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Structure–activity relationships and molecular modelling of 5-arylidene-2,4-thiazolidinediones active as aldose reductase inhibitors

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Abstract—The structure–activity relationships (SARs) of 5-arylidene-2,4-thiazolidinediones active as aldose reductase inhibitors (ARIs) were extended by varying the substitution pattern on the 5-arylidene moiety and on N-3. In particular, the introduction of an additional aromatic ring or an H-bond donor group on the 5-benzylidene ring enhanced ALR2 inhibitory potency. Moreover, the presence of a carboxylic anionic chain on N-3 was shown to be an important, although not essential, structural requisite to produce high levels of ALR2 inhibition. The length of this carboxylic chain was critical and acetic acids **4** were the most effective inhibitors among the tested derivatives. Molecular docking simulations into the ALR2 active site accorded with the in vitro inhibition data. They allowed the rationalization of the observed SARs and provided a pharmacophoric model for this class of ARIs.

1. Introduction

Aldose reductase (EC 1.1.1.21, ALR2) is an aldo-keto reductase involved in the development of degenerative long-term complications of diabetes mellitus. It is the first enzyme of the polyol pathway and catalyses the NADPH-dependent reduction of glucose to sorbitol. An increased flux of glucose through the polyol pathway occurs under conditions of hyperglycaemia, such as in diabetes, in tissues possessing insulin-independent glucose transport (retina, lenses, kidney, nerve). It was demonstrated that the activation of this metabolic pathway is linked to the onset and progression of chronic diabetic complications (neuropathy, nephropathy, retinopathy, cataracts), in consequence of the deprivation of NADPH and NAD^+ and intracellular accumulation of sorbitol with subsequent altered cellular redox potentials and osmotic imbalance.[1–5]

Therefore, ALR2 has been considered as a target enzyme to develop drugs able to prevent the onset and to check the progression of diabetic complications, even in the presence of imperfect control of glycaemia.

Over the last three decades numerous ALR2 inhibitors (ARIs) have been identified, most of which belong to the carboxylic acids (such as zopolrestat, ponalrestat, epalrestat) and hydantoins (such as sorbinil) classes of compounds (Fig. 1).[1a,2,6–9] Nevertheless, many of them have shown to be clinically inadequate because of adverse pharmacokinetics or toxic side-effects.[8] At present, epalrestat is the only ARI available on the market.[9,10]

Due to the shortage of drugs currently available for the treatment of diabetic complications, the search for new ARIs endowed with more favourable biological properties is still a major pharmaceutical challenge.

We have recently reported several (*Z*)-5-arylidene-2,4-thiazolidinediones (**1**) (Fig. 1) designed in view of the structural requisites essential for ALR2 inhibition (an acidic proton, H-bond acceptor groups and a lipophilic aromatic moiety);[9,11–17] in fact, they were shown to be effective as in vitro ARIs.[18] Some N-unsubstituted

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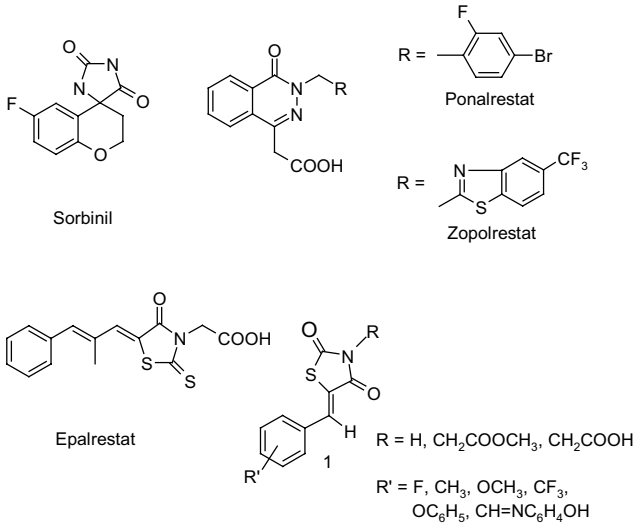
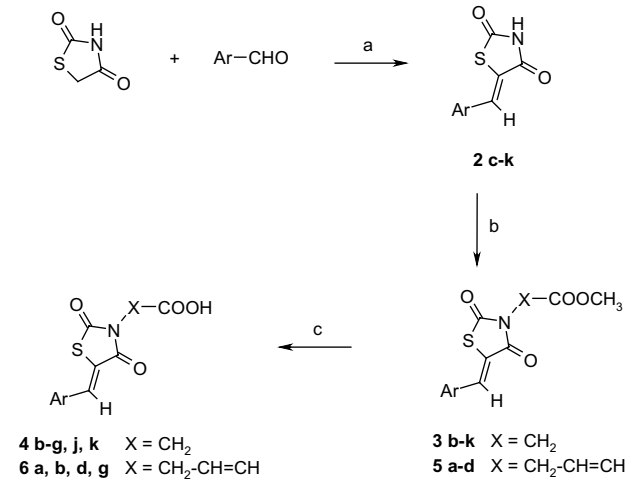


Figure 1.



Scheme 1. Reagents: (a) $C_5H_{11}N$, refluxing C_2H_5OH; (b) K_2CO_3, acetone, $BrCH_2COOCH_3$ or $BrCH_2CH{=}CHCOOCH_3$; (c) AcOH, HCl, reflux.

derivatives possessed the same inhibitory potency of sorbinil, the prototype of hydantoins, which was withdrawn from clinical trials because its in vivo efficacy as ARI was limited by hypersensitivity reactions.[2,6,8] In fact, 2,4-thiazolidinediones can be considered as hydantoin bioisosteres potentially devoid of these adverse effects, which are related to the hydantoin ring.[2,8,18]

In our compounds the introduction of an acetic chain on N-3 of the thiazolidinedione ring led to a marked increase in inhibitory potency.[18] Moreover, some of the corresponding methyl esters, although devoid of any acidic functionality, showed appreciable inhibitory properties; this finding suggested that the polar N-3 acetate chain was able to reach and bind the polar positively charged recognition region of the ALR2 active site formed by Tyr48, His110, Trp111 residues and the nicotinamide ring of cofactor $NADP^+$.[2,8,9,13,14,19] In any case, the higher inhibitory potency of the analogous carboxylic acids confirmed the importance of an anionic head in the interaction with the recognition region of the enzyme. However, in vivo the ester function could improve the crossing of biomembranes and, analogously, the N-unsubstituted derivatives might display more favourable pharmacokinetics due to their pK_a values higher than carboxylic acids.[20,21]

On the basis of these results, in this work we amplified the SARs of this class of ARIs.

Firstly, we varied the 5-arylidene moiety by synthesizing: (a) derivatives endowed with a larger lipophilic 5-arylidene moiety; (b) analogues bearing an H-bond donor group on the 5-benzylidene ring. In fact, it is known that, except for the polar positively charged recognition region, the nature of the ALR2 active site is highly hydrophobic and thus large lipophilic moieties can favour the interaction of inhibitors with the enzyme.[2,13–15,22] Otherwise, the enzyme–inhibitor complex stability could be increased by means of further interactions through H-bonds.[14,15,18]

The N-3 position was maintained unsubstituted or substituted with an acetic chain (compounds **2**, **3**, **4**, Scheme 1). Afterwards the N-3 acetic chain was replaced by the residue of 2-butenoic acid (compounds **5**, **6**, Scheme 1). We also decided to synthesize and assay in our experimental conditions some 2,4-thiazolidinedione analogues already reported as ARIs (compounds **4b**, **d**, **e**, **k** and **3b**, **d**, **e**, **k**)[23] with the aim of depicting an extensive SARs picture.

The ALR2 inhibitory activity of 2,4-thiazolidinediones **2**–**6** was evaluated in vitro using partially purified ALR2 from bovine lenses.

The results were rationalized by means of docking simulations into the ALR2 active site.

2. Chemistry

5-Arylidene-2,4-thiazolidinediones **2** were synthesized, according to a known procedure,[18,24] by the condensation of commercially available 2,4-thiazolidinedione with suitable benzaldehydes in refluxing ethanol in the presence of piperidine (Scheme 1).

