crude product (acid chloride) was dissolved in THF (200 mL). Triethylamine (27.64 mL, 198.4 mmol) was added and the mixture was stirred for 30 minutes. The yellowish suspension was poured into HCl (1N, 1000 mL), extracted with EtOAc and the organic extracts were dried over $MgSO_4$. Evaporation and crystallization from acetone/ether/hexane (at -20°C) gave a white solid (87.6 g, 94.4%).

^1H NMR (DMSO-d_6, 400 MHz): δ 3.82 (s, 3H, -CO_2Me), 4.03 (s, 3H, -OMe), 7.42 (t, J=7.26 Hz, 1H, Ar-H), 7.8 (t, J=8.2 Hz, 1H, Ar-H), 7.9 (d, J=8.3 Hz, 1H, Ar-H), 8.1 (d, J=7.26 Hz, 1H, Ar-H)

IR (KBr, cm^{-1}):	1740 (C=O), 1685 (C=O)
MS (m/e):	234 (16, M^+), 206 (38.5, M^+-CO), 203 (12, M^+-OMe)
Anal. Calcd.:	C, 61.59; H, 4.30
Found:	C, 61.82; H, 4.29

M.P. 129-130°C.

The following compound was prepared in substantially the same manner as that of Example 1, Step b):

6-Fluoro-3-methoxy-1-oxo-1H-2-benzopyran-4-carboxylic Acid Methyl Ester

^1H NMR (DMSO-d_6, 400 MHz): δ 3.81 (s, 3H, -CO_2CH_3), 4.06 (s, 3H, -OCH_3), 7.27 (dt, J=8.3 Hz, 1H, Ar-H), 7.8 (dd, J=11.83 Hz, 2.49 Hz, 1H, Ar-H), 8.16 (dd, J=8.92 Hz, 6.2 Hz, 1H, Ar-H)

IR (KBr, cm^{-1}):	1750 (C=O), 1685 (C=O)
MS (m/e):	252 (24, M^+), 224 (54, M^+-CO)

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© Anal. Calcd.: C, 57.15; H, 3.60
Found: C, 57.19; H, 3.57

M.P. 142-143°C.

Step c)

2-(Cyanomethyl)-1,2,3,4-tetrahydro-1,3-dioxo-4-isoquinoline-carboxylic Acid Methyl Ester

To a solution of 3-methoxy-1-oxo-1H-2-benzopyran-4-carboxylic acid methyl ester (8.0 g, 34.19 mmol) in DMF (100 mL) was added aminoacetonitrile hydrochloride (6.32 g, 68.37 mmol) and the suspension was stirred until all the materials have dissolved. Triethylamine (14.3 mL, 102.57 mmol) was added and the mixture was stirred at 100°C for 30 minutes, and then poured into H_2O, acidified with HCl (2N) and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and crystallization from acetone/ether/hexane (at -20°C) gave a yellowish solid (6.5 g, 73.7%).

^1H NMR (DMSO-d_6, 400 MHz): δ [3.7 (s), 3.98 (s), 3H, -$CO_2\underline{CH_3}$, tautomeric], [4.92 (s), 5.44 (s), 2H, -$N\underline{CH_2}CN$, tautomeric], 7.2-8.4 (m, 4H, Ar-$\underline{H}$, tautomeric)

IR (KBr, cm^{-1}): 3400 (OH), 1670 (C=O)

MS (m/e): 258 (20, M^+), 226 (43, M^+-MeOH), 199 (13, M^+-CO_2Me)

Anal. Calcd.: C, 60.47; H, 3.90; N, 10.85
Found: C, 60.27; H, 3.77; N, 10.69

M.P. 169-171°C.

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The following compound was prepared in substantially the same manner as that of Example 1, Step c):

2-(Cyanomethyl)-6-fluoro-1,2,3,4-tetrahydro-1,3-dioxo-4-isoquinoline-carboxylic Acid Methyl Ester

Anal. Calcd.: C, 56.53; H, 3.28; N, 10.14
Found: C, 56.45; H, 3.22; N, 10.13

M.P. 178-179°C.

