Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 14 (2006) 567–574
Bioorganic & Medicinal Chemistry

In vitro aldose reductase inhibitory activity of 5-benzyl-2,4-thiazolidinediones

Dietmar Rakowitz,[a] Rosanna Maccari,[b,*] Rosaria Ottanà[b] and Maria Gabriella Vigorita[b]

[a]*Institute of Pharmacy, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria*
[b]*Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università di Messina, Viale SS. Annunziata, 98168 Messina, Italy*

Received 22 June 2005; revised 6 August 2005; accepted 19 August 2005
Available online 3 October 2005

Abstract—Several 5-benzyl-2,4-thiazolidinediones (**5**–**7**) were synthesised and tested as in vitro aldose reductase (ALR2) inhibitors. Most of them, particularly N-unsubstituted 5-benzyl-2,4-thiazolidinediones **5** and (5-benzyl-2,4-dioxothiazolidin-3-yl)acetic acids **7**, displayed moderate to high inhibitory activity levels. In detail, the insertion of an acetic chain on N-3 significantly enhanced ALR2 inhibitory potency, leading to acids **7** which proved to be the most effective among the tested compounds. In addition, in N-unsubstituted derivatives **5** the presence of an additional aromatic ring on the 5-benzyl moiety was generally beneficial. In fact, the ALR2 inhibition results of compounds **5**–**7**, compared to those of the previously assayed corresponding 5-arylidene-2,4-thiazolidinediones, indicated that N-unsubstituted derivatives **5b**, **c** and **d**, which bore an additional aromatic group in the para position of the 5-benzyl residue, were significantly more effective than their 5-arylidene counterparts; in all other cases, the saturation of the exocyclic double bond C=C in 5 brought about a moderate decrease in activity.

1. Introduction

Diabetes mellitus is a widespread chronic disease, whose current worldwide prevalence of 150 million is predicted to double by 2025.[1–3] It is always associated with long-term complications (retinopathies, nephropathies, neuropathies, angiopathies, atherosclerosis and cataracts) that make it one of the leading causes of blindness, renal failure and neuronal pathologies. Diabetic complications also increase the risk of myocardial infarction and stroke and are thus largely responsible for the morbidity and mortality observed in these patients.[1,4]

The increased flux of glucose through the polyol pathway that occurs in hyperglycaemic conditions in tissues possessing insulin-independent glucose transport (nerve, retina, lenses and kidney) is a well-examined factor involved in the onset and progression of such chronic complications.[4–12]

Aldose reductase (EC 1.1.1.21, ALR2) is the first enzyme of the polyol pathway and catalyses the NADPH-dependent reduction of glucose to sorbitol. The deprivation of NADPH and NAD^+ and the intracellular accumulation of sorbitol result in biochemical imbalances which cause damage in target tissues. ALR2 inhibition thus represents an attractive approach to prevent or control the progression of chronic diabetic complications.[4–12]

A variety of ALR2 inhibitors (ARIs) have been reported; however, in clinical studies many of them have exhibited low efficacy or a narrow spectrum of tissue activity, generally because of unfavourable pharmacokinetics, or have proved to produce toxic side-effects.[10,12–15] Currently, epalrestat (Fig. 1) is the only ARI available on the market.[15,16]

The ARIs that have been clinically tested can be divided into two main classes: (a) cyclic imides, mostly hydantoins (such as sorbinil, Fig. 1) which often have good in vivo activity but can cause hypersensitivity reactions related to the hydantoin moiety, and (b) carboxylic acids (such as ponalrestat, epalrestat, Fig. 1), which are generally less effective in vivo than in vitro.[10,12,14]

In this context, 2,4-thiazolidinedione derivatives can be considered bioisosteres of hydantoins potentially devoid of the toxic side-effects linked to the presence of the hydantoin system.[10,15] They have also drawn extensive

Keywords: 2,4-Thiazolidinediones; Aldose reductase; ALR2 inhibitors; Structure–activity relationships.
* Corresponding author. Tel.: +39 90 6766406; fax: +39 90 355613; e-mail: rmaccari@pharma.unime.it

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.08.056

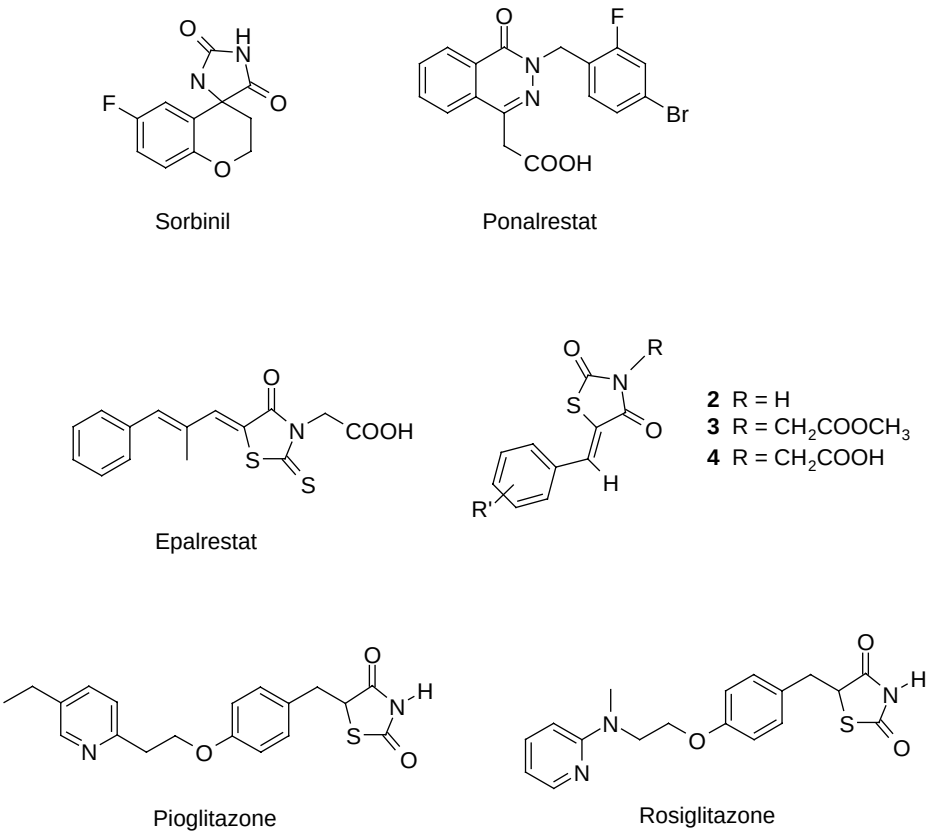


Figure 1.

interest as antihyperglycaemic compounds.[17–22] In fact, many 2,4-thiazolidinediones have been reported as insulin-sensitizing agents and some of them (pioglitazone, rosiglitazone, Fig. 1) have been marketed for the treatment of non-insulin-dependent diabetes mellitus (NIDDM or type 2 diabetes), which accounts for 90–95% of all diabetes.[1,23] In addition, some other 2,4-thiazolidinediones with dual activity, having both antihyperglycaemic and ALR2 inhibitory effects, have been patented;[10,15] they might serve to treat NIDDM and to control the progression of its long-term complications.