Esters **3** and **5** were prepared by the reaction between N-unsubstituted 2,4-thiazolidinediones **2** and methyl bromoacetate or 4-bromocrotonate, respectively, using potassium carbonate as base. Alternatively, acetates **3** could be produced by the reaction of **2** with methyl bromoacetate in DMF in the presence of sodium hydride,[18] with generally lower yields.

The synthesis of 5-(3-hydroxybenzylidene)- and 5-(4-hydroxybenzylidene) substituted acetates **3f** and **3g** was carried out by adding methyl bromoacetate drop wise to a solution of **2f** or **2g**, respectively, and potassium carbonate in acetone over 1.5 h, in order to prevent the nucleophilic attack of hydroxyl group to bromoacetate; after refluxing of the mixture for 24 h and

crystallization from methanol, only the desired 5-(3-hydroxybenzylidene)- and 5-(4-hydroxybenzylidene) substituted acetates were obtained in high yields.

5-(3-Aminobenzylidene)- and 5-(4-aminobenzylidene)-2,4-thiazolidinediones (**2h** and **2i**) were synthesized starting from 3- and 4-nitrobenzylidene substituted analogues **2j** and **2k** by the reduction of the nitro group with tin(II) chloride in refluxing ethanol. Analogously 3-aminobenzylidene and 4-aminobenzylidene substituted acetates **3h** and **3i** were obtained starting from acetates **3j** and **3k**, respectively.

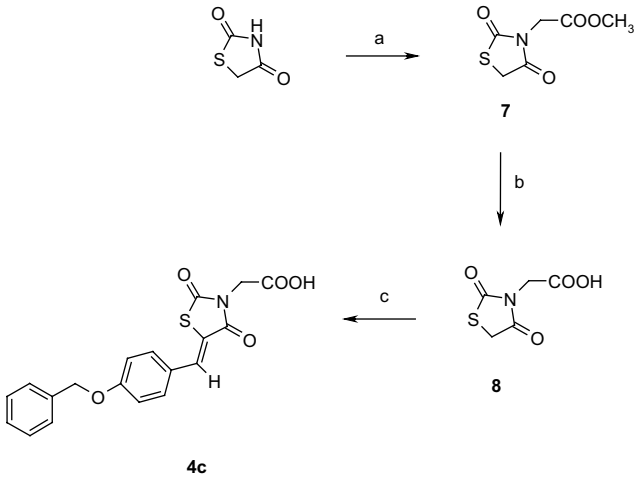
The acidic hydrolysis of **3** and **5** provided corresponding carboxylic acids **4** and **6** (Scheme 1).

The acidic hydrolysis of 5-(4-benzyloxybenzylidene) substituted methyl esters **3c** and **5c** led to [5-(4-hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (**4g**) and 4-[5-(4-hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl]-2-butenoic acid (**6g**), respectively. Therefore [5-(4-benzyloxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (**4c**) was prepared starting from the reaction between commercially available 2,4-thiazolidinedione and methyl bromoacetate in acetone in the presence of potassium carbonate, followed by acidic hydrolysis. (2,4-Dioxothiazolidin-3-yl)acetic acid (**8**) was then converted to **4c** by condensation with 4-benzyloxybenzaldehyde (Scheme 2).

Attempts to obtain [5-(3-aminobenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid and its 4-amino substituted isomer, through different synthetic pathways (hydrolysis of corresponding methyl esters **3h** and **3i**, reduction of acids **4j** and **4k** with tin(II) chloride) failed.

Analytical and spectroscopic data (IR, ^{1}H and ^{13}C NMR) confirmed the structures assigned to compounds **2**–**6**.

Analogously to their previously reported analogues **1**,[18] 5-arylidene-2,4-thiazolidinediones **2**–**6** were obtained



Scheme 2. Reagents: (a) K_2CO_3, acetone, $BrCH_2COOCH_3$; (b) AcOH, HCl, reflux; (c) 4-benzyloxybenzaldehyde, $C_5H_{11}N$, refluxing C_2H_5OH.

only as *Z* isomers; in fact, their ^{1}H NMR spectra showed only one signal attributable to the resonance of the 5-methylidene proton in the range 7.50–8.70 ppm. In their ^{13}C NMR spectra, the 5-methylidene carbon and C_5 of the thiazolidinedione ring resonated in the ranges 130.5–140.7 and 117.5–128.0 ppm, respectively.

In the ^{1}H and ^{13}C NMR spectra of esters **3**, **5** and acids **4**, **6** a signal attributable to N–CH_2 resonance was diagnostic: in particular ^{1}H NMR spectra showed a singlet at 4.35–4.55 ppm for acetic derivatives **3**, **4** and a double doublet at 4.42–4.52 ppm for 2-butenoic derivatives **5**, **6**. In their ^{13}C NMR spectra, besides two signals attributable to the resonances of 2- and 4-carbonylic groups of the thiazolidinedione ring at 164.5–168.9 ppm, another signal due to the resonance of the carboxylic carbon appeared in the same range.

The structure of acids **4**, **6** was also confirmed by means of their IR spectra, which showed a very broad band in the region of 3450–2450 cm^{-1} attributable to the stretching of carboxylic OH.

3. Results and discussion

3.1. Aldose reductase inhibition

Newly synthesized compounds **2**–**6** were evaluated for their ability to inhibit the in vitro reduction of D,L-glyceraldehyde by partially purified ALR2 from bovine lenses, using sorbinil and ponalrestat[25] as reference drugs (Table 1).

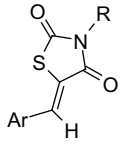
Our previous results had indicated that the presence of an additional aromatic ring on the 5-benzylidene group increased the ALR2 inhibitory activity of 5-arylidene-2,4-thiazolidinediones, very likely through further van der Waals interactions with the hydrophobic region of the active site of the enzyme.[18]

Thus, starting from the significant activity of [2,4-dioxo-5-(3-phenoxybenzylidene)thiazolidin-3-yl]acetic acid (**4a**, $IC_{50} = 0.13$ μM),[18] we first synthesized its 4-phenoxy substituted analogue (**4b**). The displacement of the 3-phenoxy group to position 4 of the 5-benzylidene moiety reduced activity and, in fact, acid **4b** was shown to be about six times less potent than **4a**; corresponding methyl ester **3b** was also significantly less active than **3a** (Table 1).

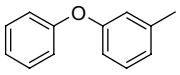
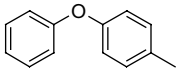
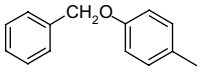
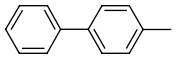
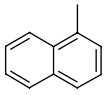
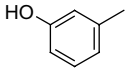
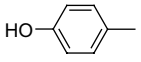
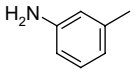
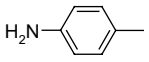
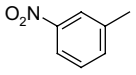
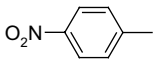
The replacement of the 4-phenoxy with the 4-benzyloxy group enhanced activity leading to a good ALR2 inhibitor (**4c**, $IC_{50} = 0.28$ μM). Similar inhibitory effect was shown by 4-phenyl substituted analogue **4d** ($IC_{50} = 0.26$ μM), whereas N-unsubstituted derivatives **2c**, **2d** and methyl esters **3c**, **3d** were almost inactive, producing 10–35% inhibition at 50 μM dose (Table 1).

