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Identification of new non-carboxylic acid containing inhibitors of aldose reductase

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ABSTRACT

Non-carboxylic acid containing bioisosteres of (5-arylidene-2,4-dioxothiazolidin-3-yl)acetic acids, which are active as aldose reductase (ALR2) inhibitors, were designed by replacing the carboxylic group with the trifluoromethyl ketone moiety. The in vitro evaluation of the ALR2 inhibitory effects of these trifluoromethyl substituted derivatives led to the identification of two inhibitors effective at low micromolar doses. It was further confirmed that a carboxylic chain on N-3 of the thiazolidinedione scaffold is a determining requisite to obtain the highest efficacy levels; however, it is not essential for the interaction with the target enzyme and it can be replaced by different polar groups, thus obtaining less ionised or unionised inhibitors.

1. Introduction

Diabetes mellitus (DM) is a very common metabolic disease whose actual worldwide prevalence is estimated at about 250 million and expected to rise to 400 million by 2030.[1,2] Chronic hyperglycaemia is the typical metabolic disorder of DM. This condition, through the activation of established biochemical mechanisms, can induce tissue injury, mainly micro- and macrovascular damage which can lead to atherosclerosis, coronary and cerebrovascular pathologies, blindness, renal failure, even in the early stages of the disease.[3,4] These debilitating pathologies represent a serious, and sometimes fatal, health threat for diabetic patients. Effective drugs aimed to prevent or delay the onset and progression of diabetes complications are required and their identification still represents a challenge for the research.

Out of the biochemical mechanisms that have been recognized as being implicated in the etiopathogenesis of hyperglycaemia-induced tissue damage, such as increased intracellular formation of advanced glycation-end products (AGEs), abnormal activation of protein kinase C, increased flux of glucose through both the polyol and the hexosamine pathways, the metabolization of glucose through the polyol pathway plays an important role.[5,6]

Aldose reductase (EC 1.1.1.21, ALR2) is the first and rate-limiting enzyme of the polyol pathway. It catalyses the NADPH-dependent reduction of glucose to sorbitol which, in turn, is oxidized to fructose by sorbitol dehydrogenase with concomitant reduction of NAD^+. Under euglycaemic conditions, ALR2 does not metabolize significant amounts of glucose, due to its low affinity for this substrate. In contrast, under hyperglycaemic conditions, up to about one-third of the total metabolized amount of glucose is reduced by ALR2 in tissues possessing insulin-independent uptake of glucose, such as kidney, lens, retina, peripheral nerves. This leads to intracellular accumulation of sorbitol with a consequent osmotic imbalance, especially in the lens. In addition, increased fructose levels contribute to protein glycation and formation of AGEs which, in turn, can cause pathological changes in the functions of intracellular proteins as well as in signalling pathways. At the same time, NADPH and NAD^+ deprivation causes changes in cellular redox potentials, reduces the activity of other NADPH-dependent enzymes, such as nitric oxide synthase and glutathione reductase, and induces intracellular oxidative stress. These metabolic and biochemical changes result in inflammation, chronic vascular damage, pro-atherogenic effects and decrease in nerve conduction velocity.[5–12]

It has been demonstrated that the increased flux of glucose through the polyol pathway is implicated in the development of diabetic cataracts, nephropathy, retinopathy, neuropathy, coronary damage, atherosclerosis lesions. Moreover, ALR2 inhibitors (ARIs) have been shown to be able to prevent or slow down the progression of certain long-term diabetic disorders.[7,13–16] Therefore, ALR2 inhibition has been considered as an attractive strategy for therapeutic intervention to attenuate the pathogenetic consequences of chronic hyperglycaemia associated with DM, independently of glycaemic control.

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In addition, there is compelling evidence that ALR2 upregulation can also occur under euglycaemic conditions as a result of different factors and it may be involved in the etiology of other pathologies that are not related to DM, such as inflammatory diseases, cancer, renal insufficiency and mood disorders.[10,17,18]

Many ARIs have been reported in the last three decades, many of which belonging to the classes of carboxylic acids (such as epalrestat, lidorestat, tolrestat, zopolrestat, Fig. 1) and cyclic imides, especially spirohydantoins (such as sorbinil, fidarestat, Fig. 1). However, many of them proved to be clinically inadequate, mainly due to adverse effects or low in vivo efficacy. Currently, epalrestat, which has been approved in Japan for the management of diabetic neuropathy, is the only ARI in therapy.[18–23]

In this context, 2,4-thiazolidinediones (2,4-TZDs) have received attention as hydantoin bioisosteres potentially devoid of the phenytoin-like hypersensitivity reactions that have been imputed to the hydantoin ring and caused the withdrawn of sorbinil (Fig. 1) from clinical trials.[24] The discovery of glitazones as oral antidiabetic drugs also encouraged the search for 2,4-TZDs that could be useful for the treatment of both type 2 DM and its long-term complications.[19,22]