We have recently reported the in vitro ALR2 inhibitory activity of numerous (*Z*)-5-arylidene-2,4-thiazolidinediones (**2**–**4**, Fig. 1); they possessed the essential structural requisites for ALR2 inhibition (an acidic proton, H bond acceptor groups and a lipophilic aromatic moiety)[12,15,24–28] and, in fact, most of them were shown to be effective as ARIs.[29,30] In particular, some N-unsubstituted derivatives (**2**) exerted the same activity as sorbinil; moreover, the introduction of an acetic chain on N-3 brought about a marked increase (from 10 to 100 times) in inhibitory potency, leading to acids **4**, which proved to be very effective ARIs.[29,30] Although devoid of any acidic functionality, some of the corresponding methyl esters **3** also displayed appreciable ALR2 inhibitory activity similar to that of the N-unsubstituted derivatives. However, acids **4** proved to be more effective than corresponding esters **3**, clearly due to the presence of the carboxylic anionic head of the N-3 acetic chain, which favours the binding with the positively charged recognition region of the ALR2 active site formed by Tyr48, His110, Trp111 residues and the nicotinamide ring of $NADP^+$.[10,14,15,24,25,30] Besides, the presence of an additional aromatic ring or an H-bond donor group in the 5-benzylidene moiety enhanced ALR2 inhibitory effect; in fact, except for the polar recognition region, the ALR2 active site is highly hydrophobic and thus large lipophilic moieties can favour the interaction of inhibitors with the enzyme; otherwise, an H-bond donor group can stabilize the enzyme–inhibitor complex through further H-bonds.[30] Molecular docking simulations into the ALR2 active site allowed the rationalization of the observed SARs and suggested a pharmacophoric model, consisting of a lipophilic region and two hydrogen-bond acceptor features, for this class of ARIs.[30]

We also found that 5-(3-phenoxybenzylidene)- and 5-(4-phenoxybenzylidene)-2,4-thiazolidinediones, belonging to series **2**, displayed moderate antihyperglycaemic activity in a preliminary in vivo assay.[31]

The next step of our ongoing research was to extend the SARs picture by assessing the significance of the exocyclic double bond C=C in position 5. Therefore, we synthesised and assayed as ARIs 5-benzyl-2,4-thiazolidinediones **5**–**7**, which retained the pharmacophoric elements of the previously explored 5-arylidene analogues.[29,30]

At the same time, the reduction of the exocyclic double bond C=C of 5-arylidene-2,4-thiazolidinediones **2–4** might enhance their potential antihyperglycaemic activity, similarly to known 5-benzyl-2,4-thiazolidinediones which are often more effective than their 5-arylidene-substituted analogues in improving blood glucose levels in NIDDM.[18,19] Recently, the antihyperglycaemic activity was also reported of several 2,4-thiazolidinediones having a carboxylic chain inserted on N-3.[32]

It seemed likely, therefore, that compounds **5–7** could be potentially endowed with both antihyperglycaemic and ALR2 inhibitory effects. We here report on their in vitro ALR2 inhibitory activity, which was explored using partially purified ALR2 from bovine lenses.

2. Chemistry

5-Benzyl-2,4-thiazolidinediones **5** were prepared by the reaction of corresponding 5-benzylidene derivatives **2** with lithium borohydride in pyridine and tetrahydrofuran at reflux for 3–4 h (Scheme 1). Compounds **2** were in turn obtained, according to a known procedure, by the Knoevenagel condensation of commercially available 2,4-thiazolidinedione with suitable benzaldehydes (Scheme 1).[29] The reduction of the exocyclic double bond C=C of several 5-benzylidene-2,4-thiazolidinediones (including compounds **2c** and **2f**) by $LiBH_4$ was reported by Giles and colleagues; it was shown to be a rapid and selective hydrogenation method of the double bond C=C and required simple equipment.[33]

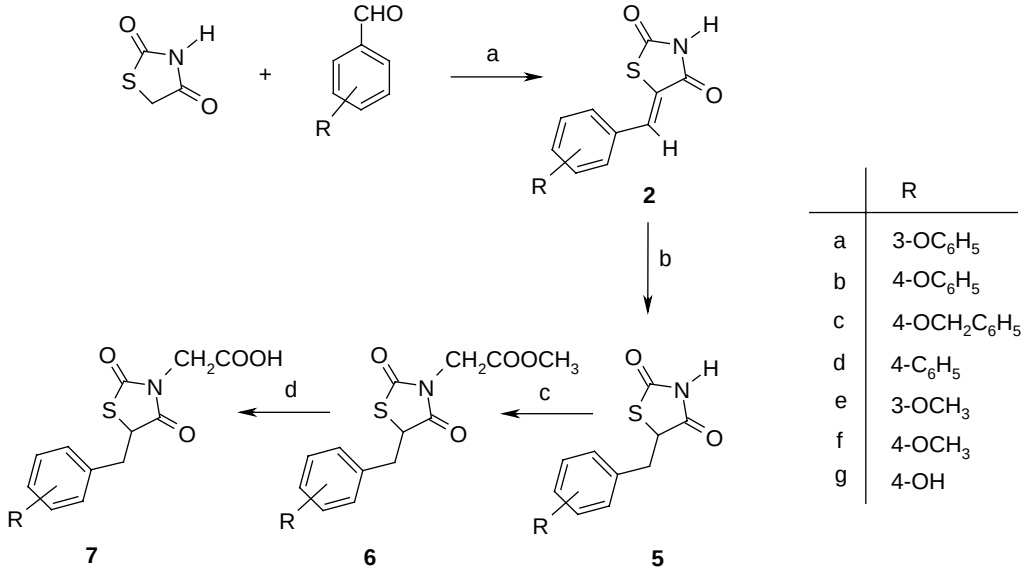
In an attempt to increase the yields of compounds **5**, we also tried carrying out the same reduction by irradiation with microwaves (MW). In fact, although reduction reactions were the last to appear on the scene of MW-assisted chemistry, it has been demonstrated that MW-assisted hydrogenation reactions can be successfully performed in a rapid and safe way.[34,35] Upon MW-irradiation, the reduction of representative compound **2b** by $LiBH_4$ in pyridine and THF was accomplished in less than 10 min. However, the yield of the desired derivative (**5b**) was similar to that obtained under conventional conditions.

