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Evaluation of in vitro aldose redutase inhibitory activity of 5-arylidene-2,4-thiazolidinediones

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Abstract—A number of 5-arylidene-2,4-thiazolidinediones containing a hydroxy or a carboxymethoxy group in their 5-benzylidene moiety have been synthesised and evaluated as in vitro aldose reductase (ALR2) inhibitors. Most of them exhibited strong inhibitory activity, with IC_{50} values in the range between 0.20 and 0.70 μM. Molecular docking simulations into the ALR2 active site highlighted that the phenolic or carboxylic substituents of the 5-benzylidene moiety can favourably interact, in alternative poses, either with amino acid residues lining the lipophilic pocket of the enzyme, such as Leu300, or with the positively charged recognition region of the ALR2 active site.

Diabetes mellitus (DM) is a common chronic metabolic disease characterized by hyperglycaemia and various metabolic imbalances; its prevalence is about 6% worldwide and the number of cases, presently estimated at more than 150 million, is predicted to double by 2025.[1–3]

DM is always associated with serious long-term complications, such as neuropathy, nephropathy, retinopathy, cataracts, accelerated atherosclerosis and increased risk of myocardial infarction, stroke and limb amputation, which negatively affect the quality of life and life expectancy of diabetic patients.[4,5] These pathologies are the consequences of microvascular and macrovascular damage induced, at least in part, by hyperglycaemia.[6–10] Despite the availability of different insulin preparations and oral antidiabetic drugs, glycaemic levels cannot be completely normalized in diabetic patients and, thus, the onset of long-term complications is unavoidable.[11] Therefore, the prevention and control of these pathologies is still a challenging problem in the therapeutic treatment of diabetes.

The increased flux of glucose through the polyol pathway, which occurs under conditions of hyperglycaemia in tissues possessing insulin-independent glucose transport (retina, lenses, kidney, nerve), has been recognized to play a major role in the development of diabetic complications.[6,7,9–15]

Aldose reductase (EC 1.1.1.21, ALR2) catalyses the NADPH-dependent reduction of glucose to sorbitol in the first step of this metabolic pathway. It has received great attention as a target enzyme in the search for drugs able to prevent or delay the onset and progression of diabetic complications, independently of glycaemic levels.

Over the last three decades, numerous ALR2 inhibitors (ARIs) have been identified; most of them belong to either carboxylic acid (such as epalrestat; Fig. 1) or hydantoin (such as sorbinil, fidarestat; Fig. 1) classes of compounds.[9,11,13,14,16] However, many of the clinically tested ARIs proved to be inadequate as drug candidates because of adverse pharmacokinetics, toxic side-effects or low efficacy. At present, epalrestat is the only ARI available on the market.[9,11,17]

In this context, 2,4-thiazolidinediones (2,4-TZDs) have aroused interest as hydantoin bioisosteres that are potentially devoid of the hypersensitivity reactions related to the hydantoin ring, which caused the

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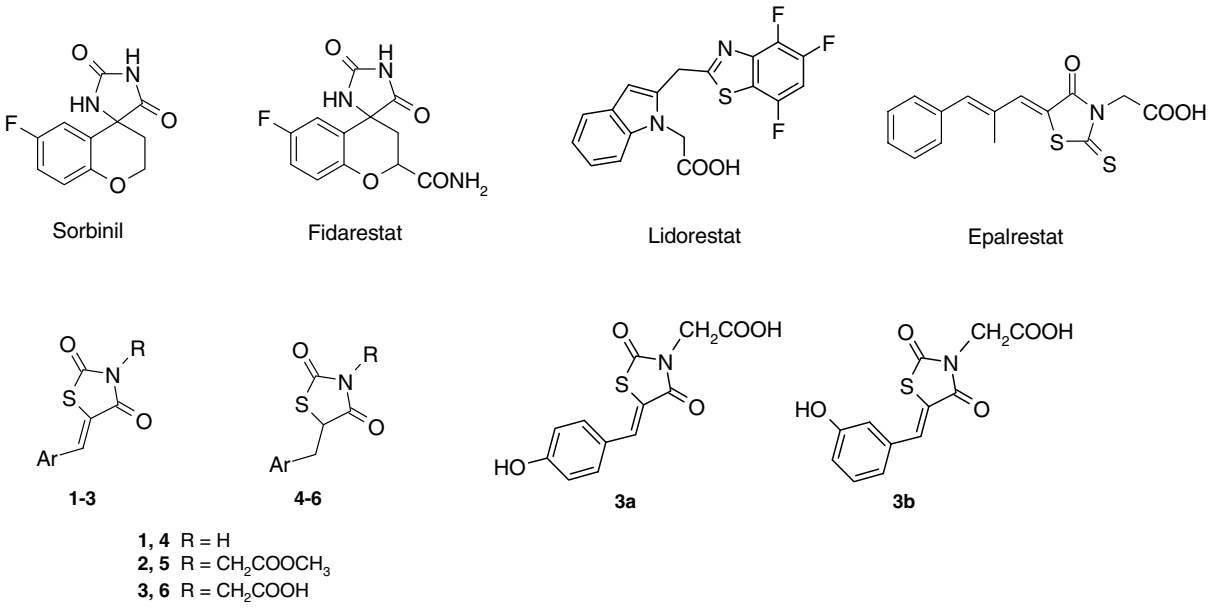