In the last few years, we have designed and evaluated numerous 5-arylidene-2,4-thiazolidinedione derivatives (series **1**–**5**, Fig. 1) as ARIs. Many of them have been shown to inhibit bovine lens ALR2 in vitro at low micromolar or submicromolar doses.[25–28] Their structures include features which are essential for ALR2 inhibition, that is, (a) an acidic hydrogen and/or H-bond acceptor groups, which can bind the positively charged polar recognition region of the ALR2 active site formed by Tyr48, His110, Trp111 residues and by the nicotinamide ring of cofactor $NADP^+$, and (b) an aromatic moiety, which can establish hydrophobic interactions with a lipophilic zone of the catalytic cleft localized among Trp111, Thr113, Phe122, Ala299, Leu300, Ser302 and Cys303.[20,22,29–31]

The structure–activity relationships that we have detected so far for these 2,4-TZDs have shown that the presence of the acetic acid chain on N-3 (compounds **3**) is related to the best ALR2 inhibitory effects with IC_{50} values in the submicromolar range.[25–27] Several N-unsubstituted analogues **1** were also found to be good ALR2 inhibitors, although they are from 10 to 100 times less potent than corresponding carboxylic acids **3**. In addition, few analogous methyl esters **2**, although devoid of any acidic functionality, were shown to produce interesting in vitro ALR2 inhibitory effects, sim-

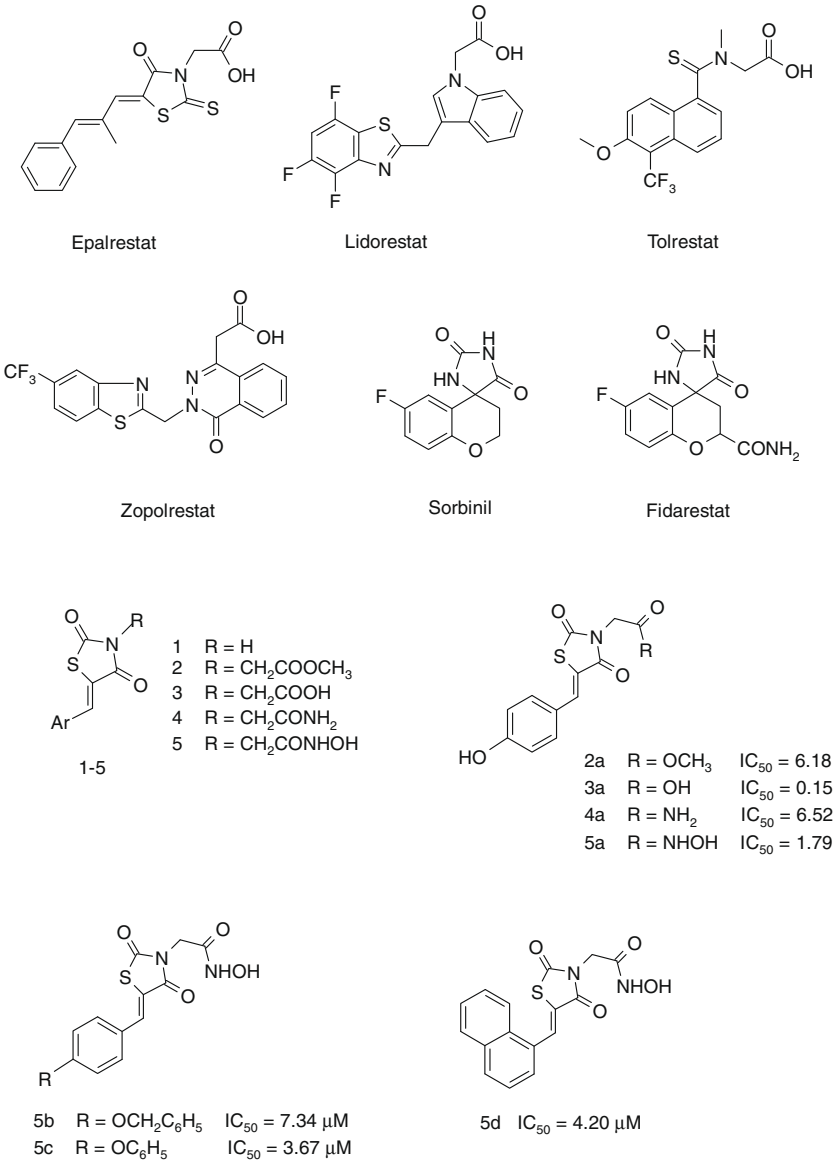
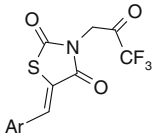


Figure 1. Structures of some ARIs.

ilar to those of N-unsubstituted 2,4-TZDs **1**.[25–27] Among other non-carboxylic acid containing 5-arylidene-2,4-thiazolidinedione derivatives, the 2-[5-(4-hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetamide (**4a**, Fig. 1), the corresponding *N*-hydroxyacetamide (**5a**, Fig. 1) and analogues **5b–d** (Fig. 1) effectively inhibited ALR2 with IC_{50} values in the low micromolar range.[28]

Indeed, among ARIs, carboxylic acids are known as potent in vitro inhibitors. However, their effectiveness has generally been shown to decrease in vivo, probably because of their poor capability to penetrate key target tissues, in particular peripheral nerves.[19–21] Thus, the identification of new low acidity ARIs, which are mainly non-ionised at physiological pH values, would be a desirable goal.[32]