The condensation of a further benzene ring to the 5-benzylidene group led to 5-naphthalen-1-ylmethylene substituted derivatives endowed with different inhibitory

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



2 R = H
3 R = CH_2COOCH_3
4 R = CH_2COOH

Ar	Compd	IC_{50}[a]	Compd	IC_{50}[a]	Compd	IC_{50}[a]
	**2a**[b]	6.14 (5.54–6.81)	**3a**[b]	1.32 (1.00–1.74)	**4a**[b]	0.13 (0.11–0.15)
	**2b**[b]	41% (37 μM)	**3b**	33% (50 μM)	**4b**	0.82 (0.76–0.88)
	**2c**	10% (50 μM)	**3c**	12% (50 μM)	**4c**	0.28 (0.12–0.65)
	**2d**	20% (50 μM)	**3d**	35% (50 μM)	**4d**	0.26 (0.18–0.37)
	**2e**	10.7 (9.58–11.97)	**3e**	12% (50 μM)	**4e**	0.17 (0.11–0.27)
	**2f**	10.7 (9.91–11.65)	**3f**	43% (50 μM)	**4f**	0.66 (0.48–0.91)
	**2g**	8.96 (6.64–12.09)	**3g**	6.18 (5.74–6.66)	**4g**	0.15 (0.10–0.22)
	**2h**	20.2 (18.4–22.2)	**3h**	39.1 (38.6–39.6)		
	**2i**	44% (50 μM)	**3i**	25% (50 μM)		
	**2j**	21% (12.5 μM)	**3j**	22% (12.5 μM)	**4j**	0.49 (0.45–0.54)
	**2k**	37% (12.5 μM)	**3k**	8% (12.5 μM)	**4k**	1.19 (1.08–1.30)
	Sorbinil	1.10 (1.01–1.19)				
	Ponalrestat	0.08 (0.073–0.087)				

[a] IC_{50} (μM) (95% C.L.) or % inhibition at the given concentration.
[b] Ref. 18.

potency: (5-naphthalen-1-ylmethylene-2,4-dioxothiazolidin-3-yl)acetic acid (**4e**) was shown to be a potent ALR2 inhibitor (IC_{50} = 0.17 μM); N-unsubstituted analogue **2e** displayed activity about 60 times lower than that of **4e**, whereas corresponding methyl ester **3e** was ineffective (Table 1). Compound **4e** was shown to be slightly more potent than (5-naphthalen-2-ylmethylene-2,4-dioxothiazolidin-3-yl)acetic acid, already known,[26] which in our assay displayed IC_{50} value (0.22 μM) very close to the reported one (0.27 μM).[26]

In the meantime, we synthesized 5-arylidene-2,4-thiazolidinediones bearing a hydroxy group on the 5-arylidene moiety as H-bond donor group.

5-(4-Hydroxybenzylidene) substituted derivatives produced appreciable ALR2 inhibition: N-unsubstituted 2,4-thiazolidinedione **2g** and methyl ester **3g** showed micromolar IC_{50} values, whereas, as expected, acid **4g** exerted significant inhibitory activity (IC_{50} = 0.15 μM), which was 60 and 40 times higher than **2g** and **3g**, respectively. Compound **4g** proved to be the most active among the 5-arylidene-2,4-thiazolidinediones reported here, along with the above-mentioned 5-naphthalen-1-ylmethylene substituted analogue (**4e**), which exhibited the same inhibitory potency (Table 1).

The displacement of the hydroxy group to position 3 of the 5-benzylidene ring decreased inhibitory effectiveness; also in this case, acid **4f** was the most active among the tested 5-(3-hydroxybenzylidene) substituted analogues, exhibiting a submicromolar IC_{50} value, which was 4 times higher than that of **4g**.

The isosteric replacement of the hydroxy group with the amino group, which is a less efficacious H-bond donor group, led to less active compounds (**2**, **3 h**, **i**). The decrease in inhibitory effectiveness was more marked in 3- and 4-nitro substituted analogues (**2**–**4 j**, **k**), which were devoid of any H-bond donor group in the 5-arylidene moiety.

On the whole these results confirmed that in 5-arylidene-2,4-thiazolidinediones, active as ARIs, the presence of an anionic carboxylic group on N-3 is an important, even though not essential, structural requisite to produce high levels of enzyme inhibition. In fact, all acetic acids **4** exerted high levels of ALR2 inhibition; they exhibited submicromolar IC_{50} values and proved to be more active than sorbinil (with the only exception of **4k**), nearly reaching the inhibitory potency of ponalrestat (Table 1).

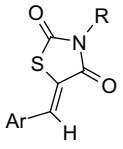
The length of the carboxylic chain on N-3 was also shown to be critical for activity. In fact, the replacement of the N-3 acetic chain with the residue of 2-butenoic acid (esters **5** and acids **6**) was detrimental for activity (Table 2). Only 5-(4-hydroxybenzylidene) substituted derivative **6g** exerted appreciable ALR2 inhibition at micromolar doses (IC_{50} = 4.17 μM).

3.2. Molecular modelling

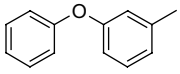
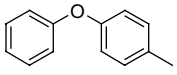
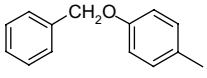
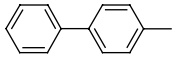
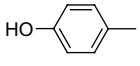
Six compounds were selected for computational analysis, namely **2a**, **3a**, **4a**, **4b**, **4g** and **6g**. Acetic acids **4a**, **4b**, **4g** and acetate **3a** are among the most active ARIs tested in our laboratories (Table 1). Compound **2a** was chosen to represent the series of N-unsubstituted 5-arylidene-2,4-thiazolidinediones **2**. Acid **6g** was the most active among the tested 2-butenoic acids **6** (Table 2).

In order to find a crystallographic structure of ALR2 suitable for the docking experiments, the Brookhaven Protein Database was searched and several entries of aldose reductase co-crystallized with different inhibitors were studied in detail. Although biological testing was performed using the enzyme purified from bovine lens, the choice of the structure of human aldose reductase is justified since there is 82% sequence identity and 89% similarity (BLAST analysis, score 556 bits) of the human enzyme with the bovine one. Finally, the PDB entry 1EL3, containing the structure of human aldose reductase complexed with the inhibitor Idd384 (Fig. 2),[27] was selected because of its relatively high resolution value (1.7 Å). Moreover, the enzyme was co-crystallized with Idd384, which shows a structural resemblance to our ARIs; in fact, the structures of the selected 2,4-thiazolidinediones do not include a benzothiazole ring able to induce large conformational changes and to open a new hydrophobic pocket in the ALR2 active site, as in the case of zopolrestat.[8,28] Thus we assumed that our ARIs and Idd384 might bind to the enzyme in a similar way. The LigandFit module implemented in the software package Cerius[2] was used for the docking and scoring procedure.[29] The final docked pose of Idd384 was found to be in good agreement with the orientation of the ligand in the crystal structure, confirming the ability of the method to accurately predict the binding conformation.

Table 2. In vitro bovine lenses ALR2 inhibitory activity of 2,4-thiazolidinediones **5**, **6**



5 R = $CH_2CH{=}CHCOOCH_3$
6 R = $CH_2CH{=}CHCOOH$

Ar	Compd	IC_{50}[a]	Compd	IC_{50}[a]
	**5a**	25% (50 μM)	**6a**	19% (50 μM)
	**5b**	0% (50 μM)	**6b**	57% (50 μM)
	**5c**	23% (50 μM)		
	**5d**	6% (50 μM)	**6d**	21% (50 μM)
			**6g**	4.17 (3.05–5.71)
	Sorbinil	1.10 (1.01–1.19)		
	Ponalrestat	0.08 (0.073–0.087)		

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

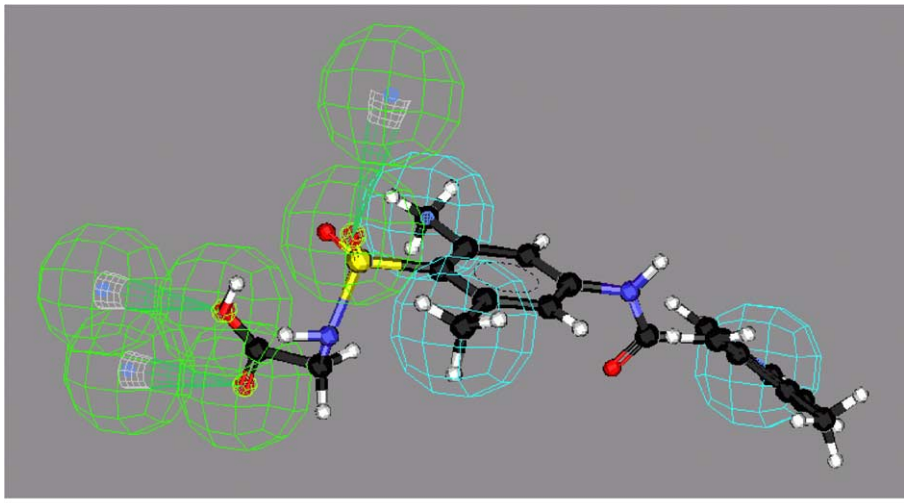


Figure 2. Pharmacophore model representing the interactions of Idd384 with the ALR2 active site.