Step d)

2-(Cyanomethyl)-1,2,3,4-tetrahydro-4-(methoxycarbonyl)-1,3-dioxo-4-isoquinolineacetic Acid 1,1-Dimethylethyl Ester

To a suspension of 2-(cyanomethyl)-1,2,3,4-tetrahydro-1,3-dioxo-4-isoquinoline carboxylic acid methyl ester (6.5 g, 25.19 mmol), K_2CO_3 (6.95 g, 50.38 mmol) and anhydrous DMF (100 mL) was added tert-butyl bromoacetate (6.1 mL, 37.79 mmol). After stirring at 85°C for 3 hours, the mixture was poured into H_2O, acidified with HCl (2N) and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography on silica gel (hexane/EtOAc 4/1) gave a white solid (8.5 g, 90.7%).

^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.03 (s, 9H, -CO_2 $\underline{CMe_3}$), 3.58 (s, 3H, -$CO_2\underline{CH_3}$), 3.64 (s, 2H, -$\underline{CH_2}CO_2$-), 5.05 (s, 2H, -N$\underline{CH_2}$CN), 7.64 (m, 2H, Ar-$\underline{H}$), 7.78 (dd, J=7.4 Hz, 2.0 Hz, 1H, Ar-$\underline{H}$), 8.24 (dd, J=8.2 Hz, 1.6 Hz, 1H, Ar-$\underline{H}$)

IR (KBr, cm^{-1}): 1745 (C=O), 1730 (C=O), 1670 (C=O)

MS (m/e): 373 (38, M^+ + H), 317 (100, M^+ + H, -CMe_3)

Anal. Calcd.: C, 61.28; H, 5.41; N, 7.52
Found: C, 61.61; H, 5.49; N, 7.13

M.P. 48-50°C.

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The following compound was obtained in substantially the same manner as that of Example 1, Step d):

2-(Cyanomethyl)-6-fluoro-1,2,3,4-tetrahydro-4-(methoxycarbonyl)-1,3-dioxo-4-isoquinolineacetic Acid 1,1-Dimethylethyl Ester

Anal. Calcd.: C, 58.46; H, 4.91; N, 7.18
Found: C, 58.65; H, 4.98; N, 7.08
M.P. 133-135°C.

Step e)

1,2,3,4-Tetrahydro-4-(methoxycarbonyl)-1,3-dioxo-2-[[(5-trifluoromethyl)-2-benzothiazolyl]methyl]-4-isoquinolineacetic Acid

To a mixture of 3-amino-4-mercaptobenzotrifluoride hydrochloride (6.1 g, 26.2 mmol) and EtOH (150 mL) was added Et_3N (3.65 mL). After stirring for 10 minutes, 2-(cyanomethyl)-1,2,3,4-tetrahydro-4-(methoxycarbonyl)-1,3-dioxo-4-isoquinoline-acetic acid 1,1-dimethylethyl ester (6.5 g, 17.47 mmol) was added and the mixture was refluxed for 15 hours, poured into H_2O, acidified with HCl (2N) and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation gave an oil (9.6 g) which was dissolved in CH_2Cl_2 (80 mL). Trifluoroacetic acid (20 mL) was added and the mixture was stirred at room temperature for 8 hours. The volatiles were removed in vacuo and the residue was purified by flash chromatography on acid washed (5%, H_3PO_4 in MeOH) silica gel to give a white solid (6.3 g, 73.3%).

^1H NMR (DMSO-d_6, 400 MHz): δ 3.57 (s, 3H, -$CO_2\underline{CH_3}$), 3.68 (dd, J=17.85 Hz, 2H, -$\underline{CH_2}CO_2H$), 5.61 (s, 2H, -$N\underline{CH_2}$-), 7.62 (m, 2H, Ar-H), 7.81 (m, 2H, Ar-H), 8.2 (dd, J=7.9 Hz, 1.04 Hz, 1H, Ar-H), 8.33 (dd, J=8.5 Hz, 0.92 Hz, 1H, Ar-H), 8.34 (d, J=0.83 Hz, 1H, Ar-H)

IR (KBr, cm^{-1}): 3200-2500 (CO_2H), 1750 (C=O), 1710 (C=O), 1670 (C=O)
MS (m/e): 492 (6, M^+), 448 (6, M^+-CO_2), 416 (62, M^+-CO_2, -MeOH)

Anal. Calcd.: C, 53.66; H, 3.07; N, 5.69
Found: C, 53.40; H, 3.01; N, 5.54
M.P. 199-201°C.