The treatment of compounds **5** with methyl bromoacetate, using potassium carbonate as base, provided esters **6** in high yields; these latter were hydrolysed in acidic medium to give acids **7** (Scheme 1). [5-(4-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid (**7g**) was prepared by the hydrolysis of the methyl ester of [5-(4-benzyloxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid (**6c**) in acidic medium.

The structures of compounds **5–7** were assigned on the basis of their analytical and spectroscopic data. NMR spectroscopy proved to be particularly useful for their characterization. In fact, ^1H NMR spectra of all compounds **5–7** showed a diagnostic ABX system, in the range 3.05–4.62 ppm, consisting of three double doublets due to the resonances of the enantiotopic methylene protons and the methinic proton in 5. In addition, the absence of any singlet in the range 7.75–7.85 ppm, attributable to the resonance of the 5-methylidene proton of parent compounds **2**,[29,30] irrefutably confirmed the reduction of the exocyclic double bond C=C.

In ^{13}C NMR spectra, the methylene carbon and the thiazolidinedione C-5 of compounds **5–7** resonated at 36.7–38.8 and 51.6–54.0 ppm, respectively.

The ^1H and ^{13}C NMR spectra of esters **6** and acids **7** showed an additional signal due to the resonance of N-CH_2 (the proton in the range 4.30–4.38 ppm and the carbon at 41.5–41.9 ppm); in their ^{13}C NMR spectra, besides two signals attributable to the resonances of 2- and 4-carbonylic groups at 170.0–175.0 ppm,



	R
a	3-OC_6H_5
b	4-OC_6H_5
c	4-$OCH_2C_6H_5$
d	4-C_6H_5
e	3-OCH_3
f	4-OCH_3
g	4-OH

Scheme 1. Reagents and conditions: (a) $C_5H_{11}N$, C_2H_5OH, Δ; (b) $LiBH_4$, Py, THF, Δ; (c) K_2CO_3, acetone, $BrCH_2COOCH_3$, Δ; (d) AcOH, HCl, Δ.

another signal due to the resonance of the carboxylic carbon appeared in the same range.

3. Results and discussion

Compounds **5**–**7** were tested in vitro for their ability to inhibit partially purified ALR2 from bovine lenses, using sorbinil and ponalrestat as reference drugs (Table 1). The enzyme activity was assayed by spectrophotometrically monitoring NADPH oxidation, which accompanies the reduction of D,L-glyceraldehyde used as substrate.

Taking into account the SARs defined in our previous studies relevant to 2,4-thiazolidinediones **2**–**4**,[29,30] we prepared analogues **5**–**7 a**–**d** which had an additional aromatic moiety, that is, a phenoxy, benzyloxy or phenyl group, on the 5-benzyl residue (Scheme 1). In addition, we synthesised derivatives **5**–**7 e**,**f** in which these additional aromatic groups were replaced by a methoxy group. Finally, we also prepared [5-(4-hydroxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid (**7g**) as the 5-benzyl analogue of **4g**, which in turn had proved to be one of the most active among the previously explored 5-arylidene-2,4-thiazolidinediones, with IC_{50} = 0.15 μM.[30]

Among N-unsubstituted 5-benzyl-2,4-thiazolidinediones, derivatives **5 a**–**d** displayed IC_{50} values ranging from 31.4 to 78.9 μM, whereas **5e** and **5f** produced only moderate ALR2 inhibition at 50 μM dose (Table 1). Compounds **5b**, **5c** and **5d**, which bore an aromatic substituent in the para position of the 5-benzyl group, were significantly more potent than the corresponding 5-arylidene analogues (**2b**, **2c** and **2d**), which in turn had produced 41%, 10%, and 20% ALR2 inhibition, respectively, at 50 μM dose.[30] This appreciable increase in potency was not observed either when the phenoxy group was displaced in meta position (**5a**) or when it was replaced by a methoxy group (**5e** and **5f**); in fact, compounds **5a**, **5e** and **5f** proved to be less effective than corresponding compounds **2**.[29,30]

The insertion of an acetic chain on N-3 led to (5-benzyl-2,4-dioxothiazolidin-3-yl)acetic acids **7**, which, analogously to what had been observed in the series of 5-arylidene-2,4-thiazolidinediones,[29,30] were shown to be from 15- to 80-fold more effective than N-unsubstituted analogues **5**; they displayed IC_{50} values ranging between 1.01 and 2.96 μM (Table 1).

The ALR2 inhibitory potency of acids **7** appeared to be independent of the pattern substitution on the 5-benzyl moiety; indeed, the most active derivatives were [5-(3-phenoxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid (**7a**, IC_{50} = 1.01 μM) and [5-(4-methoxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid (**7f**, IC_{50} = 1.07 μM).

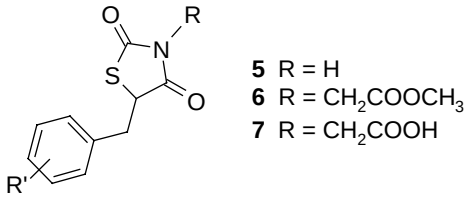
In comparison with previously studied acids **4**, analogues **7a**, **b** and **d**–**f** were shown to be from 3- to almost 8-fold less effective, whereas 5-(4-hydroxybenzyl) substituted acid **7g** was 14 times less active than its 5-arylidene counterpart.[30]

Among methyl esters **6**, only compound **6e** produced appreciable ALR2 inhibition (IC_{50} = 21.1 μM), proving to be at least 2.5-fold more active than the corresponding N-unsubstituted 2,4-thiazolidinedione **5e** (IC_{50} > 50 μM) but 7-fold less effective than acid **7e** (IC_{50} = 2.96 μM) (Table 1). Ester **6a**, which was the 5-benzyl analogue of compound **3a** (IC_{50} = 1.32 μM),[29,30] was also shown to be inactive (Table 1).

In conclusion, although the saturation of the exocyclic double bond C=C in position 5 of 5-arylidene-2,4-thiazolidinediones generally appeared to be not very beneficial for ALR2 inhibitory activity, 5-benzyl-2,4-thiazolidinediones **5** and (5-benzyl-2,4-dioxothiazolidin-3-yl)acetic acids **7** proved to be moderately to highly effective in vitro ARIs. Ester **6e** also displayed ALR2 inhibitory properties.