Figure 1.

withdrawal of sorbinil from clinical trials. In particular, several 2,4-TZDs have been reported or patented because they were recognized as having an antihyperglycaemic effect and ALR2 inhibitory activity.[9,11,13]

Over the last few years we have synthesised numerous 2,4-thiazolidinediones (**1**–**6**) (Fig. 1) designed and in vitro assayed as ARIs.[18–20] They possess the structural requisites essential for ALR2 inhibition: (a) an acidic hydrogen and 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 $NADP^+$; (b) an aromatic moiety, which can establish hydrophobic interactions with a lipophilic contact zone of the catalytic cleft lined by Leu300 and Trp111.[9,11,13,14,16,21–23]

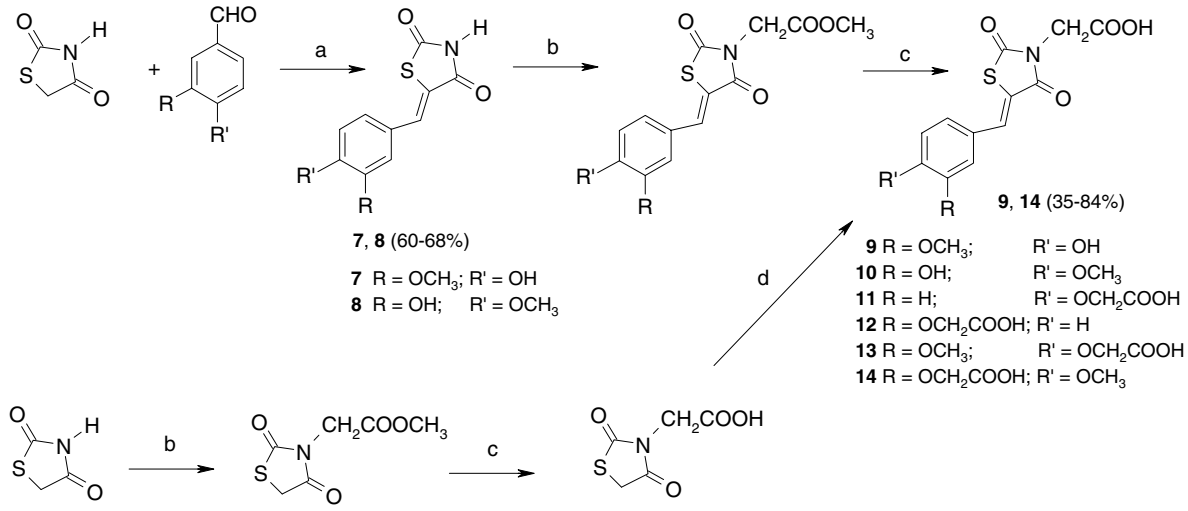
Most of the 2,4-TZDs **1**–**6** were shown to be effective in vitro ARIs.[18–20] In particular, several N-unsubstituted derivatives **1** and **4** exhibited appreciable activity at micromolar doses. The insertion of an acetic chain on N-3 of the thiazolidinedione ring (compounds **3** and **6**) gave a 10–100-fold gain in potency and led to the highest inhibition levels. In addition, certain methyl esters **2** and **5**, although devoid of any acidic functionality, showed inhibitory properties similar to those of N-unsubstituted analogues.

The inhibitory potency of 2,4-TZDs **1**–**6** was also shown to be significantly influenced by the lipophilic aryl moiety in the position 5 and by its substitution pattern. The saturation of the exocyclic C,C-double bond in 5 generally brought about a moderate decrease in activity and we found that most of the tested 5-benzylidene substituted 2,4-TZDs (**1**–**3**) were more effective ARIs than the corresponding 5-benzyl analogues (**4**–**6**).[20]

Moreover, the insertion of an additional aromatic ring or an H-bond donor group in this moiety enhanced ALR2 inhibitory effect. In particular, the presence of a hydroxy group, especially in the para position of the 5-benzylidene ring, proved to be very favourable.[19] In fact, [5-(4-hydroxybenzylidene)-2,4-dioxothyazolidin-3-yl]acetic acid (**3a**, Fig. 1) was shown to be more potent (IC_{50} = 0.15 μM) than its 3-hydroxybenzylidene substituted isomer **3b** (Fig. 1) (IC_{50} = 0.66 μM), which, however, also displayed appreciable inhibitory activity.[19]