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The following compounds were prepared in substantially the same manner as that of Example 1, Step e):

1,2,3,4-Tetrahydro-4-(methoxycarbonyl-1,3-dioxo-2-[(2-benzothiazolyl)methyl]-4-isoquinolineacetic Acid

Anal. Calcd.: C, 59.43; H, 3.80; N, 6.60
Found: C, 59.37; H, 3.74; N, 6.43

M.P. 189-190°C.

6-Fluoro-1,2,3,4-tetrahydro-4-(methoxycarbonyl-1,3-dioxo-2-[[(5-trifluoromethyl)-2-benzothiazolyl]methyl]-4-isoquinolineacetic Acid

Anal. Calcd.: C, 51.77; H, 2.76; N, 5.49
Found: C, 51.62; H, 2.97; N, 5.18

M.P. 103-105°C.

Step f)

4-[(Aminocarboxyl)methyl]-1,2,3,4-tetrahydro-1,3-dioxo-2-[[(5-trifluoromethyl)-2-benzothiazolyl]methyl]-4-isoquinoline-carboxylic Acid Methyl Ester

A mixture of 1,2,3,4-tetrahydro-4-(methoxycarbonyl)-1,3-dioxo-2-[[(5-trifluoromethyl)-2-benzothiazolyl)methyl]-4-isoquinolineacetic acid (2.5 g, 5.08 mmol) and $SOCl_2$ (25 g) was refluxed for 1 hour. The volatiles were removed in vacuo and the product (acid chloride) was taken in THF (20 mL) and added to a freshly prepared, saturated NH_3, THF solution (20 mL). After stirring for 10 minutes, the mixture was poured into HCl (1N, 500 mL), extracted with EtOAc and dried over $MgSO_4$. Evaporation and purification by flash chromatography on silica gel (hexane/EtOAc, 1/1) gave a light yellow solid (2.1 g, 84%).

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^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.53 (dd, J=16.6 Hz, 2H, -$\underline{CH_2}CONH_2$), 3.56 (s, 3H, $CO_2\underline{CH_3}$), 5.53 (d, J=15.77 Hz, 1H, -N$\underline{H}$CH-), 5.6 (d, J=15.77 Hz, 1H, -NHC$\underline{H}$-), 6.84 (br s, 1H, -CONH-), 7.49 (m, 2H, Ar-H, -CON$\underline{H}$-), 7.6 (t, J=7.47 Hz, 1H, Ar-$\underline{H}$), 7.68 (m, 2H, Ar-$\underline{H}$), 8.17 (dd, J=7.9z, 1.45 Hz, 1H, Ar-$\underline{H}$), 8.34 (m, 2H, Ar-$\underline{H}$)

IR (KBr, cm^{-1}): 3420 (NH), 3180 (NH), 1745 (C=0), 1710 (C=0), 1660 (C=0)

MS(m/e): 491 (10, M^+)

Anal. Calcd.: C, 53.77; H, 3.28; N, 8.55
Found: C, 54.23; H, 3.26; N, 8.39

M.P. 198-200°C.

The following compound was prepared in substantially the same manner as that of Example 1, Step f)

4-[(Aminocarbonyl)methyl]-6-fluoro-1,2,3,4-tetrahydro-1,3-dioxo-2-[[(5-trifluoromethyl)-2-benzothiazolyl]methyl]-4-isoquinoline-carboxylic Acid Methyl Ester

Anal. Calcd.: C, 51.87; H, 2.97; N, 8.25
Found: C, 51.87; H, 2.93; N, 8.01

M.P. 144-146°C.

Step g)

2-[[(5-(Trifluoromethyl)-2-benzothiazolyl]methyl]spiro-[isoquinoline-4(1H), 3'-pyrrolidine]-1,2',3,5'(2H)-tetrone

To a mixture of 4-[(aminocarbonyl)methyl]-1,2,3,4-tetrahydro-1,3-dioxo-2-[[(5-trifluoromethyl]-2-benzothiazolyl]methyl]-4-isoquinolineacetic acid methyl ester (1.6 g, 3.34 mmol) and anhydrous DMF (20 mL) was added NaH (80% dispersion in oil, 106.2 mg, 3.34 mmol) portionwise over a 5 minute period. After stirring for 30

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minutes, the mixture was poured into H_2O (1000 mL), acidified with HCl (2N), extracted with EtOAc and dried over $MgSO_4$. Evaporation and purification by flash chromatography on silica gel (hexane/EtOAc 1/2) gave a white solid (1.2 g, 80.3%).