On the basis of the biological results so far acquired, we could identify some structural features which influenced their effectiveness as ARIs. In N-unsubstituted derivatives **5**, the presence of an additional aromatic ring in the 5-benzyl moiety, especially in the para position, appeared to have a beneficial influence, whereas the methoxy group was a less favourable substituent. As expected, the insertion of an acetic chain on N-3 increased activity up to almost 100 times and led to acids **7**, which were the most effective among the tested compounds.

Table 1. In vitro bovine lenses' ALR2 inhibitory activity of 2,4-thiazolidinediones **5**–**7**



5 R = H
6 R = CH_2COOCH_3
7 R = CH_2COOH

R′	Compound	IC_{50}[a]
3-OC_6H_5	**5a**	78.9 (62.3–99.9)
4-OC_6H_5	**5b**	40.4 (34.8–47.1)
4-$OCH_2C_6H_5$	**5c**	31.4 (29.4–33.5)
4-C_6H_5	**5d**	65.8 (47.8–90.7)
3-OCH_3	**5e**	16% (50 μM)
4-OCH_3	**5f**	21% (50 μM)
3-OC_6H_5	**6a**	26% (50 μM)
4-OC_6H_5	**6b**	1% (50 μM)
4-$OCH_2C_6H_5$	**6c**	24% (50 μM)
4-C_6H_5	**6d**	15% (50 μM)
3-OCH_3	**6e**	21.1 (16.4–27.0)
4-OCH_3	**6f**	24% (50 μM)
3-OC_6H_5	**7a**	1.01 (0.92–1.11)
4-OC_6H_5	**7b**	2.83 (2.68–3.00)
4-C_6H_5	**7d**	1.68 (1.09–2.59)
3-OCH_3	**7e**	2.96 (2.37–3.70)
4-OCH_3	**7f**	1.07 (0.96–1.20)
4-OH	**7g**	2.10 (1.81–2.43)
	Sorbinil	3.42 (3.39–3.46)
	Ponalrestat	0.08 (0.073–0.087)

[a] IC_{50} (μM) (95% CL) or % inhibition at the given concentration.

Taking into account that compounds **5b** and **5f** are already present in the literature as antihyperglycaemic agents,[17,36] the logical continuation of this research may be the evaluation, in a suitable animal model of NIDDM, of the antihyperglycaemic activity of the most promising ARIs among the 5-benzyl-2,4-thiazolidinediones here reported, in order to assess their ability to act as potential antidiabetic agents with dual effect.

4. Experimental section

4.1. Chemistry

Melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. TLC controls were carried out on precoated silica gel plates (F 254 Merck). Elemental analyses (C, H, N), determined by means of a C. Erba mod. 1106 elem. Analyzer, were within ±0.4% of theory. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 magnetic resonance spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are given in δ units (ppm) relative to internal standard Me_4Si and refer to $CDCl_3$ solutions. Coupling constants (J) are given in hertz (Hz). ^{13}C NMR spectra were determined by Attached Proton Test (APT) experiments and the resonances were always attributed by proton–carbon heteronuclear chemical shift correlation. A microwave oven Discover (CEM) was used to carry out microwave-assisted reactions. Unless stated otherwise, all materials were obtained from commercial suppliers and used without further purification. All synthesised compounds were assayed as racemic mixtures.

4.2. General method for the synthesis of 5-benzyl-2,4-thiazolidinediones 5

Lithium borohydride (1.54 g, 70.6 mmol) was slowly added to a stirred solution of **2**[29,30] (32 mmol) in pyridine (60 ml) and anhydrous THF (22 ml) at room temperature; effervescence was controlled by the addition rate. When the addition of $LiBH_4$ was completed and effervescence finished, the stirred mixture was refluxed for 3–4 h. A solution of hydrochloric acid 12 N (16 ml) and deionised water (100 ml) was carefully added to the stirred mixture, which was cooled on an ice bath, and then the mixture was heated to reflux for 1 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in chloroform, washed with water (3×100 ml) and dried with anhydrous Na_2SO_4. After evaporation of the solvent in vacuo, the solid was purified by column chromatography on silica gel, using diethyl ether/light petroleum 40–60 °C (4:6) as eluant.

4.2.1. 5-(3-Phenoxybenzyl)-2,4-thiazolidinedione (5a). Yield 43%; mp 98 °C. ^{1}H NMR ($CDCl_3$): δ 3.11 (dd, J = 13.8 and 9.9 Hz, 1H, part A of ABX system, CH_2); 3.52 (dd, J = 13.8 and 3.8 Hz, 1H, part B of ABX system, CH_2); 4.52 (dd, J = 9.9 and 3.8 Hz, 1H, part X of ABX system, 5-CH); 6.88–7.39 (m, 9H, CH arom); 8.68 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 38.4 (CH_2); 53.1 (5-CH); 117.9, 119.1, 119.3, 123.6, 123.8, 129.8 and 130.2 (9 CH arom); 137.7, 156.8 and 157.8 (3 Cq arom); 170.1 and 173.9 (2 CO). Anal. ($C_{16}H_{13}NO_3S$) C, H, N.

4.2.2. 5-(4-Phenoxybenzyl)-2,4-thiazolidinedione (5b). Yield 41%; mp 120–123 °C. ^{1}H NMR ($CDCl_3$): δ 3.18 (dd, J = 14.1 and 9.9 Hz, 1H, part A of ABX system, CH_2); 3.55 (dd, J = 14 and 3.6 Hz, 1H, part B of ABX system, CH_2); 4.57 (dd, J = 9.9 and 3.6 Hz, 1H, part X of ABX system, 5-CH); 7.01–7.53 (m, 9H, CH arom); 8.57 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 37.9 (CH_2); 53.5 (5-CH); 118.9, 119.2, 123.6, 129.8 and 130.6 (9 CH arom); 130.3, 156.8 and 157.0 (3 Cq arom); 170.5 and 174.3 (2 CO). Anal. ($C_{16}H_{13}NO_3S$) C, H, N.