In continuing our search for non-carboxylic acid containing 2,4-TZDs, we selected the trifluoromethyl ketone moiety as a bioisoster of the carboxylic group of acetic acids **3** to obtain new derivatives in which the pharmacophoric elements that we previously defined for 5-arylidene-2,4-thiazolidinediones (i.e., two H-bond acceptor groups and a lipophilic region)[26] were maintained (compounds **6**, Fig. 2). In most of derivatives **6**, the 5-arylidene moiety contained two aromatic rings or a phenyl ring bearing a hydroxy group, since in the previously studied series of 5-arylidene-2,4-thiazolidinediones this was found to be a favourable feature for ALR2 inhibition.[26] Methoxy substituted analogues were also included for comparison. Here we present the synthesis and evaluation of the in vitro ALR2 inhibitory activity of these new non-carboxylic acid containing derivatives.



6 a-j

a Ar = 3-OC_6H_5-C_6H_4
b Ar = 4-OC_6H_5-C_6H_4
c Ar = 4-$OCH_2C_6H_5$-C_6H_4
d Ar = 4-C_6H_5-C_6H_4
e Ar = 1-Naphthyl
f Ar = 2-Naphthyl
g Ar = 3-OCH_3-C_6H_4
h Ar = 4-OCH_3-C_6H_4
i Ar = 3-OH-C_6H_4
j Ar = 4-OH-C_6H_4

Figure 2. General structure of the newly synthesised 2,4-TZDs.

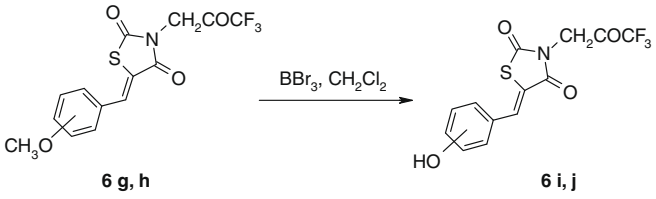
2. Chemistry

The synthetic route which was followed to obtain the designed trifluoromethyl substituted derivatives (Scheme 1) started from the already reported synthesis of 2,4-TZDs **1**, which were N-alkylated by the reaction with methyl bromoacetate to give esters **2**.[25,26] These latter were hydrolysed to acids **3** in acidic medium and, subsequently, converted into the corresponding chlorides **7** by reacting with thionyl chloride. According to a reported procedure,[33] the reaction of compounds **7** with trifluoroacetic anhydride and pyridine provided 5-arylidene-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinediones **6** (Scheme 1). 5-Hydroxybenzylidene substituted derivatives **6i, j** were obtained by the reaction of the corresponding methoxybenzylidene analogues with boron tribromide (Scheme 2).

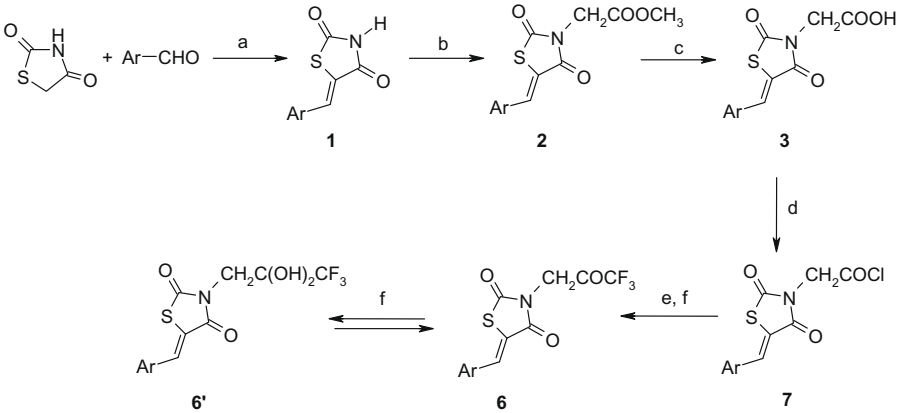
Mass spectra of all compounds **6** showed, along with the molecular peak, a diagnostic intense peak (which is often the base peak) due to the loss of a fragment with $m/z = 181$ corresponding to $CON(CH_2COCF_3)CO$. Fragmentation modes by loss of CF_3 or $COCF_3$ were also detected which, however, produced weak peaks (generally with relative abundance $\leqslant 2\%$).

It is well-known that trifluoromethyl ketones can be readily hydrated. In fact, analogously to what was observed by other authors for different triflouromethyl ketones,[33,34] compounds **6** were found to exist in an equilibrium with the corresponding hydrates (**6′**, Scheme 1) under proper conditions.

In ^1H NMR spectra recorded in $CDCl_3$ solution, signals attributable to hydrates **6′**, along with those due to related ketones **6**, were



Scheme 2. Synthesis of compounds **6i**, **j**.



(a) $C_5H_{11}N$, C_2H_5OH, Δ; (b) $BrCH_2COOCH_3$, K_2CO_3, acetone, Δ; (c) AcOH, HCl, Δ; (d) $SOCl_2$, Δ; (e) $(CF_3CO)_2O$, pyridine, anhydrous Et_2O, r.t.; (f) H_2O.