^1H NMR (DMSO-d_6, 400 MHz) δ 3.44 (dd, J=18.26 Hz, 2H, -$\underline{CH_2}$CO-), 5.56 (s, 2H, N$\underline{CH_2}$), 7.66 (dt, J=7.26 Hz, 1.04 Hz, 1H, Ar-$\underline{H}$), 7.75 (m, 2H, Ar-$\underline{H}$), 7.82 (dt, J=7.88 Hz, 1.66 Hz, 1H, Ar-$\underline{H}$), 8.22 (dd, J=7.88 Hz, 1.45 Hz, 1H, Ar-$\underline{H}$), 8.33 (m, 2H, Ar-$\underline{H}$), 12.05 (s, 1H, -CON$\underline{H}$CO-)

IR (KBr, cm^{-1}): 3400 (NH), 3240 (NH), 1725 (C=0), 1680 (C=0)

MS(m/e): 459 (82, M^+)

Anal. Calcd.:	C, 54.90; H, 2.63; N, 9.15
Found:	C, 55.17; H, 2.71; N, 9.06

M.P. 122-124°C.

The following compounds were prepared in substantially the same manner as that of Example 1, Step g)

2-(2-Benzothiazolylmethyl)spiro[isoquinoline-4(1H),3'-pyrrolidine)-1,2',3,5'(2H)-tetrone

Anal. Calcd.:	C, 61.37; H, 3.35; N, 10.74
Found:	C, 61.09; H, 3.47; N, 10.59

M.P. 225-227°C.

6-Fluoro-2-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]spiro-[isoquinoline-4(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrone

Anal. Calcd.:	C, 52.84; H, 2.32; N, 8.80
Found:	C, 52.76; H, 2.43; N, 8.53

M.P. 123-125°C.

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2-(2-Benzothiazolylmethyl)-6-fluorospiro[isoquinoline-4(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrone

Anal. Calcd.:	C, 58.68; H, 2.95; N, 10.26
Found:	C, 58.77; H, 3.12; N, 10.11

M.P. 198-199°C.

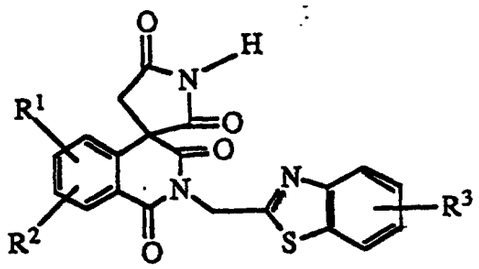
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CLAIMS

1. A compound of formula (I)



(I)

or a salt thereof, wherein R^1 and R^2 are independently hydrogen, alkyl containing 1 to 6 carbon atoms, halogen, lower alkoxy containing 1 to 6 carbon atoms, trifluoromethyl, nitro, aryl or aryl(lower alkyl)oxy wherein aryl contains 6 to 10 carbon atoms and lower alkyl contains 1 to 6 carbon atoms; and R^3 is hydrogen, halogen, alkyl containing 1 to 6 carbon atoms, lower alkoxy containing 1 to 6 carbon atoms, trifluoromethyl, nitro, aryl or aryl(lower alkyl)oxy wherein aryl contains 6 to 10 carbon atoms and lower alkyl contains 1 to 5 carbon atoms.

2. A compound according to Claim 1 wherein R^1 and R^2 are independently hydrogen or halogen; and R^3 is hydrogen, halogen or trifluoromethyl.

3. A compound as claimed in Claim 1 or Claim 2 wherein R^1 is in the 6-position.

4. A compound as claimed in anyone of Claims 1 to 3 wherein R^3 is in the 5-position.

5. A compound which is
2-[(2-benzothiazolylmethyl)spiro[isoquinoline-4

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(1H),3′-pyrrolidine]-1,2′,3,5′(2H)-tetrone or a pharmaceutically acceptable salt thereof.

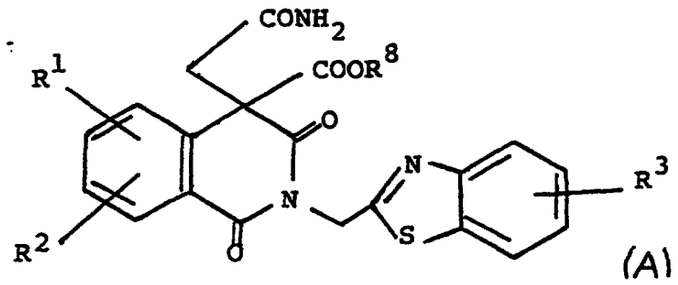
6. A compound according to Claim 2 which is 2-[(2-benzothiazolylmethyl)-6-fluorospiro-[isoquinoline-4(1H)3′-pyrrolidine]-1,2′,3,5′(2H)-tetrone or a pharmaceutically acceptable salt thereof.