Compound **5b** was also synthesised according to the following procedure: a mixture of **2b** (0.25 g, 0.84 mmol), $LiBH_4$ (0.04 g, 1.85 mmol), pyridine (1.6 ml) and anhydrous THF (0.58 ml), in a hermetically sealed vial, was irradiated with microwaves (300 W, 120 °C, max pressure 300 psi) for 5–6 min. The vial was cooled on an ice bath and a solution of HCl 12 N (0.42 ml) and H_2O (2.6 ml) was slowly introduced by means of a syringe. The mixture was again irradiated with microwaves (300 W, 110 °C, max pressure 300 psi) for 2 min. After evaporation of the solvent in vacuo, the crude solid was treated as above described in the general method for the synthesis of compounds **5**, to give pure **5b** (yield 42%).

4.2.3. 5-(4-Benzyloxybenzyl)-2,4-thiazolidinedione (5c). Yield 42%; mp 115–118 °C. ^{1}H NMR ($CDCl_3$): δ 3.11 (dd, J = 14.1 and 9.3 Hz, 1H, part A of ABX system, CH_2); 3.47 (dd, J = 14.1 and 3.9 Hz, 1H, part B of ABX system, CH_2); 4.51 (dd, J = 9.3 and 3.9 Hz, 1H, part X of ABX system, 5-CH); 5.06 (s, 2H, OCH_2); 6.96 (m, 2H, CH arom); 7.16 (m, 2H, CH arom); 7.35–7.47 (m, 5H, CH arom); 8.72 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 36.7 (CH_2); 53.6 (5-CH); 69.5 (OCH_2); 115.1, 127.5, 128.0, 128.6 and 130.3 (9 CH arom); 130.7, 138.9 and 158.0 (3 Cq arom); 171.3 and 175.0 (2 CO). Anal. ($C_{17}H_{15}NO_3S$) C, H, N.

4.2.4. 5-Biphenyl-4yilmethyl-2,4-thiazolidinedione (5d). Yield 46%; mp 138–140 °C. ^{1}H NMR ($CDCl_3$): δ 3.24 (dd, J = 14.1 and 9.9 Hz, 1H, part A of ABX system, CH_2); 3.65 (dd, J = 14.1 and 3.6 Hz, 1H, part B of ABX system, CH_2); 4.62 (dd, J = 9.9 and 3.6 Hz, 1H, part X of ABX system, 5-CH); 7.34–7.64 (m, 9H, CH arom); 8.36 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 38.3 (CH_2); 53.3 (5-CH); 127.0, 127.4, 127.5, 128.8 and 129.6 (9 CH arom); 134.7, 140.4 and 140.6 (3 Cq arom); 170.1 and 173.9 (2 CO). Anal. ($C_{16}H_{13}NO_2S$) C, H, N.

4.2.5. 5-(3-Methoxybenzyl)-2,4-thiazolidinedione (5e). Yield 40%; mp 115 °C. ^{1}H NMR ($CDCl_3$): δ 3.08 (dd, J = 14.1 and 10.2 Hz, 1H, part A of ABX system, CH_2); 3.55 (dd, J = 14.1 and 3.9 Hz, 1H, part B of ABX system, CH_2); 3.81 (s, 3H, OCH_3); 4.53 (dd, J = 10.2 and 3.9 Hz, 1H, part X of ABX system, 5-CH); 6.78–6.85 (m, 3H, CH arom); 7.26 (dd, J = 7.8 and 7.8 Hz, 1H, CH arom); 9.36 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 38.7 (CH_2); 53.4 (5-CH); 55.2

(OCH_3); 112.9, 114.8, 121.3 and 129.9 (4 CH arom); 137.5 and 159.8 (2 Cq arom); 170.9 and 174.6 (2 CO). Anal. ($C_{11}H_{11}NO_3S$) C, H, N.

4.2.6. 5-(4-Methoxybenzyl)-2,4-thiazolidinedione (5f). Yield 47%; mp 110 °C. ^1H and ^{13}C NMR data were reported in Ref. 33. Anal. ($C_{11}H_{11}NO_3S$) C, H, N.

4.3. General method for the synthesis of (5-benzyl-2,4-dioxothiazolidin-3-yl)acetic acids methyl esters 6

A mixture of 5-benzyl-2,4-thiazolidinedione **5** (17 mmol), methyl bromoacetate (5.2 g, 34 mmol) and potassium carbonate (4.7 g, 34 mmol) in acetone (150 ml) was refluxed for 24 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in chloroform, washed with water (3×100 ml) and dried with anhydrous Na_2SO_4. After evaporation of the solvent, the solid was recrystallized from ethanol providing pure ester **6**.

4.3.1. [2,4-Dioxo-5-(3-phenoxybenzyl)thiazolidin-3-yl]acetic acid methyl ester (6a). Yield 60%; mp 80 °C. ^1H NMR ($CDCl_3$): δ 3.06 (dd, $J = 14.1$ and 10.2 Hz, 1H, part A of ABX system, CH_2); 3.59 (dd, $J = 14.1$ and 3.9 Hz, 1H, part B of ABX system, CH_2); 3.77 (s, 3H, OCH_3); 4.33 (s, 2H, N-CH_2); 4.52 (dd, $J = 10.2$ and 3.9 Hz, 1H, part X of ABX system, 5-CH); 6.88–7.03 (m, 5H, CH arom); 7.15 (m, 1H, CH arom); 7.30–7.39 (m, 3H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.8 (CH_2); 41.9 (N-CH_2); 51.7 (5-CH); 52.9 (OCH_3); 117.9, 119.1, 119.2, 123.6, 123.7, 129.9 and 130.3 (9 CH arom); 138.0, 157.1 and 158.6 (3 Cq arom); 170.1, 170.4 and 173.2 (3 CO). Anal. ($C_{19}H_{17}NO_5S$) C, H, N.

4.3.2. [2,4-Dioxo-5-(4-phenoxybenzyl)thiazolidin-3-yl]acetic acid methyl ester (6b). Yield 94%; mp 70 °C. ^1H NMR ($CDCl_3$): δ 3.10 (dd, $J = 13.8$ and 9.3 Hz, 1H, part A of ABX system, CH_2); 3.58 (dd, $J = 13.8$ and 3.0 Hz, 1H, part B of ABX system, CH_2); 3.76 (s, 3H, OCH_3); 4.33 (s, 2H, N-CH_2); 4.52 (dd, $J = 9.3$ and 3.0 Hz, 1H, part X of ABX system, 5-CH); 6.94–7.34 (m, 9H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.2 (CH_2); 41.9 (N-CH_2); 52.0 (5-CH); 52.8 (OCH_3); 118.9, 119.1, 123.5, 129.8 and 130.5 (9 CH arom); 130.4, 155.2 and 157.0 (3 Cq arom); 170.3, 173.0 and 173.8 (3 CO). Anal. ($C_{19}H_{17}NO_5S$) C, H, N.