Scheme 1. General method for the synthesis of 5-arylidene-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinediones **6a–h**.

present (ratios **6**:**6′** ranged from 1:1.5 to 1:3 at 25 °C). Two singlets at 4.25–4.30 ppm and 4.91–4.96 ppm were diagnostic, which are attributable to the resonance of the methylene protons of hydrates 6′ and ketones **6**, respectively. In addition, two singlets, which were due to the resonance of the two different 5-methylidene protons of species **6** and **6′**, were present in the range from 8.00 to 8.05 ppm.

When ^{1}H NMR spectra were recorded in hygroscopic DMSO-d_6, we observed only one set of signals, which can be attributed to hydrates **6′**. In this case, the resonance of the methylene protons gave rise to a singlet at 3.88–3.94 ppm, whereas the signal attributable to the methylidene proton appeared in the range between 7.82 ppm and 8.55 ppm.

^{13}C NMR spectra of compounds **6** were recorded in DMSO-d_6 solution, with the exception of few cases, because of the generally moderate solubility of these derivatives in $CDCl_3$. ^{13}C NMR spectra in DMSO-d_6 solution showed a diagnostic quartet at 92.0–92.4 ppm (J_{CF} = 30–32 Hz) that was due to the resonance of C-2 of the dihydroxypropylic side chain of geminal diols **6′**, whereas no signal attributable to the carbonyl group of corresponding compounds **6** was detected. Another quartet was present at 121.2–124.0 ppm (J_{CF} = 277.5–288.0 Hz) which was due to the resonance of the trifluoromethyl group (see Section 4). In the $CDCl_3$ solution of compound **6d**, besides the signals attributable to hydrate **6′d**, a quartet at 183 ppm (J_{CF} = 37.5 Hz) was observed, which was due to the resonance of the carbonyl group adjacent to the CF_3 group of the ketone. In addition, two distinct signals were present at 44.6 ppm and 45.0 ppm, which were originated by the resonance of the methylene carbon atoms of compounds **6d** and **6′d**.

Variable temperature NMR experiments in $CDCl_3$ solution confirmed the existence of an equilibrium between compounds **6** and **6′**, which was more and more shifted towards the hydrate form as temperature was increased whereas it was restored by cooling.

The configuration *Z* of the exocyclic C=C bond of compounds **6** was assigned on the analogy of that of previously reported thiazolidinones, which was determined by means of X-ray diffractometry,[25,35] as well as on the bases of the NMR data.

3. Results and discussion

Compounds **6** were evaluated for their ability to inhibit the in vitro reduction of D,L-glyceraldehyde by highly purified ALR2 from bovine lenses, using sorbinil as a reference drug (Table 1).

On the basis of the above reported findings, it is reasonable to expect that hydrates **6′** are the predominant species under the experimental conditions of the enzymatic assay as well as in the biological environment. Moreover, given their calculated pK_a values (7.94–7.95),[36] geminal diols **6′** are expected to be ionised for 22–23% at physiological pH values, in comparison with ketones **6** which are completely non-ionised. This aroused our interest with regard to their inhibitory activity since, analogously to *N*-hydroxyacetamides **5** (calculated pK_a values ranging from 8.64 to 8.75),[28] compounds **6′** could bind ALR2 in their deprotonated form which can be stabilized by the positive charge of $NADP^+$ and by hydrogen bonds donated by the amino acid residues lining the polar recognition region of the ALR2 active site.

Table 1
In vitro bovine lens ALR2 inhibitory activity of compounds **6**/**6′**

Compd	Ar	IC_{50}[a]
6/**6′a**	3-OC_6H_5–C_6H_4	64%
6/**6′b**	4-OC_6H_5–C_6H_4	8.3 (3.0–23.4)
6/**6′c**	4-$OCH_2C_6H_5$–C_6H_4	N.D.
6/**6′d**	4-C_6H_5–C_6H_4	45%
6/**6′e**	1-Naphthyl	52%
6/**6′f**	2-Naphthyl	59%
6/**6′g**	3-OCH_3–C_6H_4	23.3 (13.8–39.3)
6/**6′h**	4-OCH_3–C_6H_4	17.4 (12.5–24.1)
6/**6′i**	3-OH–C_6H_4	12.31 (9.65–14.28)
6/**6′j**	4-OH–C_6H_4	3.0 (2.2–4.0)
Sorbinil		2.0 (1.7–3.5)

N.D. = not detectable.
[a] IC_{50} (μM) (95% CL) or %inhibition at 25 μM.

Out of the tested compounds, the 5-(4-hydroxybenzylidene) substituted derivative (**6**/**6′j**) was the most active ARI, with an IC_{50} value (3.0 μM) very similar to that of sorbinil (Table 1). Although 20-fold less potent than the [5-(4-hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (**3a**, Fig. 1),[26] **6**/**6′j** was twice more active than amide **4a** and almost as effective as *N*-hydroxyacetamide **5a** (Fig. 1).[28] The 3-hydroxybenzylidene isomer (**6**/**6′i**) also showed a reasonable IC_{50} value (12.31 μM), but it was fourfold less effective (Table 1). The replacement of the hydroxy group of both compounds **6**/**6′i** and **6**/**6′j** with a methoxy one led to less active derivatives (**6**/**6′g** and **6**/**6′h**, respectively) (Table 1) and, in particular, the loss in potency was more marked for the *para* substituted analogue than for the *meta* substituted isomer.