7. A compound according to Claim 2 which is 2-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-spiro[isoquinoline-4-(1H),3′-pyrrolidine]-1,2′,3,5′(2H)-tetrone or a pharmaceutically acceptable salt thereof.

8. A compound according to Claim 2 which is 6-fluoro-2-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]spiro[isoquinoline-4(1H),3′-pyrrolidine]-1,2′,3,5′(2H)-tetrone or a pharmaceutically acceptable salt thereof.

9. A process for preparing a compound of formula I as claimed in Claim 1 which comprises one of the following:

a) cyclising an amide of formula (A)



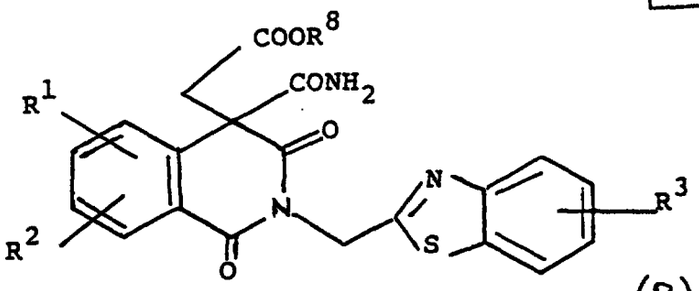
(A)

wherein R^1, R^2 and R^3 are as defined above and $COOR^8$ is ester group, using a suitable base such as sodium hydride to give a compound of formula I;
or

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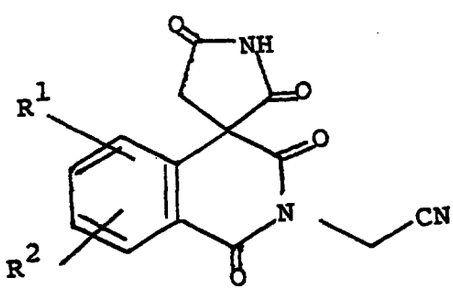
b) cyclising a compound of formula (B)



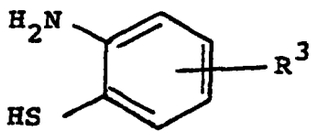
(B)

wherein R^1, R^2, R^3 and $COOR^8$ are as defined above using a suitable base such as sodium hydride or lithium bis-(trimethylsilyl)amide to give a compound of formula I; or

c) reacting a compound of formula



with a 2-aminothiophenol of formula

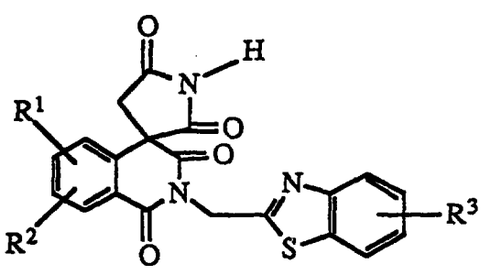


in which formulue R^{1-3} are as defined above, to give a compound of formula I .

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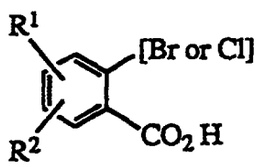
10. A process for preparing a compound of formula (I)



(I)

wherein R^1 and R^2 are independently hydrogen, alkyl containing 1 to 6 carbon atoms, halogen, lower alkoxy containing 1 to 6 carbon atoms, trifluoromethyl, nitro, aryl or aryl(lower alkyl)oxy wherein aryl contains 6 to 10 carbon atoms and lower alkyl contains 1 to 6 carbon atoms; and R^3 is hydrogen, alkyl containing 1 to 6 carbon atoms, lower alkoxy containing 1 to 6 carbon atoms, trifluoromethyl, nitro, aryl or aryl(lower alkyl)oxy wherein aryl contains 6 to 10 carbon atoms and lower alkyl contains 1 to 5 carbon atoms which comprises:

a) reacting the compound of formula (II)