On the basis of these findings, we continued our search aimed at amplifying the structure–activity relationships of 2,4-TZDs active as ARIs and at identifying new active analogues. In particular, starting from the promising activity of compounds **3a** and **3b**, we synthesised and evaluated 5-arylidene analogues **7**–**14** (Scheme 1), which retained the pharmacophoric elements previously identified for 5-arylidene-2,4-thiazolidinediones active as ARIs.[19] Compounds **7**–**14** bore a hydroxy, a methoxy and/or a carboxymethoxy group in their 5-benzylidene moiety. Such substituents are potentially able to enhance enzyme/inhibitor complexes stability. This can be achieved via further hydrogen bonds and/or electrostatic interactions with amino acid residues in the lipophilic pocket of the ALR2 active site, analogous to what has recently been reported for fidarestat.[24] In fact, the carbamoyl group of fidarestat has been shown to be hydrogen bonded to the main-chain nitrogen atom of Leu300, which lines part of this lipophilic pocket, and it was suggested that this interaction was responsible for the high affinity and selectivity of fidarestat for ALR2.[24] Moreover, the substitution of this carbamoyl moiety with a carboxylic group enhanced the predicted binding energy of the enzyme/inhibitor complex.[25]

Among 2,4-TZDs **7**–**14**, compounds **7** and **8** were already known in the literature[26] and **9**, **11** and **13** are



Scheme 1. Reagents and conditions: (a) $C_5H_{11}N$, C_2H_5OH, Δ; (b) $BrCH_2COOCH_3$, K_2CO_3, acetone; (c) AcOH, HCl, Δ; (d) 3-hydroxy-4-methoxybenzaldehyde or 4-hydroxy-3-methoxybenzaldehyde, $C_5H_{11}N$, C_2H_5OH, Δ.

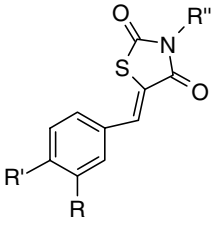
commercially available products. However, to the best of our knowledge, their ALR2 inhibitory activity has never been reported.

5-Arylidene-2,4-thiazolidinediones **7** and **8** were synthesised, following a known procedure,[18,19] by Knoevenagel condensation of commercially available 2,4-thiazolidinedione with suitable aldehydes (Scheme 1). The treatment of **7** and **8** with methyl bromoacetate, in acetone in the presence of potassium carbonate as base, produced N/O-alkylation; after acidic hydrolysis, only acids **13** and **14** were obtained with good yields.[27] Acids **11** and **12** were synthesised, following the same procedure, starting from 5-(4-hydroxybenzylidene)-2,4-thiazolidinedione and its 3-hydroxybenzylidene isomer. In order to prevent O-alkylation, the preparation of acids **9** and **10** started from the synthesis of (2,4-dioxothiazolidin-3-yl)acetic acid[19] followed by Knoevenagel condensation with the corresponding benzaldehydes (Scheme 1). Analytical and spectroscopic data (^1H and ^{13}C NMR) confirmed the structures assigned to compounds **7**–**14**.[28]

The ALR2 inhibitory activities of 2,4-TZDs **7**–**14** were evaluated in vitro using partially purified ALR2 from bovine lenses; sorbinil was used as a reference drug (Table 1).[29–31]

5-(4-Hydroxy-3-methoxybenzylidene)-2,4-thiazolidinedione (**7**) displayed moderate ALR2 inhibitory activity, in the micromolar range (IC_{50} = 11.8 μM), similar to that of the 5-(3-methoxybenzylidene) and 5-(4-hydroxybenzylidene) monosubstituted analogues previously

Table 1. In vitro bovine lenses ALR2 inhibitory activity of 2,4-thiazolidinediones **7**–**14**



Compound	R	R′	R″	IC_{50}[a]
3a[b]	H	OH	CH_2COOH	0.15 (0.10–0.22)
3b[b]	OH	H	CH_2COOH	0.66 (0.48–0.91)
7	OCH_3	OH	H	11.8 (9.64–14.4)
8	OH	OCH_3	H	33% (12.5 μM)
9	OCH_3	OH	CH_2COOH	0.70 (0.68–0.72)
10	OH	OCH_3	CH_2COOH	0.56 (0.46–0.69)
11	H	OCH_2COOH	CH_2COOH	0.60 (0.40–0.90)
12	OCH_2COOH	H	CH_2COOH	0.23 (0.092–0.57)
13	OCH_3	OCH_2COOH	CH_2COOH	0.26 (0.10–0.70)
14	OCH_2COOH	OCH_3	CH_2COOH	0.20 (0.096–0.43)
Sorbinil				1.41 (1.12–1.79)

[a] IC_{50} (μM) (95% C.L.) or % inhibition at the given concentration.
[b] Ref. 19.

reported (IC_{50} = 13.28 μM and 8.96 μM, respectively).[18,19] In contrast, its isomer **8** was less effective, producing 33% inhibition at 12.5 μM dose (Table 1); it also displayed lower activity than previously assayed 5-(3-hydroxybenzylidene)-2,4-thiazolidinedione (IC_{50} = 10.7 μM).[19]

Acids **9**–**14** were shown to be potent in vitro ARIs, displaying IC_{50} values in the range between 0.20 and 0.70 μM (Table 1).