Compound **6**/**6′b** stood out of 2,4-TZDs **6**/**6′a–f**, which possess two aromatic rings in their 5-arylidene moiety, with its IC_{50} value of 8.3 μM (Table 1), whereas analogues **6**/**6′a, d–f** produced from 45% to 64% ALR2 inhibition at 25 μM dose. The 4-benzyloxybenzylidene derivative (**6**/**6′c**) strongly affected NADPH oxidation, making impossible the exact evaluation of its IC_{50} value, which, however, appeared to be higher than 9 μM.

On the whole, the tested trifluoromethyl derivatives **6**/**6′** resulted to be significantly more potent ARIs than acetamides **4**, whereas their inhibitory effects were generally less marked than those of corresponding *N*-hydroxyacetamides **5**. In addition, in comparison with the corresponding N-unsubstituted 2,4-TZDs,[25,26] compounds **6**/**6′b**, **h** and **j**, which bear different groups linked to an oxygen atom in the position 4 of the benzylidene ring, were found to be from 2.4-fold to 4.5-fold more effective ARIs, whereas their *meta* substituted isomers **6**/**6′a**, **g** and **i** resulted to be less active.

Moreover, the comparison of the inhibitory potency of *para* substituted analogues **6**/**6′b**, **h** and **j** showed an efficacy order **6**/**6′j** > **6**/**6′b** > **6**/**6′h**. Thus, the replacement of the phenoxy group of **6**/**6′b** with a methoxy one proved to be detrimental since it halved the inhibitory effectiveness (compound **6**/**6′h**), whereas, in contrast, its replacement with a hydroxy group provided a threefold gain in potency (compound **6**/**6′j**). The removal of the oxygen bridge in the *para* position of the 5-benzylidene moiety produced a marked decrease in potency (compound **6**/**6′d**).

These data are in accordance with the SARs that we previously observed for 5-arylidene-2,4-thiazolidinediones active as ARIs.[26,28] In fact, analogously to **6**/**6′j**, the 5-(4-hydroxybenzylidene) moiety led to 2,4-TZDs of series **1–5** also endowed with good ALR2 inhibitory activity. The above-mentioned phenol derivative **4a** (Fig. 1) was the only compound out of previously evaluated acetamides **4** which showed an appreciable affinity towards ALR2.[28] The corresponding *N*-hydroxyamide (compound **5a**, Fig. 1) was also found to be the most effective inhibitor in the series of *N*-hydroxyacetamides that we investigated.[28] Analogously, in the class of (5-arylidene-2,4-dioxothiazolidin-3-yl)acetic acids **3**, 5-(4-hydroxybenzylidene) substituted derivative **3a** (Fig. 1) is one of the most potent ARIs.[26] A similar observation can be made for methyl esters **2**, among which the 4-hydroxybenzylidene analogue (**2a**, Fig. 1) was one of few derivatives with a micromolar IC_{50} value.[26] A reasonable explanation for the appreciable affinity of these 5-(4-hydroxybenzylidene) substituted derivatives towards ALR2 emerged from our recent docking results that retrieved a favourable binding mode in which the deprotonated phenol group interacts with the positively charged polar recognition region of the ALR2 catalytic centre, in particular with Tyr48 and His110, whereas the side chain on N-3 and the carbonyl group at position

2 of the thiazolidinedione ring can establish hydrogen bonds with Leu300, Leu301 and Ser302 in the lipophilic pocket of the enzyme. Thus, these compounds appeared to bind ALR2 in a flipped pose compared with the more generally expected one which, however, was shown to be adopted by more lipophilic analogues devoid of any acidic group in the 5-arylidene moiety.[28] Therefore, it is reasonable to assume that the wide lipophilic arylidene moiety of 4-phenoxybenzylidene derivative **6**/**6′b** fits the lipophilic pocket of the ALR2 active site, whereas the acetic chain on N-3 interacts with the polar recognition region. Accordingly, the replacement of the distal phenyl ring of **6**/**6′b** with a methyl group led to a less lipophilic analogue (**6**/**6′h**) which can interact less effectively with the lipophilic region of the active site.

The results of the in vitro testing here reported further confirmed the SAR picture that we have detected so far. In fact, the presence of a carboxylic chain on N-3 of the thiazolidinedione scaffold is a determining requisite to obtain the highest ALR2 inhibition levels. However, it is not essential for the inhibition of the enzyme, provided that it is replaced by groups that can effectively interact with the ALR2 polar recognition region, such as the *N*-hydroxycarboxamide or trifluoromethyl ketone groups.

The in vitro evaluation of this series of 5-arylidene-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinediones as ARIs allowed the identification of two new non-carboxylic acid containing 5-arylidene-2,4-thiazolidinedione derivatives (**6**/**6′b** and **6**/**6′j**) that are active at low micromolar doses. This work is part of a project which is targeted to identify new ARIs endowed with favourable pharmacological profiles. The best inhibitors selected among compounds of series **1**–**6**, which contain groups having different degrees of ionization on the critical portions of the 5-arylidene-2,4-thiazolidinedione skeleton, will be included in further studies to assess their in vivo efficacy.