4.3.3. [5-(4-Benzyloxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid methyl ester (6c). Yield 80%; mp 86 °C. ^1H NMR ($CDCl_3$): δ 3.07 (dd, $J = 14.4$ and 10.2 Hz, 1H, part A of ABX system, CH_2); 3.56 (dd, $J = 14.4$ and 3.9 Hz, 1H, part B of ABX system, CH_2); 3.78 (s, 3H, OCH_3); 4.33 (s, 2H, N-CH_2); 4.51 (dd, $J = 10.2$ and 3.9 Hz, 1H, part X of ABX system, 5-CH); 5.07 (s, 2H, OCH_2); 6.95 (m, 2H, CH arom); 7.17 (m, 2H, CH arom); 7.34–7.46 (m, 5H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.1 (CH_2); 41.8 (N-CH_2); 52.2 (5-CH); 52.8 (OCH_3); 70.0 (OCH_2); 115.2, 127.5, 128.0, 128.6 and 130.3 (9 CH arom); 130.2, 138.4 and 160.7 (3 Cq arom); 170.0, 172.3 and 172.5 (3 CO). Anal. ($C_{20}H_{19}NO_5S$) C, H, N.

4.3.4. (5-Biphenyl-4-ylmethyl-2,4-dioxothiazolidin-3-yl)acetic acid methyl ester (6d). Yield 80%; mp 95 °C. ^1H NMR ($CDCl_3$): δ 3.14 (dd, $J = 14.4$ and 10.5 Hz, 1H, part A of ABX system, CH_2); 3.66 (dd, $J = 14.4$ and 3.9 Hz, 1H, part B of ABX system, CH_2); 3.76 (s, 3H, OCH_3); 4.34 (s, 2H, N-CH_2); 4.57 (dd, $J = 10.5$ and 3.9 Hz, 1H, part X of ABX system, 5-CH); 7.30–7.37, 7.42–7.46, 7.55–7.59 (3m, 9H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.6 (CH_2); 41.9 (N-CH_2); 51.9 (5-CH); 52.8 (OCH_3); 127.1, 127.5, 127.6, 128.8 and 129.6 (9 CH arom); 135.0, 140.5 and 140.6 (3 Cq arom); 171.6, 172.9 and 173.8 (3 CO). Anal. ($C_{19}H_{17}NO_4S$) C, H, N.

4.3.5. [5-(3-Methoxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid methyl ester (6e). Yield 60%; mp 92 °C. ^1H NMR ($CDCl_3$): δ 3.06 (dd, $J = 14.1$ and 10.5 Hz, 1H, part A of ABX system, CH_2); 3.65 (dd, $J = 14.1$ and 3.6 Hz, 1H, part B of ABX system, CH_2); 3.78 and 3.82 (2s, 6H, 2 OCH_3); 4.35 (s, 2H, N-CH_2); 4.57 (dd, $J = 10.5$ and 3.6 Hz, 1H, part X of ABX system, 5-CH); 6.80–6.86 (m, 3H, CH arom); 7.26 (dd $J = 7.5$ and 7.5 Hz, 1H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.8 (CH_2); 41.8 (N-CH_2); 51.8 (5-CH); 52.8 and 55.2 (2 OCH_3); 113.0, 115.1, 123.3 and 129.9 (4 CH arom); 132.1 and 161.5 (2 Cq arom); 170.8, 171.3 and 173.6 (3 CO). Anal. ($C_{14}H_{15}NO_5S$) C, H, N.

4.3.6. [5-(4-Methoxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid methyl ester (6f). Yield 75%; mp 88 °C. ^1H NMR ($CDCl_3$): δ 3.05 (dd, $J = 13.8$ and 9.9 Hz, 1H, part A of ABX system, CH_2); 3.54 (dd, $J = 13.8$ and 3.9 Hz, 1H, part B of ABX system, CH_2); 3.76 and 3.79 (2s, 6H, 2 OCH_3); 4.31 (s, 2H, N-CH_2); 4.50 (dd, $J = 9.9$ and 3.9 Hz, 1H, part X of ABX system, 5-CH); 6.85 (m, 2H, CH arom); 7.15 (m, 2H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.2 (CH_2); 41.5 (N-CH_2); 51.9 (5-CH); 52.8 and 55.2 (2 OCH_3); 113.9 and 129.9 (4 CH arom); 128.0 and 159.2 (2 Cq arom); 170.4, 171.0 and 173.4 (3 CO). Anal. ($C_{14}H_{15}NO_5S$) C, H, N.

4.4. General method for the synthesis of (5-benzyl-2,4-dioxothiazolidin-3-yl)acetic acids 7

A mixture of ester **6** (10 mmol), glacial AcOH (120 ml) and HCl 12N (30 ml) was refluxed for 1.5 h. After evaporation in vacuo, the residue was refluxed again with AcOH (120 ml) and HCl (30 ml) for 1.5 h. After evaporation to dryness in vacuo, the crude solid was washed with H_2O and recrystallized from ethanol providing pure carboxylic acid **7**.

4.4.1. [2,4-Dioxo-5-(3-phenoxybenzyl)thiazolidin-3-yl]acetic acid (7a). Yield 60%; mp 150 °C. ^1H NMR ($CDCl_3$): δ 3.07 (dd, $J = 14.1$ and 10.2 Hz, 1H, part A of ABX system, CH_2); 3.57 (dd, $J = 14.1$ and 3.9 Hz, 1H, part B of ABX system, CH_2); 4.36 (s, 2H, N-CH_2); 4.53 (dd, $J = 10.2$ and 3.9 Hz, 1H, part X of ABX system, 5-CH); 6.88–7.03 (m, 5H, CH arom); 7.15 (m, 1H, CH arom); 7.29–7.39 (m, 3H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.6 (CH_2); 41.6 (N-CH_2); 51.6 (5-CH); 117.9, 119.1, 119.3, 123.6, 123.8, 129.8 and 130.2 (9 CH arom); 137.7, 156.8 and 157.7 (3 Cq arom);

170.3, 170.7 and 173.0 (3 CO). Anal. ($C_{18}H_{15}NO_5S$) C, H, N.