As expected, compounds **9** and **10** were appreciably more effective ARIs than the corresponding N-unsubstituted analogues (**7** and **8**). Once again these data demonstrate that the presence of a carboxylic anionic head on N-3 is an important structural requisite to produce high levels of enzyme inhibition. However, acid **9** proved to be less active than 5-(4-hydroxybenzylidene) substituted analogue **3a** (Table 1).

The replacement of the hydroxy group in compounds **9** and **10** with a carboxymethoxy one led to derivatives **13** and **14** and gave a 3-fold gain in ALR2 inhibitory potency (Table 1).

The removal of the methoxy group of **13** and **14** led to different results. In fact, [5-(3-carboxymethoxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (**12**) displayed an inhibitory effect equal to that of the parent compound (**14**); moreover, it was shown to be 3-fold more active than its 5-(3-hydroxybenzylidene) analogue **3b** (Table 1). In contrast, [5-(4-carboxymethoxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (**11**), although displaying appreciable ALR2 inhibitory activity (IC_{50} = 0.60 μM), proved to be about twice less potent than the parent compound (**13**); it was also shown to be about 3-fold less active than its isomer **12** and 4-fold less effective than 5-(4-hydroxybenzylidene) substituted analogue **3a** (Table 1).

Indeed, 5-(3-carboxymethoxybenzylidene) substituted acids **12** and **14** proved to be the most active ARIs out of the 2,4-TZDs here reported, followed by [5-(4-carboxymethoxy-3-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (**13**) (Table 1). The latter also exhibited potency twice as high as the 5-(3-methoxybenzylidene) substituted analogue (IC_{50} = 0.48 μM), which we had previously evaluated.[18]