4. Experimental

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 DMSO-d_6 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. Mass spectra were recorded on a Shimadzu GC17A/MSQP5050 spectrometer.

Unless stated otherwise, all materials were obtained from commercial suppliers and used without further purification.

4.2. General method for the synthesis of 5-arylidene-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinediones 6a–h

(5-Arylidene-2,4-dioxothiazolidin-3-yl)acetic acids **3** were obtained according to a reported procedure.[25,26] Acid **3** (1.4 mmol) was dissolved in 5–6 ml of thionyl chloride and the reaction mixture was refluxed for 1 h. The crude mixture was evaporated under reduced pressure to dryness, providing pure compound **7**. Acid chloride **7** (1.5 mmol) was dissolved in anhydrous diethyl ether (7 ml). The ether solution of **7** and anhydrous pyridine (0.95 g, 12 mmol) were added to a solution of trifluoroacetic anhydride (1.9 g, 9 mmol) in anhydrous diethyl ether (7 ml). The reaction mixture was stirred at room temperature for 2 h and the reaction course was monitored by means of TLC. Then H_2O (5 ml) was added cautiously and the mixture was stirred until CO_2 disappeared. The mixture was poured into H_2O (70 ml) and extracted with diethyl ether. The organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure to provide compound **6**.

^{1}H and ^{13}C NMR data reported below for all compounds **6** refer to DMSO-d_6 solutions, in which (as described above) only signals due to the hydrate forms (**6′**) were detected.

4.2.1. (*Z*)-5-[(3-Phenoxyphenyl)methylidene]-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6a)

Yield 34%; mp 93–95 °C ^{1}H NMR (DMSO-d_6): δ 3.88 (s, 2H, NCH_2); 7.07–7.22 (m, 5H, CH arom); 7.36–7.58 (m, 4H, CH arom); 7.97 (s, 1H, CH methylidene). ^{13}C NMR (DMSO-d_6): δ 45.3 (NCH_2); 92.0 (q J_{CF} = 30.75 Hz, $C(OH)_2$); 119.4, 119.8, 120.7, 124.7, 125.3, 130.7, 131.6, 132.5 (CH arom + CH methylidene); 122.8 (C-5); 123.7 (q J_{CF} = 285.0 Hz, CF_3); 135.4, 156.3, 158.1 (Cq arom); 165.6, 167.0 (C=O). MS (EI), *m/z* (%): 407 (63) [M^+], 226 (100). Anal. Calcd for $C_{19}H_{12}F_3NO_4S$: C, 56.02; H, 2.97; N, 3.44. Found: C, 56.37; H, 3.21; N, 3.46.

4.2.2. (*Z*)-5-[(4-Phenoxyphenyl)methylidene]-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6b)

Yield 23%; mp 128–131 °C ^{1}H NMR (DMSO-d_6): δ 3.90 (s, 2H, NCH_2); 7.10–7.25 (m, 5H, CH arom); 7.44 (m, 2H, CH arom); 7.63 (m, 2H, CH arom); 7.89 (s, 1H, CH methylidene). ^{13}C NMR (DMSO-d_6): δ 45.5 (NCH_2); 92.3 (q J_{CF} = 31.0 Hz, $C(OH)_2$); 119.1, 120.6, 125.4, 131.1, 132.9, 133.1 (CH arom + CH methylidene); 120.3 (C-5); 123.9 (q J_{CF} = 285.0 Hz, CF_3); 128.5, 155.9, 159.8 (Cq arom); 166.2, 167.5 (C=O). MS (EI), *m/z* (%): 407 (52) [M^+], 226 (100). Anal. Calcd for $C_{19}H_{12}F_3NO_4S$: C, 56.02; H, 2.97; N, 3.44. Found: C, 56.21; H, 2.78; N, 3.51.

4.2.3. (*Z*)-5-[(4-Benzyloxyphenyl)methylidene]-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6c)

Yield 37%; mp 118–120 °C. ^{1}H NMR (DMSO-d_6): δ 3.90 (s, 2H, NCH_2); 5.17 (s, 2H, OCH_2); 7.17–7.49 (m, 7H, CH arom); 7.59 (m, 2H, CH arom); 7.86 (s, 1H, CH methylidene). ^{13}C NMR (DMSO-d_6): δ 45.5 (NCH_2); 70.4 (OCH_2); 92.4 (q J_{CF} = 31.35 Hz, $C(OH)_2$); 116.5, 117.8, 129.2, 132.9, 133.5, 134.7 (CH arom + CH methylidene); 123.7 (q J_{CF} = 285.0 Hz, CF_3); 124.7 (C-5); 126.4, 155.1, 158.6 (Cq arom); 166.2, 167.4 (C=O). MS (EI), *m/z* (%): 421 (15) [M^+], 91 (100). Anal. Calcd for $C_{20}H_{14}F_3NO_4S$: C, 57.01; H, 3.35; N, 3.32. Found: C, 57.21; H, 3.45; N, 3.03.