4.4.2. [2,4-Dioxo-5-(4-phenoxybenzyl)thiazolidin-3-yl]acetic acid (7b). Yield 50%; mp 160 °C. ^{1}H NMR ($CDCl_3$): δ 3.11 (dd, $J = 14.1$ and 9.9 Hz, 1H, part A of ABX system, CH_2); 3.56 (dd, $J = 14.1$ and 3.9 Hz, 1H, part B of ABX system, CH_2); 4.38 (s, 2H, N-CH_2); 4.53 (dd, $J = 9.9$ and 3.9 Hz, 1H, part X of ABX system, 5-CH); 6.94–7.03, 7.10–7.21, 7.32–7.38 (3m, 9H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.0 (CH_2); 41.6 (N-CH_2); 51.9 (5-CH); 118.9, 119.2, 123.6, 129.8 and 130.5 (9 CH arom); 130.3, 156.8 and 157.0 (3 Cq arom); 170.3, 170.9 and 173.1 (3 CO). Anal. ($C_{18}H_{15}NO_5S$) C, H, N.

4.4.3. (5-Biphenyl-4-ylmethyl-2,4-dioxothiazolidin-3-yl)acetic acid (7d). Yield 56%; mp 145 °C. ^{1}H NMR ($CDCl_3$): δ 3.18 (dd, $J = 13.8$ and 9.6 Hz, 1H, part A of ABX system, CH_2); 3.65 (dd, $J = 13.8$ and 3.0 Hz, 1H, part B of ABX system, CH_2); 4.38 (s, 2H, N-CH_2); 4.57 (dd, $J = 9.6$ and 3.0 Hz, 1H, part X of ABX system, 5-CH); 7.30–7.60 (m, 9H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.6 (CH_2); 41.6 (N-CH_2); 51.9 (5-CH); 127.0, 127.5, 127.6, 128.8 and 129.5 (9 CH arom); 134.9, 140.7 and 140.8 (3 Cq arom); 170.4, 171.4 and 172.3 (3 CO). Anal. ($C_{18}H_{15}NO_4S$) C, H, N.

4.4.4. [5-(3-Methoxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid (7e). Yield 57%; mp 88–90 °C. ^{1}H NMR ($CDCl_3$): δ 3.05 (dd, $J = 13.8$ and 10.5 Hz, 1H, part A of ABX system, CH_2); 3.60 (dd, $J = 13.8$ and 3.6 Hz, 1H, part B of ABX system, CH_2); 3.80 (s, 3H, OCH_3); 4.37 (s, 2H, N-CH_2); 4.55 (dd, $J = 10.5$ and 3.6 Hz, 1H, part X of ABX system, 5-CH); 6.81 (m, 3H, CH arom); 7.25 (m, 1H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.8 (CH_2); 41.7 (N-CH_2); 51.8 (5-CH); 55.2 (OCH_3); 113.0, 114.7, 121.3 and 129.9 (4 CH arom); 137.4 and 159.8 (2 Cq arom); 170.5, 170.8 and 173.2 (3 CO). Anal. ($C_{13}H_{13}NO_5S$) C, H, N.

4.4.5. [5-(4-Methoxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid (7f). Yield 55%; mp 84 °C. ^{1}H NMR ($CDCl_3$): δ 3.08 (dd, $J = 13.8$ and 9.6 Hz, 1H, part A of ABX system, CH_2); 3.53 (dd, $J = 13.8$ and 3.9 Hz, 1H, part B of ABX system, CH_2); 3.81 (s, 3H, OCH_3); 4.37 (s, 2H, N-CH_2); 4.53 (dd, $J = 9.6$ and 3.9 Hz, 1H, part X of ABX system, 5-CH); 6.87 (m, 2H, CH arom); 7.16 (m, 2H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.0 (CH_2); 41.6 (N-CH_2); 52.2 (5-CH); 55.3 (OCH_3); 114.3 and 130.3 (4 CH arom); 127.7 and 159.1 (2 Cq arom); 170.5, 170.9 and 173.2 (3 CO). Anal. ($C_{13}H_{13}NO_5S$) C, H, N.

4.4.6. [5-(4-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl]acetic acid (7g). was synthesised as above described, strarting from compound **6c**: yield 60%; mp 130–132 °C. ^{1}H NMR ($CDCl_3$): δ 3.08 (dd, $J = 13.8$ and 9.3 Hz, 1H, part A of ABX system, CH_2); 3.46 (dd, $J = 13.8$ and 3.9 Hz, 1H, part B of ABX system, CH_2); 4.30 (s, 2H, N-CH_2); 4.51 (dd, $J = 9.3$ and 3.9 Hz, 1H, part X of ABX system, 5-CH); 6.76 (m, 2H, CH arom); 7.29 (m, 2H, CH arom). ^{13}C NMR ($CDCl_3$): δ 38.1 (CH_2); 41.7 (N-CH_2); 51.9 (5-CH); 115.8 and 131.9 (4 CH arom); 129.9 and 159.8 (2 Cq arom); 170.2, 170.5 and 172.8 (3 CO). Anal. ($C_{12}H_{11}NO_5S$) C, H, N.

4.5. Enzyme section

NADPH, DL-glyceraldehyde and dithiothreitol (DTT) were purchased from Sigma Chemical Co. DEAE-cellulose (DE-52) was obtained from Whatman. Sorbinil was a gift from Professor L. Costantino, University of Modena (Italy). All other chemicals were commercial samples of good grade. Calf lenses for the purification of ALR2 were obtained locally from freshly slaughtered animals. The enzyme was purified by a chromatographic procedure as previously described.[37] Briefly, ALR2 was released by carving the capsule and the frozen lenses were suspended in potassium phosphate buffer, pH 7, containing 5 mM DTT and stirred in an ice-cold bath for 2 h. The suspension was centrifuged at 4000 rpm at 4 °C for 30 min and the supernatant was subjected to ion exchange chromatography on DE52. Enzyme activity was assayed spectrophotometrically on a Cecil Super Aurius CE 3041 spectrophotometer by measuring the decrease in absorption of NADPH at 340 nm which accompanies the oxidation of β-NADPH catalysed by ALR2. The assay was performed at 37 °C in a reaction mixture containing 0.25 M sodium phosphate buffer, pH 6.8, 0.38 M ammonium sulfate, 0.11 mM NADPH and 4.7 mM DL-glyceraldehyde as substrate in a final volume of 1.5 ml. All inhibitors were dissolved in DMSO. The final concentration of DMSO in the reaction mixture was 1%. To correct for the non-enzymatic oxidation of NADPH, the rate of NADPH oxidation in the presence of all of the reaction mixture components, except the substrate, was subtracted from each experimental rate. Each dose–effect curve was generated using at least three concentrations of inhibitor causing an inhibition between 20% and 80%. Each concentration was tested in duplicate, and IC_{50} values as well as the 95% confidence limits (95% CL) were obtained by using CalcuSyn software for dose effect analysis.[38]

Acknowledgments

The authors acknowledge Schlachthof Salzburg-Bergheim (Austria) (KR Ing. Sebastian Grießner and Mag. Verena Klob) for providing calf lenses.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.08.056.