Information about the interactions of Idd384 within the ALR2 active site was used in the pharmacophore design process by means of the Catalyst program.[30] The carboxylate group interacts with the positively charged nitrogen of the $NADP^+$ and additionally contributes to binding by the formation of hydrogen bonds with Tyr48. Further hydrogen bonds with Trp111 and His110 are accepted by the sulfone oxygen atoms. The lipophilic moieties of the inhibitor interact with the ALR2 active site through hydrophobic bonds. Thus this pharmacophore consists of three H-bond acceptor features and three hydrophobic areas. In Figure 2, the superposition of Idd384 with the pharmacophore model is shown.

The molecular models of the selected 2,4-thiazolidinediones were built using Catalyst, which was also used to generate conformational models, feature-based pharmacophore hypotheses, and search for conformers that fit these hypotheses best. This procedure was used in order to detect beforehand those conformers that closely resemble the protein-bound conformation of Idd384 and most likely are the binding conformations of our ARIs.

Common pharmacophoric features shared by all selected 5-arylidene-2,4-thiazolidinediones were identified as follows: two H-bond acceptors representing the carboxylic group and one sulfone oxygen atom of Idd384 together with a hydrophobic region representing the dimethylphenyl system (Fig. 3). Starting from the initial model derived from the active protein-bound conformation of Idd384, three pharmacophore hypotheses were manually built, which slightly varied in size and location of the features in order to cover the flexibility of the side chains within the active site. The selected 2,4-thiazolidinediones were fitted on the pharmacophore models and all the conformers that were shown to map the pharmacophores in a Idd384 like mode by at least two of the three hypotheses were selected for docking and imported into the Cerius[2] software package. This preselection procedure of conformers within the Catalyst program proved to be more valuable than flexible ligand docking, since the algorithm in Cerius[2] could not provide satisfying conformational sampling for this target resulting in conformations and poses to be considerably diverse from the known binding mode of Idd384.

The files containing the most suitable 38 conformers were used for a rigid fit docking procedure followed by a final energy minimization step in the protein, which ensured that optimal results were obtained even from non-ideal conformations. Thereby, 230 different orientations of the 38 conformers were retrieved. These 230 ligand–enzyme complexes were evaluated via the computation of scores using all the scoring functions implemented in Cerius[2]: Ligscore1, Ligscore2, PLP1, PLP2, JAIN, PMF and LUDI. The concept of consensus scoring was applied, which proved to be a valuable tool for improving the prediction efficiency of scoring functions.

Especially LigScore1 and LigScore2 reflected very well the ranking order of measured in vitro activities. We therefore suggest that LigScore1 and LigScore2 were the scoring functions of choice for this structural class of ARIs in our experimental conditions. Nevertheless, it must be mentioned that differences in binding affinities of the compounds were rather small in some cases and therefore hardly elusive by means of scoring.

In Figure 4 the superposition of docked structures of compounds **3a**, **4a** and Idd384 is shown together with

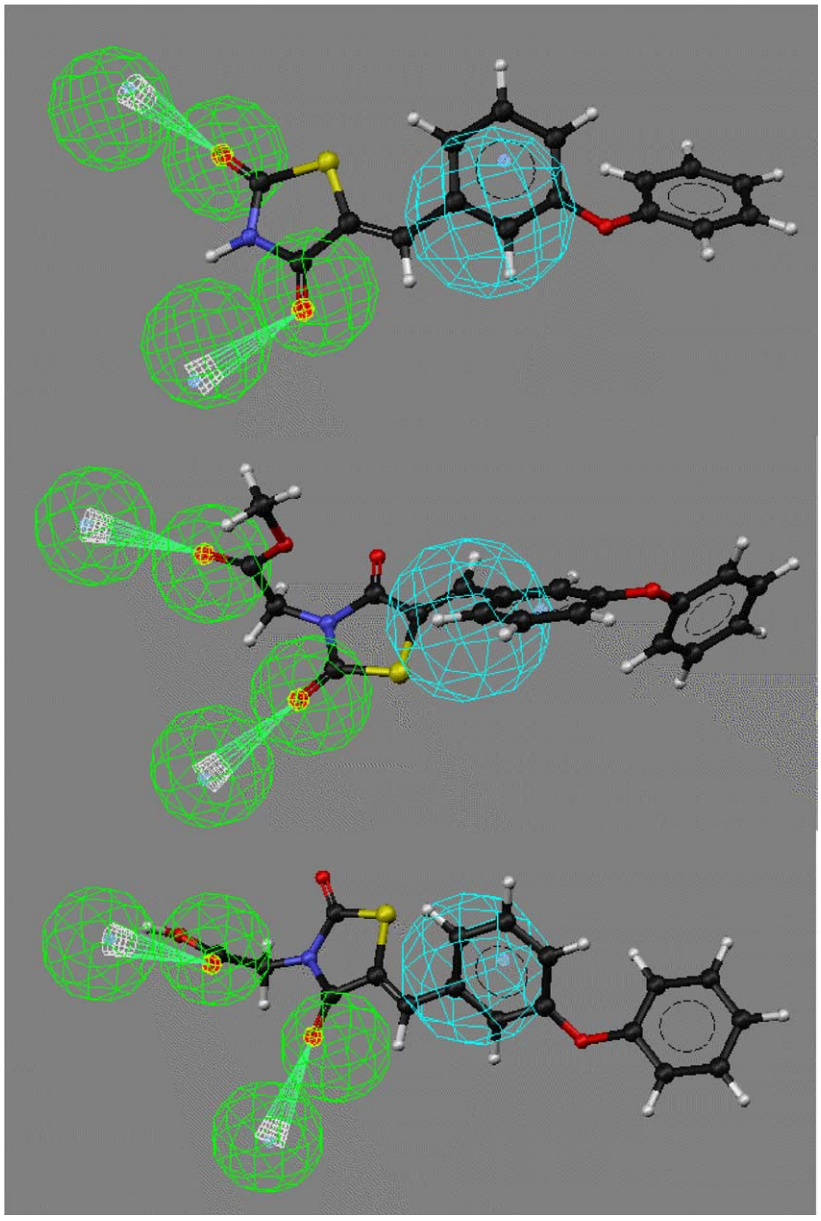


Figure 3. Compounds **2a**, **3a** and **4a** (from the top to the bottom) mapped on a common features pharmacophore model (Green: H bond acceptor; Cyan: lipophilic region).

the most important interacting amino acids within the binding site. As known, ARL2 inhibition strongly depends on charge–charge interactions and hydrogen bonds of inhibitors with the recognition region formed by Tyr48, His110, Trp111 and $NADP^+$ and on aromatic–aromatic interactions with the hydrophobic region of the ALR2 active site.[2,8,9,13,14,19] Most of these bonds as well as new possibilities for interaction were evidenced when the 5-arylidene-2,4-thiazolidinediones under study were docked into the active site of the enzyme (Figs. 4–6). When measuring the distances between the atoms belonging to their polar part and the surrounding amino acids, additional and alternative possibilities for the formation of hydrogen bonds could be observed. Two novel hydrogen bonds for inhibitor **4a** were enabled by Cys298; the median phenyl ring was centred between the hydrophobic Pro218, Phe122 and Trp219 (Fig. 5).

Figure 6 shows the image of the docked compound **4g**. This complex revealed Trp20 a new hydrogen bond partner for one of the cyclic imide carbonyl oxygens; Cys298 and Tyr48 could interact with the carboxylate moiety and the phenyl substituent was surrounded by the hydrophobic side chains of Trp20, Pro218 and Trp219.