4.2.4. (*Z*)-5-[(4-Phenylphenyl)methylidene]-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6d)

Yield 30%; mp 184–186 °C. ^{1}H NMR (DMSO-d_6): δ 3.92 (s, 2H, NCH_2); 7.30 (br s., 2H, 2 OH, exchangeable with D_2O); 7.41–7.52 (m, 3H, CH arom); 7.71–7.76 (m, 4H, CH arom); 7.86 (m, 2H, CH arom); 7.97 (s, 1H, CH methylidene). ^{13}C NMR (DMSO-d_6): δ 45.3 (NCH_2); 92.1 (q J_{CF} = 30.75 Hz, $C(OH)_2$); 121.6 (C-5); 123.7 (q J_{CF} = 287.2 Hz, CF_3); 127.4, 128.0, 128.8, 129.6, 131.3, 132.8 (CH arom + CH methylidene); 132.6, 139.3, 142.5 (Cq arom); 165.9, 167.2 (C=O). MS (EI), *m/z* (%): 391 (61) [M^+], 210 (100). Anal. Calcd for $C_{19}H_{12}F_3NO_3S$: C, 58.31; H, 3.09; N, 3.58. Found: C, 58.57; H, 2.89; N, 3.33.

4.2.5. (*Z*)-5-(Naphthalen-1-ylmethylidene)-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6e)

Yield 35%; mp 144–145 °C. ^{1}H NMR (DMSO-d_6): δ 3.94 (s, 2H, NCH_2); 7.62–7.72 (m, 4H, CH arom); 8.02–8.13 (m, 3H, CH arom); 8.55 (s, 1H, CH methylidene). ^{13}C NMR (DMSO-d_6): δ 45.2 (NCH_2); 92.0 (q J_{CF} = 30.5 Hz, $C(OH)_2$); 123.6 (q J_{CF} = 277.5 Hz, CF_3); 123.9, 126.1, 126.8, 127.3, 128.0, 129.4, 130.1, 131.4 (CH arom + CH methylidene); 125.4 (C-5); 130.7, 131.6, 133.8 (Cq arom); 165.4,

166.2 (C=O). MS (EI), *m/z* (%): 365 (58) [M^+], 184 (100). Anal. Calcd for $C_{17}H_{10}F_3NO_3S$: C, 55.89; H, 2.76; N, 3.83. Found: C, 56.01; H, 3.00; N, 3.59.

4.2.6. (*Z*)-5-(Naphthalen-2-ylmethylidene)-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6f)

Yield 34%; mp 183–186 °C. ^{1}H NMR (DMSO-d_6): δ 3.92 (s, 2H, NCH_2); 7.25 (br s., 2H, 2 OH, exchangeable with D_2O); 7.60–8.07 (m, 7H, CH arom); 8.22 (s, 1H, CH methylidene). ^{13}C NMR (DMSO-d_6): δ 45.3 (NCH_2); 92.0 (q J_{CF} = 30.75 Hz, $C(OH)_2$); 122.0 (C-5); 123.7 (q J_{CF} = 288 Hz, CF_3); 126.4, 127.6, 128.2, 128.6, 129.4, 131.4, 132.3, 133.1 (CH arom + CH methylidene); 131.1, 132.2, 133.8 (Cq arom); 165.8, 167.8 (C=O). MS (EI), *m/z* (%): 365 (53) [M^+], 184 (100). Anal. Calcd for $C_{17}H_{10}F_3NO_3S$: C, 55.89; H, 2.76; N, 3.83. Found: C, 56.12; H, 3.07; N, 3.48.

4.2.7. (*Z*)-5-[(3-Methoxyphenyl)methylidene]-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6g)

Yield 52%; mp 120–122 °C. ^{1}H NMR (DMSO-d_6): δ 3.80 (s, 3H, OCH_3); 3.90 (s, 2H, NCH_2); 7.07 (d J = 8.1 Hz, 1H, CH arom); 7.19–7.25 (m, 2H, CH arom); 7.46 (dd J = 8.1 and 8.1 Hz, 1H, CH arom); 7.90 (s, 1H, CH methylidene). ^{13}C NMR (DMSO-d_6): δ 45.5 (NCH_2); 56.1 (OCH_3); 92.3 (q J_{CF} = 30.75 Hz, $C(OH)_2$); 116.2, 117.2, 122.5, 131.3, 133.4 (CH arom + CH methylidene); 121.2 (q J_{CF} = 277.5 Hz, CF_3); 122.4 (C-5); 135.1, 160.4 (Cq arom); 166.1, 167.5 (C=O). MS (EI), *m/z* (%): 345 (58) [M^+], 164 (100). Anal. Calcd for $C_{14}H_{10}F_3NO_4S$: C, 48.70; H, 2.92; N, 4.06. Found: C, 48.39; H, 3.11; N, 3.96.

4.2.8. (*Z*)-5-[(4-Methoxyphenyl)methylidene]-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6h)

Yield 35%; mp 112–113 °C. ^{1}H NMR (DMSO-d_6): δ 3.82 (s, 3H, OCH_3); 3.91 (s, 2H, NCH_2); 7.10 (m, 2H, CH arom); 7.22 (br s, 2H, 2 OH, exchangeable with D_2O); 7.61 (m, 2H, CH arom); 7.88 (s, 1H, CH methylidene). ^{13}C NMR (DMSO-d_6): δ 45.6 (NCH_2); 56.4 (OCH_3); 92.3 (q J_{CF} = 30.0 Hz, $C(OH)_2$); 115.8, 133.0, 133.5 (CH arom + CH methylidene); 118.8 (C-5); 124.0 (q J_{CF} = 287.7 Hz, CF_3); 126.3, 159.7 (Cq arom); 166.3, 168.3 (C=O). MS (EI), *m/z* (%): 345 (59) [M^+], 164 (100). Anal. Calcd for $C_{14}H_{10}F_3NO_4S$: C, 48.70; H, 2.92; N, 4.06. Found: C, 48.51; H, 3.17; N, 4.12.