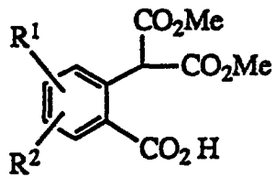


(II)

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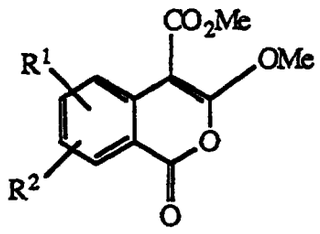
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wherein R^1 and R^2 are as defined above with dimethyl malonate and NaH in the presence of a catalytic amount of CuBr to produce the compound of formula (III)



(III)

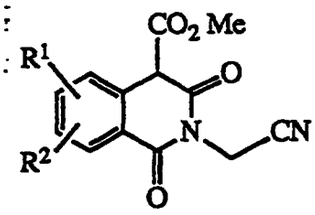
b) reacting the compound of formula (III) wherein R^1 and R^2 are as defined above with $SOCl_2$ and subsequently treating with Et_3N in a conventional solvent to produce the compound of formula (IV)



(IV)

wherein R^1 and R^2 are as defined above

c) reacting said compound of formula (IV) with aminoacetonitrile hydrochloride in the presence of Et_3N in a conventional solvent to produce the compound of formula (V)



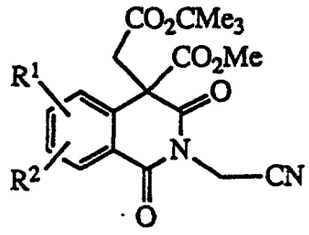
(V)

wherein R^1 and R^2 are as defined above

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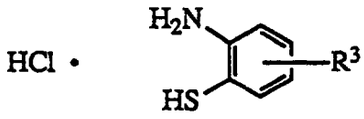
d) reacting said compound of formula (V) with an inorganic base in a conventional solvent and subsequently adding tert-butyl bromoacetate to produce the compound of formula (VI)



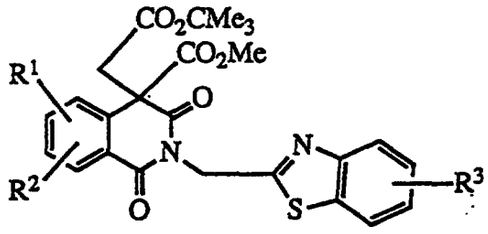
(VI)

wherein R^1 and R^2 are as defined above

e) reacting said compound of formula (VI) with a 2-aminothiophenol of structural formula



wherein R^3 is as defined above to produce the tert-butyl ester compound of structural formula

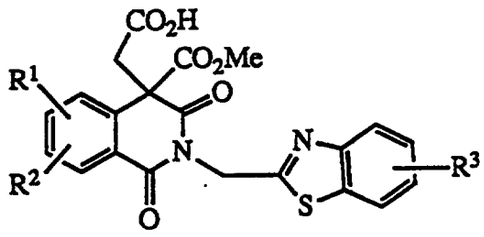


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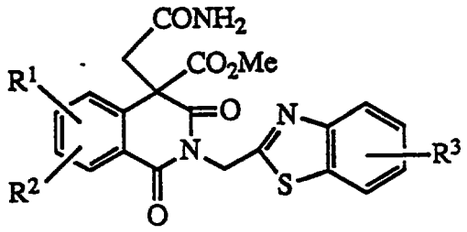
f) hydrolyzing said tert-butyl ester with an organic acid in a conventional solvent to produce compound of formula (VII)



(VII)

wherein R^1, R^2 and R^3 are as defined above

g) reacting said compound of formula (VII) with thionyl chloride and subsequently adding THF/NH_3 to produce the compound of formula (VIII)



(VIII)

wherein R^1, R^2 and R^3 are as defined above

h) reacting said compound of formula (VIII) with a base in a conventional solvent to produce the compound of formula (I).

11. A pharmaceutical composition which comprises a compound as claimed in any one of Claims 1 to 8 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

12. A compound of formula I or a pharmaceutical salt thereof as defined in any of Claims 1 to 8 for use as a pharmaceutical.

13. Use of a compound of formula I or a pharmaceutically acceptable salt thereof as defined in any one of Claims 1 to 8 in the manufacture of a medicament for the treatment or prevention of neuropathy, nephropathy, retinopathy or cataracts in a diabetic mammal.

* C L M S *