It is worth mentioning that LigandFit keeps the protein rigid during the docking procedure. Thus, other possible H-bonds between the enzyme and our inhibitors might not be evidenced in these experimental conditions and slightly enlarged distance compared to the boundary

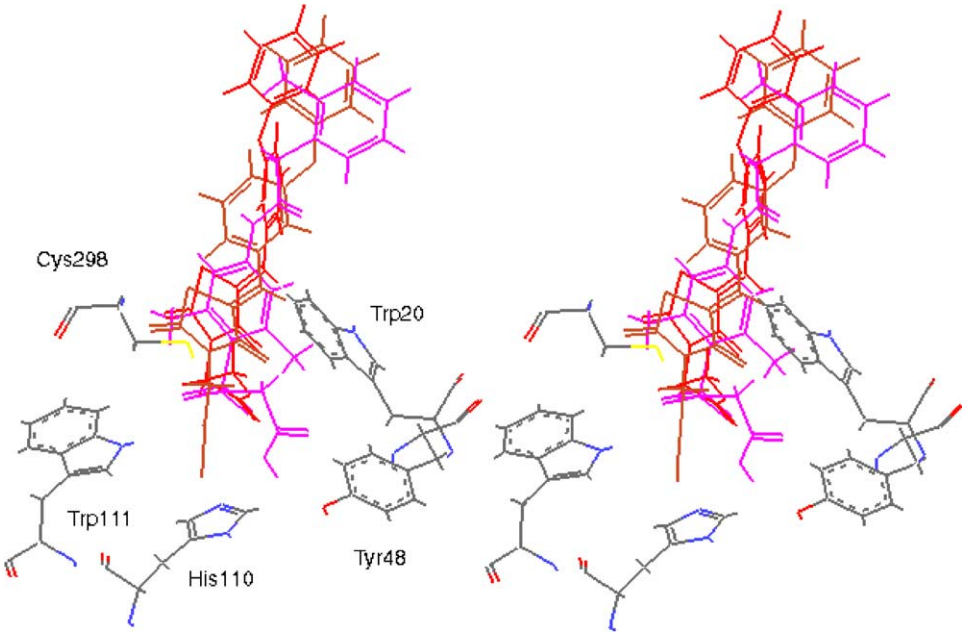


Figure 4. Superposition of docked structures of compounds **3a** (red) and **4a** (orange) with the bound conformation structure of Idd384 (purple) (relaxed stereo view).

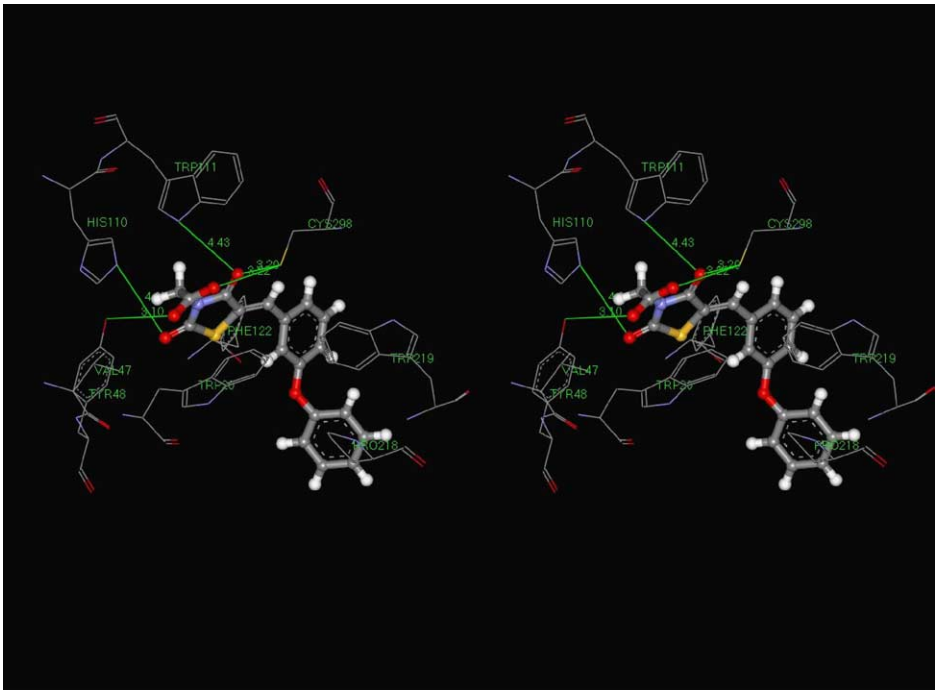


Figure 5. Possibilities of hydrogen bond interactions of docked inhibitor **4a** in the ALR2 active site.

values for hydrogen bonds had to be accepted as possible new interactions.

No explanation could be provided by this docking methodology concerning the high activity of ester derivative **3a** in comparison with free acid **4a**. Although possibilities of hydrogen bonds between the carbonyl oxygen atoms and the ALR2 active site were observed, no additional highly favourable interactions, which might compensate the obvious lack of charge–charge interaction with $NADP^+$, occurred.

4. Conclusions

The ALR2 inhibitory data reported here confirmed that 5-arylidene-2,4-thiazolidinediones can be considered as promising ARIs and allowed us to extend their structure–activity relationships.

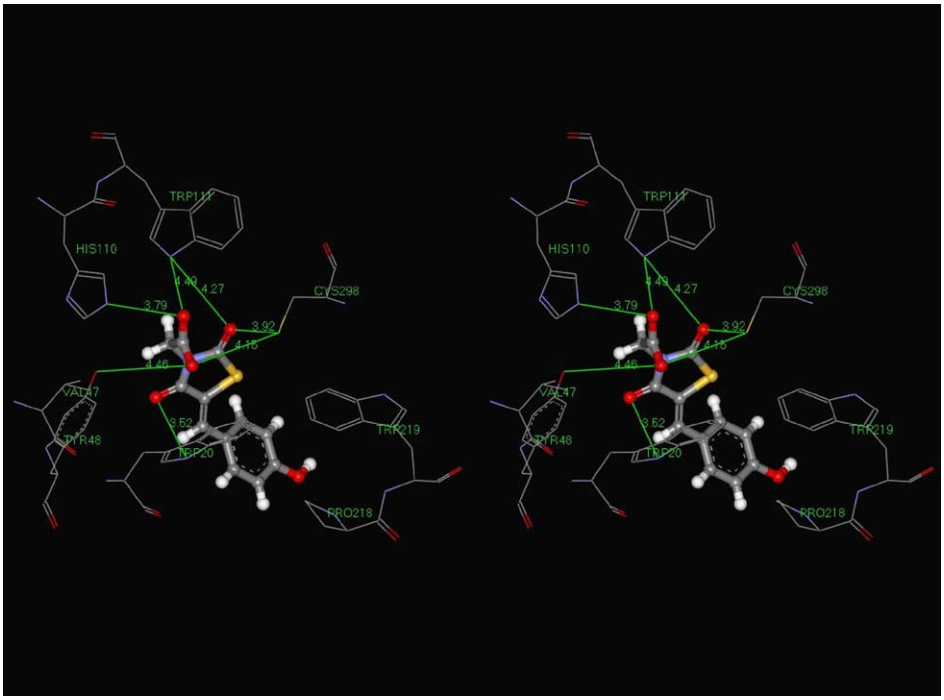


Figure 6. Possibilities of hydrogen bond interactions of docked inhibitor **4g** in the ALR2 active site.

The introduction of an additional aromatic ring or an H-bond donor group on the 5-benzylidene ring was shown to be very favourable for the ALR2 inhibitory effectiveness of these compounds.

The presence of the carboxylic anionic head of the N-3 acetic chain provided the highest activity levels and allowed the identification of some potent inhibitors, whose effectiveness was higher than that of sorbinil and similar to that of ponalrestat. In addition, our results indicated that the substituent on N-3 could be a polar group able to get to and bind the polar recognition region of ALR2 or a hydrogen atom, although in these cases the inhibitory potency decreased.

The inhibition data obtained were rationalized by means of molecular docking simulations into the ALR2 active site, which were in agreement with the above-mentioned structure–activity relationships. The molecular modelling experiments provided a pharmacophoric model for this class of ARIs.

5. Experimental

5.1. 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. IR spectra were obtained with a Perkin–Elmer 683 spectrophotometer as Nujol or esachlorobutadiene mulls. ^{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 the internal standard Me_4Si and refer to $CDCl_3$ or 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.

5.2. General method for the synthesis of 5-arylidene-2,4-thiazolidinediones 2c–g, j, k

A mixture of 2,4-thiazolidinedione (2.4 g, 20 mmol), aldehyde (20 mmol), piperidine (1.4 g, 16 mmol) and EtOH (150 mL) was refluxed for 24 h. The reaction mixture was poured into H_2O and acidified with AcOH to give **2c**–**g**, **j**, **k** as solids, which were recrystallized from methanol.