References and notes

1. Diamond, J. *Nature* **2003**, *423*, 599.
2. Zimmet, P. *Diabetes Metab.* **2003**, *29*(4 Pt. 2), 6S9.

3. King, H.; Aubert, R. E.; Herman, W. H. *Diabetes Care* **1998**, *21*, 1414.
4. Brownlee, M. *Nature* **2001**, *414*, 813.
5. Suzen, S.; Buyukbingol, E. *Curr. Med. Chem.* **2003**, *10*, 1329.
6. Porte, D., Jr.; Schwartz, M. W. *Science* **1996**, *272*, 699.
7. Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. *J. Med. Chem.* **1985**, *28*, 841.
8. Kador, P. F. *Med. Res. Rev.* **1988**, *8*, 325.
9. Yabe-Nishimura, C. *Pharmacol. Rev.* **1998**, *20*, 21.
10. Costantino, L.; Rastelli, G.; Cignarella, G.; Vianello, P.; Barlocco, D. *Exp. Opin. Ther. Patents* **1997**, *7*, 843.
11. Wolffenbuttel, B. H.; van Haeften, T. W. *Drugs* **1995**, *50*, 263.
12. Miyamoto, S. *Chem-Bio Inform. J.* **2002**, *2*, 74.
13. Larson, E. R.; Lipinski, C. A.; Sarges, R. *Med. Res. Rev.* **1988**, *8*, 159.
14. Costantino, L.; Rastelli, G.; Vianello, P.; Cignarella, G.; Barlocco, D. *Med. Res. Rev.* **1999**, *19*, 3.
15. Costantino, L.; Rastelli, G.; Gamberoni, M. C.; Barlocco, D. *Exp. Opin. Ther. Patents* **2000**, *10*, 1245.
16. Castañer, J.; Prous, J. *J. Drugs Future* **1987**, *12*, 336.
17. Sohda, T.; Mizuno, K.; Imamiya, E.; Sugiyama, Y.; Fujita, T.; Kawamatsu, Y. *Chem. Pharm. Bull.* **1982**, *30*, 3580.
18. Willson, T. M.; Cobb, J. E.; Cowan, D. J.; Wiethe, R. W.; Correa, I. D.; Prakash, S. R.; Beck, K. D.; Moore, L. B.; Kliever, S. A.; Lehmann, J. M. *J. Med. Chem.* **1996**, *39*, 665.
19. Reddy, K. A.; Lohray, B. B.; Bhushan, V.; Bajji, A. C.; Reddy, K. V.; Reddy, P. R.; Krishna, T. H.; Rao, I. N.; Jajoo, H. K.; Rao, N. V. S. M.; Chakrabarti, R.; Dileepkumar, T.; Rajagopalan, R. *J. Med. Chem.* **1999**, *42*, 1927.
20. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
21. Jones, T. A.; Sautter, M.; Van Gaal, L. F.; Jones, N. P. *Diabetes Obes. Metab.* **2003**, *5*, 163.
22. Aljabri, K.; Kozak, S. E.; Thompson, D. M. *Am. J. Med.* **2004**, *116*, 230.
23. Moller, D. E. *Nature* **2001**, *414*, 821.
24. Urzhumtsev, A.; Tete-Favier, F.; Mitschler, A.; Barbanton, J.; Barth, P.; Urzhumtseva, L.; Biellmann, J. F.; Podjarny, A.; Moras, D. *Structure* **1997**, *5*, 601.
25. El-Kabbani, O.; Wilson, D. K.; Petrash, J. M.; Quiocho, F. A. *Mol. Vis.* **1998**, *4*, 19.
26. Lee, Y. S.; Chen, Z.; Kador, P. F. *Bioorg. Med. Chem.* **1998**, *6*, 1811.
27. Singh, S. B.; Malamas, M. S.; Hohman, T. C.; Nilakantan, R.; Carper, D. A.; Kitchen, D. *J. Med. Chem.* **2000**, *43*, 1062.
28. Darmanin, C.; Chevreux, G.; Potier, N.; Van Dorsselaer, A.; Hazemann, I.; Podjarny, A.; El-Kabbani, O. *Bioorg. Med. Chem.* **2004**, *12*, 3797.
29. Bruno, G.; Costantino, L.; Curinga, C.; Maccari, R.; Monforte, F.; Nicolò, F.; Ottanà, R.; Vigorita, M. G. *Bioorg. Med. Chem.* **2002**, *10*, 1077.
30. Maccari, R.; Ottanà, R.; Curinga, C.; Vigorita, M. G.; Rakowitz, D.; Steindl, T.; Langer, T. *Bioorg. Med. Chem.* **2005**, 13.
31. Ongoing assays.
32. Bhat, B. A.; Ponnala, S.; Sahu, D. P.; Tiwari, P.; Tripathi, B. K.; Srivastava, A. K. *Bioorg. Med. Chem.* **2004**, *12*, 5857.
33. Giles, R. G.; Lewis, N. J.; Quick, J. K.; Sasse, M. J.; Urquhart, M. W. J.; Youssef, L. *Tetrahedron* **2000**, *56*, 4531.
34. Banik, B. K.; Barakat, K. J.; Wagle, D. R.; Manhas, M. S.; Bose, A. K. *J. Org. Chem.* **1999**, *64*, 5746.
35. Al-Qahtani, M. H.; Cleator, N.; Danks, T. N.; Garman, R. N.; Jones, J. R.; Stefaniak, S.; Morgan, A. D.; Simmonds, A. J. *J. Chem. Res., Synop.* **1998**, 400.
36. (a) Druzgala, P.; Milner, P. G.; Pfister, J. R. PCT Int. Appl., WO 2003024943 A2, publ. date 2003 March 27.; (b) Druzgala, P.; Milner, P. G.; Pfister, J. R. U.S. Pat. Appl. Publ., US 2003236227 A1, publ. date 2003 December 25.
37. Costantino, L.; Rastelli, G.; Vescovini, K.; Cignarella, G.; Vianello, P.; Del Corso, A.; Cappiello, M.; Mura, U.; Barlocco, D. *J. Med. Chem.* **1996**, *39*, 4396.
38. Chou, T.-C.; Hayball, M. P. CalcuSyn software version 1.1.1., Biosoft, Cambridge, UK, 1996.