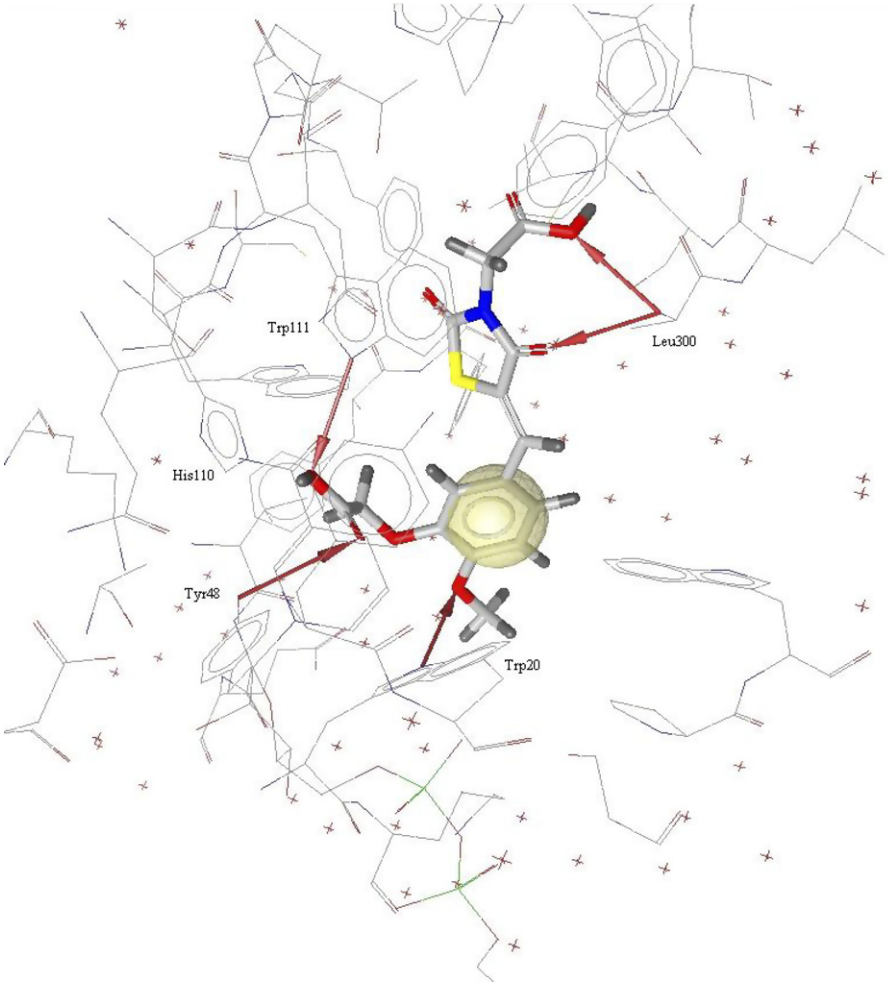


Figure 2. Docking pose of compound **14** within the binding site of ALR2 (PDB entry 1Z3N) showing possible interactions with the protein. Colour coding in LigandScout: red, hydrogen bond acceptor; yellow, hydrophobic features; magenta, ring aromatic feature.

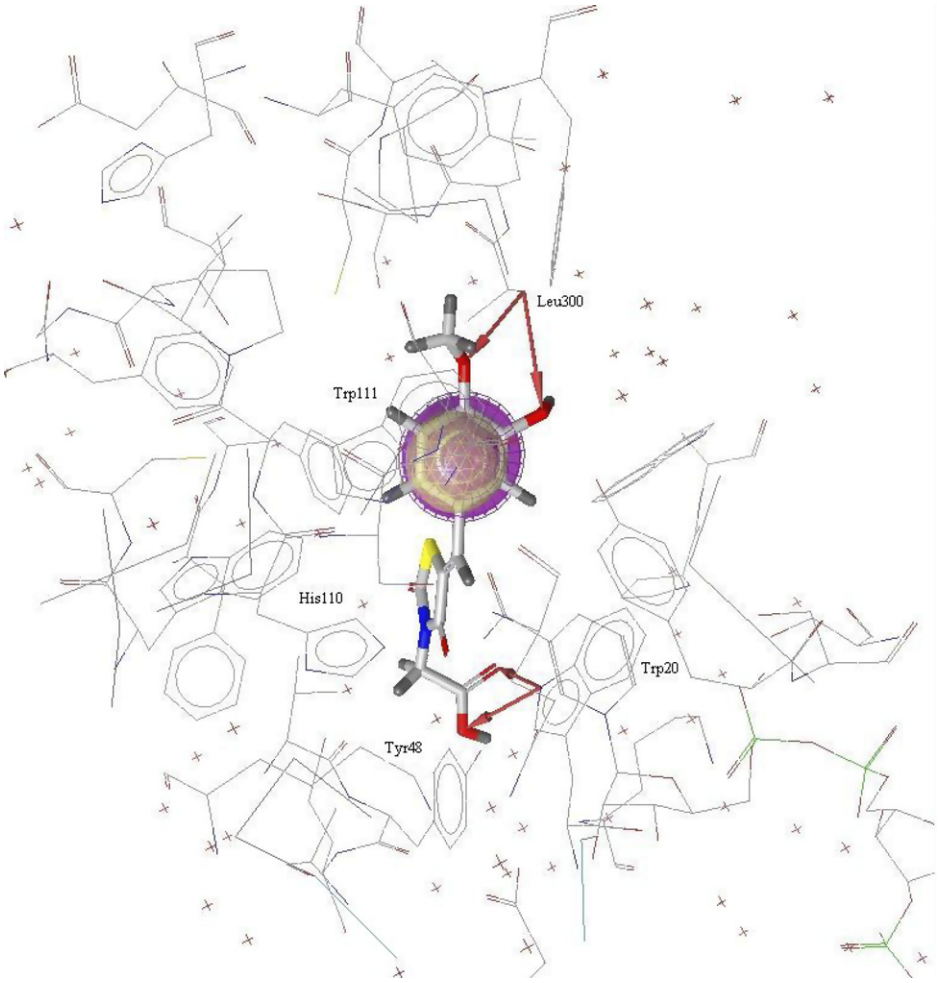


Figure 3. Docking pose of compound **10** within the binding site of ALR2 (PDB entry 1Z3N) showing possible interactions with the protein. Colour coding see Figure 2.

A docking study was performed in order to investigate how 2,4-TZDs **7**–**14** fit into the active site of ALR2 and to visualise interaction possibilities with the enzyme.[32]

PDB (Protein Brookhaven Databank)[33] entry 1N3Z, which shows a resolution of about 1 Å and has recently been released, was chosen for the docking study.[34] Its ligand, lidorestat (Fig. 1), shows the typical binding characteristics shared by many ALR2 inhibitors, thiazolidinediones included. These characteristics comprise: chemical functions enabling hydrogen bonds to Tyr48, His110 and Trp111; an acidic function for charge interactions; lipophilic areas for contacts with hydrophobic parts of the pocket and preferably also a hydrogen bonding possibility to Leu300.