5.2.1. 5-(4-Benzyloxybenzylidene)-2,4-thiazolidinedione (2c). Yield 96%; mp 199–202 °C; ^{1}H NMR (DMSO-d_6): δ 5.19 (s, 2H, CH_2); 7.18 (m, 2H, arom); 7.38–7.45 (m, 5H, arom); 7.56 (m, 2H, arom); 7.75 (s, 1H, CH); 12.41 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 69.5 (CH_2); 120.4 (5-C); 115.7, 127.9, 128.1, 128.6, 131.9 (CH arom); 132.2 (CH methylidene); 125.7, 136.5, 160.1 (Cq arom); 167.5, 168.1 (CO); Anal. ($C_{17}H_{13}NO_3S$) C, H, N.

5.2.2. 5-Biphenyl-4-ylmethylene-2,4-thiazolidinedione (2d). Yield 72%; mp 249 °C; ^{1}H NMR (DMSO-d_6): δ 7.41–7.52 (m, 3H, arom); 7.68–7.76 (m, 4H, arom); 7.87 (m, 2H, arom); 7.84 (s, 1H, CH); 11.77 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 123.6 (5-C); 127.1, 127.7, 128.6, 129.5, 131.7 (CH arom); 131.1 (CH methylidene); 132.3, 139.0, 142.1 (Cq arom); 167.7, 168.3 (CO); Anal. ($C_{16}H_{11}NO_2S$) C, H, N.

5.2.3. 5-Naphthalen-1-ylmethylene-2,4-thiazolidinedione (2e). Yield 88%; mp 202 °C; ^{1}H NMR (DMSO-d_6): δ 7.36–7.51 (m, 4H, arom); 7.74 (m, 2H, arom); 7.95 (m, 1H, arom); 8.34 (s, 1H, CH); 11.98 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 123.1, 125.3, 126.1, 126.6, 127.2, 128.4, 128.6 (CH arom); 126.9 (5-C); 130.5 (CH methylidene); 130.1, 130.8, 133.1 (Cq arom); 166.6, 167.8 (CO); Anal. ($C_{14}H_9NO_2S$) C, H, N.

5.2.4. 5-(3-Hydroxybenzylidene)-2,4-thiazolidinedione (2f). Yield 94%; mp 265 °C; ^{1}H NMR (DMSO-d_6): δ 6.89 (d, J = 7.8 Hz, 1H, arom); 6.99 (s, 1H, arom); 7.04 (d, J = 8.4 Hz, 1H, arom); 7.35 (dd, J = 8.4 and 7.8 Hz, 1H, arom); 7.70 (s, 1H, CH); 9.96 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 116.0, 117.8, 121.4, 130.5 (CH arom); 123.5 (5-C); 132.0 (CH methylidene); 134.3, 157.9 (Cq arom); 167.5, 168.1 (CO); Anal. ($C_{10}H_7NO_3S$) C, H, N.

5.2.5. 5-(4-Hydroxybenzylidene)-2,4-thiazolidinedione (2g). Yield 68%; mp 252 °C; ^{1}H NMR (DMSO-d_6): δ 6.93 (m, 2H, arom); 7.47 (m, 2H, arom); 7.72 (s, 1H, CH); 10.33 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 116.3, 132.2 (CH arom); 123.9 (5-C); 132.3 (CH methylidene); 119.0, 159.9 (Cq arom); 167.4, 168.0 (CO); Anal. ($C_{10}H_7NO_3S$) C, H, N.

5.2.6. 5-(3-Nitrobenzylidene)-2,4-thiazolidinedione (2j). Yield 47%; mp 245 °C; ^{1}H NMR (DMSO-d_6): δ 7.83 (dd, J = 7.8 and 7.5 Hz, 1H, arom); 7.94 (s, 1H, CH); 8.02 (d, J = 7.5 Hz, 1H, arom); 8.30 (d, J = 7.8 Hz, 1H, arom); 8.45 (s, 1H, arom); ^{13}C NMR (DMSO-d_6): δ 124.2, 124.3, 129.3, 135.3 (CH arom); 126.6 (5-C); 130.8 (CH methylidene); 134.7, 148.2 (Cq arom); 166.9, 167.1 (CO); Anal. ($C_{10}H_6N_2O_4S$) C, H, N.

5.2.7. 5-(4-Nitrobenzylidene)-2,4-thiazolidinedione (2k). Yield 58%; mp 280 °C; ^{1}H NMR (DMSO-d_6): δ 7.88 (s, 1H, CH); 7.91 (m, 2H, arom); 8.36 (m, 2H, arom); ^{13}C NMR (DMSO-d_6): δ 124.2, 129.0 (CH arom); 128.0 (5-C); 130.8 (CH methylidene); 139.3, 147.4 (Cq arom); 166.9, 167.2 (CO); Anal. ($C_{10}H_6N_2O_4S$) C, H, N.

5.2.8. 5-(3-Aminobenzylidene)-2,4-thiazolidinedione (2h). Tin(II) chloride (6.07 g, 32 mmol), was added to a solution of 5-(3-nitrobenzylidene)-2,4-thiazolidinedione (**2j**) (1.6 g, 6.4 mmol) in ethanol and the mixture was refluxed for 2 h. After evaporation to dryness in vacuo, a saturated solution of sodium hydrogencarbonate was added to the crude solid. The mixture was then extracted with ethyl acetate, the organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The solid was recrystallized from methanol. Yield 42%; mp 215 °C; ^{1}H NMR (DMSO-d_6): δ 7.21 (m, 1H, arom); 7.25 (m, 1H, arom); 7.29 (m, 1H, arom); 7.70 (m, 1H, arom); 8.13 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 114.2, 116.5, 118.4, 129.9 (CH arom); 122.5 (5-C); 132.9 (CH methylidene); 133.8, 149.6 (Cq arom); 167.6, 167.8 (CO); Anal. ($C_{10}H_8N_2O_2S$) C, H, N.

5.2.9. 5-(4-Aminobenzylidene)-2,4-thiazolidinedione (2i). The procedure was the same as reported for compound **2h**, starting from 5-(4-nitrobenzylidene)-2,4-thiazolidinedione (**2k**). Yield 38 %; mp 231 °C; ^{1}H NMR (DMSO-d_6): δ 6.64 (m, 2H, arom); 7.27 (m, 2H, arom); 7.51 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 114.6, 132.3 (CH arom); 122.2 (5-C); 132.5 (CH methylidene); 138.2, 151.1 (Cq arom); 167.2, 167.6 (CO); Anal. ($C_{10}H_8N_2O_2S$) C, H, N.

5.3. General method for the synthesis of (5-arylidene-2,4-dioxothiazolidin-3-yl)acetic acids methyl esters 3b–g, j, k

A mixture of 5-arylidene-2,4-thiazolidinedione **2** (10 mmol), methyl bromoacetate (3.06 g, 20 mmol) and potassium carbonate (2.76 g, 20 mmol) in acetone (120 mL) was refluxed for 24 h. After cooling, the inorganic salts were filtered off; then the solvent was evaporated under reduced pressure and the solid residue was recrystallized from methanol providing pure acetate **3**.

5.3.1. [2,4-Dioxo-5-(4-phenoxybenzylidene)thiazolidin-3-yl]acetic acid methyl ester (3b). Yield 72%; mp 113–115 °C; ^{1}H NMR ($CDCl_3$): δ 3.80 (s, 3H, CH_3); 4.51 (s, 2H, CH_2); 7.05–7.10 (m, 3H, arom); 7.21–7.25 (m, 2H, arom); 7.42–7.51 (m, 4H, arom); 7.91 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 41.8 (CH_2); 52.5 (CH_3); 119.2 (5-C); 118.3, 119.9, 124.5, 129.9, 132.1 (CH arom); 133.8 (CH methylidene); 127.5, 155.5, 159.9 (Cq arom); 165.4, 166.5, 167.1 (CO); Anal. ($C_{19}H_{15}NO_5S$) C, H, N.