4.2.9. (*Z*)-5-[(3-Hydroxyphenyl)methylidene]-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6i)

Boron tribromide (1.74 g, 7 mmol) was added to a solution of **6g** (0.4 g, 1.1 mmol) in dichloromethane on an ice bath and the reaction mixture was stirred for 2 h. Then methanol was added and the mixture was evaporated under reduced pressure to dryness. The crude solid was dissolved in ethyl acetate and the solution was washed with H_2O, dried with anhydrous Na_2SO_4 and evaporated under reduced pressure to provide pure compound **6i**. Yield 84%; mp 130–132 °C. ^{1}H NMR (DMSO-d_6): δ 3.90 (s, 2H, NCH_2); 6.88 (d J = 8.1 Hz, 1H, CH arom); 7.00 (s, 1H, CH arom); 7.06 (d J = 7.8 Hz, 1H, CH arom); 7.25 (br s, 2H, 2 OH, exchangeable with D_2O); 7.34 (dd J = 8.1 and 7.8 Hz, 1H, CH arom); 7.82 (s, 1H, CH methylidene); 9.87 (br s, 1H, OH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): δ 45.3 (NCH_2); 92.1 (q J_{CF} = 30.0 Hz, $C(OH)_2$); 116.5, 118.4, 121.8, 130.9, 133.5 (CH arom + CH methylidene); 123.7 (q J_{CF} = 287.3 Hz, CF_3); 125.6 (C-5); 134.7, 158.5 (Cq arom); 165.9, 167.4 (C=O). MS (EI), *m/z* (%): 331 (41) [M^+], 150 (100). Anal. Calcd for $C_{13}H_8F_3NO_4S$: C, 47.13; H, 2.43; N, 4.23. Found: C, 46.87; H, 2.78; N, 4.02.

4.2.10. (*Z*)-5-[(4-Hydroxyphenyl)methylidene]-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (6j)

The procedure was the same as reported for compound **6i**, starting from (*Z*)-5-[(4-methoxyphenyl)methylidene]-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinedione (**6h**). Yield 75%; mp 148–151 °C. ^{1}H NMR (DMSO-d_6): δ 3.90 (s, 2H, NCH_2); 6.91 (m, 2H, CH arom); 7.24 (br s, 2H, 2 OH, exchangeable with D_2O); 7.48 (m, 2H, CH arom); 7.82 (s, 1H, CH methylidene); 10.37 (br s, 1H, OH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): δ 45.2 (NCH_2); 92.0 (q J_{CF} = 30.0 Hz, $C(OH)_2$); 117.1 (C-5); 116.9, 132.9, 133.7 (CH arom + CH methylidene); 123.7 (q J_{CF} = 287.3 Hz, CF_3); 124.4, 160.5 (Cq arom); 166.1, 167.5 (C=O). MS (EI), *m/z* (%): 331 (40) [M^+], 150 (100). Anal. Calcd for $C_{13}H_8F_3NO_4S$: C, 47.13; H, 2.43; N, 4.23. Found: C, 46.91; H, 2.69; N, 3.98.

4.3. Determination of the in vitro aldose reductase inhibition

Bovine eyes were obtained from a local abattoir soon after slaughtering; lenses were removed and kept frozen at −20 °C until used. Aldose reductase was purified from bovine lens by an ionic exchange chromatographic step on DE-52 and an affinity chromatographic step on Matrex Orange A.[37] The final enzyme preparation (0.93 U/mg specific activity) was stored at 4 °C in 10 mM sodium phosphate buffer pH 7.0 supplemented with 2 mM dithiothreitol.

The enzyme activity was measured at 37 °C, using 4.67 mM D,L-glyceraldehyde as substrate in 0.25 M sodium phosphate, pH 6.8, 0.38 M ammonium sulfate, 0.11 mM NADPH, and 0.5 mM EDTA. One Unit of enzyme activity is the amount of enzyme that catalyses the oxidation of 1 μmol/min of NADPH in the above assay conditions.

The sensitivity of aldose reductase to different compounds was tested in the above assay conditions in the presence of inhibitors dissolved at proper concentration in DMSO. The concentration of DMSO in the assay mixture was kept constant at 1% (v/v). IC_{50} values (the concentration of the inhibitor required to produce a 50% inhibition of the enzyme catalyzed reaction) were determined by non linear regression analysis by fitting the data to the equation describing one site competition in a log dose–inhibition curve. Each log dose–inhibition curve was generated using at least five concentrations of inhibitor (causing an inhibition between 20% and 80%) and each concentration was tested at least in triplicate. The 95% confidence limits (95% CL) were calculated using GraphPad Prism software.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.016.

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