Minimised structures[35] of compounds **7**, **10**, **12**, **13** and **14** were flexibly docked into the binding site using the LigandFit module in the Cerius[2] software package.[36,37] Good poses of the five investigated new ARIs showed that the ligands either satisfied interactions in the polar part of the site very well (Tyr48, His110, Trp111), or interacted predominantly in the lipophilic region (Leu300), or both.

The most active compound **14** was frequently found in poses fulfilling the typical interaction pattern also shown by lidorestat. The software LigandScout,[38] a tool for automatic identification of interaction possibilities and structure-based pharmacophore generation from protein–ligand complexes, was used to investigate and visualise the docked ligands within the binding site. Figure 2 shows an example of a good docking pose of thiazolidinedione **14**. The substitution pattern of the benzene ring is well suited for making the typical hydrogen bonds with active site residues Tyr48 and Trp111. In addition, the carboxylic acid group is positioned facing the basic His110, enabling charge–charge interactions and probably also hydrogen bonds (Fig. 2). The latter are not visualised in LigandScout because of bad angles, but can be expected in reality, considering that the protein is treated as rigid during the docking process. As can be seen from this example, an additional interaction with Trp20 was sometimes found. Moreover, hydrogen bonds to Leu300 were also achieved by two acceptor functions of the ligand (carbonyl oxygen atoms of the thiazolidinedione ring and the second carboxylic acid moiety) (Fig. 2). Lipophilic parts of the ligand, especially the benzene ring, are surrounded

by hydrophobic binding site residues. In the proximity of the benzene ring (3–5.5 Å) the aromatic rings of Trp20, Trp219, Phe122, Trp111 and Trp79 can be found. Furthermore, looking at the overlay of compound **14** with lidorestat demonstrated that their positions in the site were very similar when spatial occupation and positioning of the chemical functions for interaction were considered.

Other docking poses also showed the ligands in 180 degree rotated orientations, that is, the orientations we originally expected on the basis of our previous results.[19] However, especially for the larger structures (**13** and **14**), these poses were found less frequently and the resultant interaction possibilities were usually more limited than the ones from the reversed poses. For example, only one out of the 20 docking poses for ligand **14** was orientated in such a way. In general the LigandFit dock_score, a measurement of the non-bonded intermolecular interactions between the ligand and the protein, was also lower for this alternative pose with a value of 56.4. In comparison, most of the other retrieved poses, which showed orientations as in Figure 2, obtained higher dock-scores starting with a value of 71.6. Good docking results with the thiazolidinedione part facing the inner polar side of the binding cavity were found, for example, for 5-(3-hydroxy-4-methoxybenzylidene) substituted compound **10** (Fig. 3), where hydrogen bonds to Trp20, Leu 300 and π–π stacking with Trp111 can be seen.

In conclusion, this work allowed us to identify a number of other 5-arylidene-2,4-thiazolidinediones active as in vitro ARIs and to extend the structure–activity relationships of this class of ALR2 inhibitors.

In particular, the introduction of a hydroxy group in the para position of the 5-benzylidene ring appeared to be beneficial. The addition of a methoxy group in position 3 or the displacement of the 4-hydroxy group to position 3 of the benzylidene ring usually led to less active analogues. Moreover, substituting the hydroxy group with a carboxymethoxy one generally enhanced activity. These results suggested that the phenolic or carboxylic substituents of the 5-benzylidene moiety played a beneficial role in the binding to ALR2. In fact, molecular docking simulations into the active site of the enzyme highlighted that these groups can favourably interact, in alternative poses, either with amino acid residues of the C-terminal loop in the lipophilic pocket of the enzyme, such as Leu300, or with the positively charged recognition region of the ALR2 active site, lined by Tyr48, His110 and Trp111; in the latter case, hydrogen bonds to Leu300 can be achieved by the acetic chain on N-3 and the carbonyl oxygen atoms of the thiazolidinedione ring.

Taking into account that the interactions between these 2,4-TZDs and Leu300 could be responsible for a selective inhibition of ALR2 over other aldo–keto reductases, the evaluation of their inhibitory effects towards related enzymes, such as aldehyde reductase, will be the subject of a future note.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.04.109.

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27. A mixture of **7** or **8** (2.51 g, 10 mmol), methyl bromoacetate (6.12 g, 40 mmol) and potassium carbonate (5.53 g,

40 mmol) in acetone (150 ml) was refluxed for 24 h. The solvent was evaporated under reduced pressure. The solid residue was washed with H_2O and then refluxed in glacial AcOH (40 ml) and HCl 12N (10 ml) for 2 h. After evaporation in vacuo, the residue was refluxed again with AcOH (40 ml) and HCl (10 ml) for 2 h. After evaporation to dryness in vacuo, the crude solid was washed with H_2O and recrystallized from ethanol providing pure carboxylic acid **13** or **14**.