5.3.2. [5-(4-Benzyloxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid methyl ester (3c). Yield 88%; mp 123–125 °C; ^{1}H NMR ($CDCl_3$): δ 3.80 (s, 3H, CH_3); 4.51 (s, 2H, CH_2); 5.15 (s, 2H, CH_2O); 7.07 (m, 2H, arom); 7.42–7.48 (m, 7H, arom); 7.91 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 41.9 (CH_2); 52.8 (CH_3); 70.2 (CH_2O); 118.0 (5-C); 115.6, 127.5, 128.3, 128.7, 132.0 (CH arom); 134.6 (CH methylidene); 125.8, 136.0, 160.8 (Cq arom); 165.7, 166.8, 167.6 (CO); Anal. ($C_{20}H_{17}NO_5S$) C, H, N.

5.3.3. (5-Biphenyl-4-ylmethylene-2,4-dioxothiazolidin-3-yl)-tic acid methyl ester (3d). Yield 90%; mp 145 °C; ^{1}H NMR ($CDCl_3$): δ 3.81 (s, 3H, CH_3); 4.52 (s, 2H, CH_2); 7.41–7.48 (m, 3H, arom); 7.61 (m, 4H, arom); 7.73 (m, 2H, arom); 7.99 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 41.9 (CH_2); 52.9 (CH_3); 120.6 (5-C); 127.1, 127.8, 128.2, 129.0, 130.8 (CH arom); 134.3 (CH methylidene); 131.9, 139.6, 143.4 (Cq arom); 165.6, 166.7, 167.4 (CO); Anal. ($C_{19}H_{15}NO_4S$) C, H, N.

5.3.4. (5-Naphthalen-1-ylmethylene-2,4-dioxothiazolidin-3-yl)acetic acid methyl ester (3e). Yield 52%; mp 124 °C; ^{1}H NMR ($CDCl_3$): δ 3.83 (s, 3H, CH_3); 4.55 (s, 2H, CH_2); 7.57–7.61 (m, 4H, arom); 7.95 (m, 2H, arom); 8.12 (m, 1H, arom); 8.69 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 41.9 (CH_2); 52.9 (CH_3); 123.4, 125.3, 126.6,

126.8, 127.4, 128.9, 131.3 (CH arom); 124.1 (5-C); 131.9 (CH methylidene); 130.3, 131.7, 133.7 (Cq arom); 165.1, 166.7, 167.8 (CO); Anal. ($C_{17}H_{13}NO_4S$) C, H, N.

5.3.5. [5-(3-Hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid methyl ester (3f). This was synthesized as above described, adding methyl bromoacetate drop wise during 1.5 h to a solution of compound **2f** and potassium carbonate in acetone. Yield 86%; mp 198 °C; ^{1}H NMR ($CDCl_3$): δ 3.82 (s, 3H, CH_3); 4.52 (s, 2H, CH_2); 6.94 (m, 1H, arom); 7.05 (m, 1H, arom); 7.28–7.36 (m, 2H, arom); 7.83 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 41.9 (CH_2); 53.0 (CH_3); 116.7, 118.1, 122.7, 130.4 (CH arom); 121.8 (5-C); 134.7 (CH methylidene); 134.4, 156.6 (Cq arom); 165.6, 166.9, 167.2 (CO); Anal. ($C_{13}H_{11}NO_5S$) C, H, N.

5.3.6. [5-(4-Hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid methyl ester (3g). This was synthesized as above described, adding methyl bromoacetate drop wise during 1.5 h to a solution of compound **2g** and potassium carbonate in acetone. Yield 78%; mp 212 °C; ^{1}H NMR ($CDCl_3$): δ 3.81 (s, 3H, CH_3); 4.51 (s, 2H, CH_2); 6.93 (m, 2H, arom); 7.37 (m, 2H, arom); 7.82 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 41.2 (CH_2); 52.1 (CH_3); 116.1, 132.0 (CH arom); 123.9 (5-C); 134.6 (CH methylidene); 118.8, 159.8 (Cq arom); 166.3, 167.0, 167.5 (CO); Anal. ($C_{13}H_{11}NO_5S$) C, H, N.

5.3.7. [5-(3-Nitrobenzylidene)-2,4-dioxothiazolidin-3-yl]-acetic acid methyl ester (3j). Yield 78%; mp 211 °C; ^{1}H NMR (DMSO-d_6): δ 3.81 (s, 3H, CH_3); 4.53 (s, 2H, CH_2); 7.71 (dd, J = 8.1 and 7.8 Hz, 1H, arom); 7.83 (d, J = 7.8 Hz, 1H, arom); 7.97 (s, 1H, CH); 8.30 (dd, J = 8.1 and 3.0 Hz, 1H, arom); 8.39 (d, J = 3.0 Hz, 1H, arom); ^{13}C NMR (DMSO-d_6): δ 42.2 (CH_2); 52.6 (CH_3); 123.5 (5-C); 124.8, 124.9, 131.9, 135.4 (CH arom); 130.9 (CH methylidene); 134.4, 148.3 (Cq arom); 164.5, 166.2, 167.0 (CO); Anal. ($C_{13}H_{10}N_2O_6S$) C, H, N.

5.3.8. [5-(4-Nitrobenzylidene)-2,4-dioxothiazolidin-3-yl]-acetic acid methyl ester (3k). Yield 82%; mp 255 °C; ^{1}H NMR ($CDCl_3$): δ 3.82 (s, 3H, CH_3); 4.53 (s, 2H, CH_2); 7.69 (m, 2H, arom); 7.97 (s, 1H, CH); 8.35 (m, 2H, arom); ^{13}C NMR ($CDCl_3$): δ 42.1 (CH_2); 52.9 (CH_3); 121.9 (5-C); 124.4, 131.3 (CH arom); 130.6 (CH methylidene); 139.0, 148.2 (Cq arom); 164.7, 165.8, 167.3 (CO); Anal. ($C_{13}H_{10}N_2O_6S$) C, H, N.

5.3.9. [5-(3-Aminobenzylidene)-2,4-dioxothiazolidin-3-yl]-acetic acid methyl ester (3h). Tin(II) chloride (6.07 g, 32 mmol), was added to a solution of [5-(3-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid methyl ester (**3j**) (2.06 g, 6.4 mmol) in ethanol and the mixture was refluxed for 2 h. After evaporation to dryness in vacuo, a saturated solution of sodium hydrogencarbonate was added to the crude solid. The mixture was then extracted with ethyl acetate, the organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The solid was recrystallized from methanol. Yield 52%; mp 240 °C; ^{1}H NMR (DMSO-d_6): δ 3.38 (s, 3H, CH_3); 4.49 (s, 2H, CH_2); 5.39 (br s, 2H, NH_2), 6.69–6.78 (m, 3H, arom); 7.16 (m, 1H, arom); 7.78 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 41.8 (CH_2), 52.5 (CH_3), 114.2, 116.7, 118.3, 129.7 (CH arom); 119.3 (5-C); 133.1, 149.3 (Cq arom); 135.1 (CH methylidene); 164.7, 164.9, 167.0 (CO); Anal. ($C_{13}H_{12}N_2O_4S$) C, H, N.

5.3.10. [5-(4-Aminobenzylidene)-2,4-dioxothiazolidin-3-yl]-acetic acid methyl ester (3i). The procedure was the same as reported for compound **3h**, starting from [5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid methyl ester (**3k**). Yield 47%; mp 244 °C; ^{1}H NMR (DMSO-d_6): δ 3.68 (s, 3H, CH_3); 4.45 (s, 2H, CH_2); 6.23 (br s, 2H, NH_2), 6.66 (m, 2H, arom); 7.33 (m, 2H, arom); 7.76 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 42.0 (CH_2), 52.6 (CH_3), 114.0, 133.1 (CH arom); 119.5 (5-C); 134.8, 150.6 (Cq arom); 135.4 (CH methylidene); 167.4, 167.7, 168.9 (CO); Anal. ($C_{13}H_{12}N_2O_4S$) C, H, N.

5.4. General method for the synthesis of (5-arylidene-2,4-dioxothiazolidin-3-yl)acetic acids 4b, d–g, j, k

A mixture of acetate **3** (10 mmol), glacial AcOH (40 mL) and HCl 12 N (10 mL) was refluxed 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 **4**.