28. *5-(4-Hydroxy-3-methoxybenzylidene)-2,4-thiazolidinedione* (***7***). Yield 68%; mp 217 °C. ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H, OCH_3); 6.95 (d, J = 8.1 Hz, 1H, CH arom); 7.09 (d, J = 8.1 Hz, 1H, CH arom); 7.19 (s, 1H, CH arom); 7.74 (s, 1H, CH); 9.97 and 12.42 (2 br s exchangeable with D_2O, 2H, OH and NH); ^{13}C NMR (DMSO-d_6): δ 56.1 (OCH_3); 114.6, 116.7, 124.6 (CH arom); 119.7 (5-C); 133.1 (CH methylidene); 124.9, 148.5, 149.9 (Cq arom); 167.9, 168.5 (CO). Anal. ($C_{11}H_9NO_4S$). Calcd.: C, 52.59%; H, 3.59%; N, 5.58%. Found: C, 52.38%; H, 3.70%; N, 5.39%.
5-(3-Hydroxy-4-methoxybenzylidene)-2,4-thiazolidinedione (***8***). Yield 60%; mp 260 °C. ^{1}H NMR (DMSO-d_6): δ 3.82 (s, 3H, OCH_3); 7.01–7.07 (m, 3H, CH arom); 7.62 (s, 1H, CH); 9.49 and 11.95 (2 br s exchangeable with D_2O, 2H, OH and NH); ^{13}C NMR (DMSO-d_6): δ 56.2 (OCH_3); 113.0, 116.4, 123.9 (CH arom); 120.6 (5-C); 132.8 (CH methylidene); 126.2, 147.5, 150.6 (Cq arom); 168.0, 168.5 (CO). Anal. ($C_{11}H_9NO_4S$). Calcd.: C, 52.59%; H, 3.59%; N, 5.58%. Found: C, 52.65%; H, 3.48%; N, 5.73%.
[5-(4-Hydroxy-3-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (***9***). Yield 35%; mp187–189 °C. ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H, OCH_3); 4.38 (s, 2H, NCH_2); 6.98 (d, J = 8.1 Hz, 1H, CH arom); 7.18 (d, J = 8.1 Hz, 1H, CH arom); 7.22 (s, 1H, CH arom); 7.88 (s, 1H, CH); 10.07 (br s exchangeable with D_2O, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 44.9 (NCH_2); 56.4 (OCH_3); 115.1, 117.0, 125.0 (CH arom); 117.7 (5-C); 134.4 (CH methylidene); 124.9, 148.8, 150.7 (Cq arom); 166.3, 168.0, 169.5 (CO). Anal. ($C_{13}H_{11}NO_6S$) Calcd.: C, 50.49%; H, 3.56%; N, 4.53%. Found: C, 50.67%; H, 3.70%; N, 4.38%.
[5-(3-Hydroxy-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (***10***). Yield 40%; mp 215–218 °C. ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H, OCH_3); 4.36 (s, 2H, NCH_2); 7.07–7.17 (m, 3H, CH arom); 7.84 (s, 1H, CH); 10.05 (br s exchangeable with D_2O, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 42.8 (NCH_2); 56.4 (OCH_3); 113.0, 116.3, 124.4 (CH arom); 117.6 (5-C); 134.9 (CH methylidene); 126.0, 147.5, 151.0 (Cq arom); 166.4, 167.6, 168.3 (CO). Anal. ($C_{13}H_{11}NO_6S$). Calcd.: C, 50.49%; H, 3.56%; N, 4.53%. Found: C, 50.31%; H, 3.29%; N, 4.68%.
[5-(4-Carboxymethoxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (***11***). Yield 78%; mp 288 °C. ^{1}H NMR (DMSO-d_6): δ 4.36 (s, 2H, NCH_2); 4.74 (s, 2H, OCH_2); 6.96 (m, 2H, CH arom); 7.40 (m, 2H, CH arom); 7.78 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 42.7 (NCH_2); 65.1 (OCH_2); 116.0, 132.8 (CH arom); 118.2 (5-C); 134.2 (CH methylidene); 126.2, 160.3 (Cq arom); 165.6, 167.5, 168.5, 170.2 (CO). Anal. ($C_{14}H_{11}NO_7S$). Calcd.: C, 49.85%; H, 3.26%; N, 4.15%. Found: C, 50.01%; H, 3.38%; N, 4.02%.
[5-(3-Carboxymethoxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (***12***). Yield 68%; mp 290 °C. ^{1}H NMR (DMSO-d_6): δ 4.41 (s, 2H, NCH_2); 4.78 (s, 2H, OCH_2); 7.10 (d, J = 9 Hz, 1H, CH arom); 7.22 (s, 1H, CH arom); 7.28 (d, J = 9 Hz, 1H, CH arom); 7.50 (dd, J = 9 and 9 Hz, 1H, CH arom); 8.00 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 42.8 (NCH_2); 65.0 (OCH_2); 116.3, 117.8, 123.1, 131.0 (CH arom); 121.7 (5-C); 134.2 (CH methylidene); 134.6, 158.7 (Cq arom); 165.4, 167.3, 168.4, 170.4 (CO). Anal. ($C_{14}H_{11}NO_7S$). Calcd.: C, 49.85%; H, 3.26%; N, 4.15%. Found: C, 49.78%; H, 3.16%; N, 4.27%.
[5-(4-Carboxymethoxy-3-methoxybenzylidene)-2,4-dioxothiazolidin-3- yl]acetic acid (***13***). Yield 67%; mp 256 °C. ^{1}H NMR (DMSO-d_6): δ 3.86 (s, 3H, OCH_3); 4.39 (s, 2H, NCH_2); 4.81 (s, 2H, OCH_2); 7.04 (d, J = 9 Hz, 1H, CH arom); 7.22 (d, J = 9 Hz, 1H, CH arom); 7.30 (s, 1H, CH arom); 7.96 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 42.7 (NCH_2); 56.2 (OCH_3); 65.4 (OCH_2); 113.8, 114.6, 124.1 (CH arom); 118.5 (5-C); 134.7 (CH methylidene); 126.6, 149.6, 150.1 (Cq arom); 165.6, 167.5, 168.5, 170.2 (CO). Anal. ($C_{15}H_{13}NO_8S$). Calcd.: C, 49.05%; H, 3.54%; N, 3.81%. Found: C, 49.27%; H, 3.73%; N, 3.75%.
[5-(3-Carboxymethoxy-4-methoxybenzylidene)-2,4-dioxothiazolidin-3- yl]acetic acid (***14***). Yield 84%; mp 246 °C. ^{1}H NMR (DMSO-d_6): δ 3.85 (s, 3H, OCH_3); 4.36 (s, 2H, NCH_2); 4.74 (s, 2H, OCH_2); 7.14–7.18 (m, 2H, CH arom); 7.28 (dd, J = 8.7 and 2.4 Hz, 1H, CH arom); 7.91 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 42.0 (NCH_2); 55.5 (OCH_3); 64.7 (OCH_2); 112.3, 114.2, 124.8 (CH arom); 117.4 (5-C); 133.8 (CH methylidene); 125.0, 147.1, 151.0 (Cq arom);165.1, 166.8, 167.7, 169.7 (CO). Anal. ($C_{15}H_{13}NO_8S$). Calcd.: C, 49.05%; H, 3.54%; N, 3.81%. Found: C, 48.91%; H, 3.35%; N, 4.01%.
29. Isolation of ALR2 from calf lenses and purification of this enzyme have been previously described in ref. 30. IC_{50} values were determined from least squares analyses of the linear portion of the log dose-inhibition curves by using CalcuSyn software. Each curve was generated using at least three concentrations of the tested compounds (added as a solution in DMSO; final concentration of DMSO in the incubation mixture was 1%) causing an inhibition between 20% and 80%, with two replicates at each concentration. For details concerning the assay procedure, see Ref. 19.
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32. Docking calculations were performed on a Pentium IV 2.8 GHz PC running Fedora Core 4 using the LigandFit module in the Cerius^2 software package, version 4.11 and the cff1.02 force field. The software tool Catalyst version 4.11 was employed for ligand building, minimisation and export as mol files. In order to select a well-suited protein–inhibitor complex for docking from the PDB, which contains about 50 entries for ALR2, several PDB search criteria were applied to filter the numerous structures: EC number 1.1.1.21, keyword Homo sapiens, existence of ligands, X-ray resolution from 0 to 2 Å and deposition between 2004 and 2007. The remaining entries were inspected for the existence of typical ALR2 inhibitor binding characteristics. Protein preparation of complex 1Z3N for docking in the Cerius^2 software package included the elimination of the inhibitor and of the solvent molecules (keeping the cofactor as part of the protein structure), the addition of hydrogen atoms from templates upon protein import and definition of the suitable tautomeric form of active site His110 for hydrogen bonding to acceptor functions in the ligands. Ligands were processed for docking by defining bond orders and charges correctly, that is, setting the partial charges on both oxygen atoms of the carboxylic acids to a value of −1/2. Site definition resulted from the co-crystallized ligand. Flexible docking runs were carried out without using interaction filters. In the docking preferences the number of Monte Carlo trials was set to a fixed value of 5000 and the maximum number of saved poses to 20, in the energy preferences the force field selection was changed to CFF. Default settings were kept for the remaining site definition and docking parameters.

The LigandScout software version 1.02 served as a tool for visualisation of the interactions of the docked ligands with the protein.

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