5.4.1. [2,4-Dioxo-5-(4-phenoxybenzylidene)thiazolidin-3-yl]acetic acid (4b). Yield 95%; mp 210 °C; ^{1}H NMR (DMSO-d_6): δ 4.38 (s, 2H, CH_2); 7.16 (m, 4H, arom); 7.28 (m, 1H, arom); 7.49 (m, 2H, arom); 7.70 (m, 2H, arom); 7.98 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 42.4 (CH_2); 118.9 (5-C); 118.3, 120.0, 124.8, 130.5, 132.7 (CH arom); 133.4 (CH methylidene); 127.5, 155.1, 159.4 (Cq arom); 165.2, 167.1, 168.1 (CO); Anal. ($C_{18}H_{13}NO_5S$) C, H, N.

5.4.2. (5-Biphenyl-4-ylmethylene-2,4-dioxothiazolidin-3-yl)-acetic acid (4d). Yield 85%; mp 228–230 °C; ^{1}H NMR (DMSO-d_6): δ 4.41 (s, 2H, CH_2); 7.42–7.52 (m, 4H, arom); 7.76 (m, 3H, arom); 7.88 (m, 2H, arom); 8.10 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 42.3 (CH_2); 120.4 (5-C); 126.8, 127.5, 128.3, 129.1, 131.0 (CH arom); 133.5 (CH methylidene); 131.8, 138.7, 142.2 (Cq arom); 165.0, 166.8, 168.0 (CO); Anal. ($C_{18}H_{13}NO_4S$) C, H, N.

5.4.3. (5-Naphthalen-1-ylmethylene-2,4-dioxothiazolidin-3-yl)acetic acid (4e). Yield 88%; mp 221 °C; ^{1}H NMR (DMSO-d_6): δ 4.44 (s, 2H, CH_2); 7.67–7.76 (m, 4H, arom); 8.11–8.14 (m, 3H, arom); 8.65 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 43.0 (CH_2); 124.1, 126.3, 127.2, 127.6, 128.2, 129.5, 131.6 (CH arom); 125.1 (5-C); 131.8 (CH methylidene); 130.7, 131.8, 133.9 (Cq arom); 165.2, 168.0, 168.6 (CO); Anal. ($C_{16}H_{11}NO_4S$) C, H, N.

5.4.4. [5-(3-Hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (4f). Yield 80%; mp 225 °C; ^{1}H NMR (DMSO-d_6): δ 4.38 (s, 2H, CH_2); 6.93 (d, J = 8.1 Hz, 1H, arom); 7.03 (s, 1H, arom); 7.09 (d, J = 7.8 Hz, 1H, arom); 7.36 (dd, J = 8.1 and 7.8 Hz, 1H, arom); 7.90 (s, 1H, CH); 9.91 (br s, 1H, OH); ^{13}C NMR

(DMSO-d_6): δ 41.7 (CH_2); 115.5, 117.6, 120.9, 129.9 (CH arom); 119.9 (5-C); 133.6 (CH methylidene); 133.4, 157.4 (Cq arom); 164.5, 166.4, 167.4 (CO); Anal. ($C_{12}H_9NO_5S$) C, H, N.

5.4.5. [5-(4-Hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (4g). Yield 74%; mp 257 °C; ^{1}H NMR (DMSO-d_6): δ 4.36 (s, 2H, CH_2); 6.97 (m, 2H, arom); 7.54 (m, 2H, arom); 7.90 (s, 1H, CH); 10.41 (br s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 42.1 (CH_2); 116.4, 132.7 (CH arom); 116.1, 160.3 (Cq arom); 123.7 (5-C); 134.3 (CH methylidene); 165.2, 167.0, 167.9 (CO); Anal. ($C_{12}H_9NO_5S$) C, H, N.

5.4.6. [5-(3-Nitrobenzylidene)-2,4-dioxothiazolidin-3-yl]-acetic acid (4j). Yield 80%; mp 235 °C; ^{1}H NMR (DMSO-d_6): δ 4.41 (s, 2H, CH_2); 7.84 (dd, J = 9.0 and 9.0 Hz, 1H, arom); 8.06 (d, J = 9.0 Hz, 1H, arom); 8.17 (s, 1H, CH); 8.32 (dd, J = 9.0 and 3.0 Hz, 1H, arom); 8.51 (s, 1H, arom); ^{13}C NMR (DMSO-d_6): δ 42.4 (CH_2); 123.6 (5-C); 124.7, 124.8, 131.6, 135.4 (CH arom); 130.9 (CH methylidene); 134.4, 148.2 (Cq arom); 164.6, 166.3, 167.8 (CO); Anal. ($C_{12}H_8N_2O_6S$) C, H, N.

5.4.7. [5-(4-Nitrobenzylidene)-2,4-dioxothiazolidin-3-yl]-acetic acid (4k). Yield 93%; mp 271 °C; ^{1}H NMR (DMSO-d_6): δ 4.44 (s, 2H, CH_2); 7.93 (m, 2H, arom); 8.10 (s, 1H, CH); 8.37 (m, 2H, arom); ^{13}C NMR (DMSO-d_6): δ 42.4 (CH_2); 124.1 (5-C); 124.2, 131.0 (CH arom); 131.3 (CH methylidene); 138.9, 147.7 (Cq arom); 164.6, 166.3, 167.6 (CO); Anal. ($C_{12}H_8N_2O_6S$) C, H, N.

5.4.8. [5-(4-Benzyloxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (4c). A mixture of 2,4-thiazolidinedione (1 g, 8.5 mmol), methyl bromoacetate (2.6 g, 17 mmol) and potassium carbonate (2.35 g, 17 mmol) was refluxed for 24 h; then the solvent was evaporated under reduced pressure. The residue was washed with methanol to provide (2,4-dioxothiazolidin-3-yl)acetic acid methyl ester (**7**) as oil (1.29 g, yield 80%); ^{1}H NMR ($CDCl_3$): δ 3.77 (s, 3H, CH_3); 4.05 (s, 2H, 5-CH_2); 4.36 (s, 2H, N–CH_2). A mixture of **7** (0.8 g, 4.23 mmol) glacial AcOH (17 mL) and HCl 2 N (4.2 mL) was refluxed for 2 h. After evaporation in vacuo, the crude mixture was refluxed again with AcOH (17 mL) and HCl (4.2 mL). After evaporation to dryness in vacuo, the crude oil was washed with H_2O and then with ethanol to provide pure (2,4-dioxothiazolidin-3-yl)acetic acid (**8**) as oil (0.44 g, yield 60%); ^{1}H NMR ($CDCl_3$): δ 4.06 (s, 2H, 5-CH_2); 4.42 (s, 2H, N–CH_2). Piperidine (0.17 g, 2.03 mmol) and 4-benzyloxybenzaldehyde (0.54 g, 2.54 mmol) were added to a solution of **8** (0.44 g, 2.54 mmol) in ethanol and the mixture was refluxed for 24 h. The reaction mixture was then poured into H_2O and acidified with AcOH to give **4c** as solid, which was recrystallized from methanol. Yield 41%; mp 262 °C; ^{1}H NMR (DMSO-d_6): δ 4.40 (s, 2H, CH_2); 5.23 (s, 2H, CH_2O); 7.22 (m, 2H, arom); 7.39–7.51 (m, 5H, arom); 7.65 (m, 2H, arom); 7.99 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 42.1 (CH_2); 69.5 (CH_2O); 117.5 (5-C); 115.8, 127.8, 128.0, 128.5, 132.4 (CH arom); 133.8 (CH methylidene); 125.5, 136.4, 160.4 (Cq arom); 165.1, 167.0, 167.9 (CO); Anal. ($C_{19}H_{15}NO_5S$) C, H, N.

5.5. General method for the synthesis of methyl 4-(5-arylidene-2,4-dioxothiazolidin-3-yl)-2-butenoic acids methyl esters 5a–d

A mixture of 5-arylidene-2,4-thiazolidinedione **2** (10 mmol), methyl 4-bromocrotonate (3.58 g, 20 mmol) and potassium carbonate (2.76 g, 20 mmol) in acetone (130 mL) was refluxed for 24 h. After cooling, the inorganic salts were filtered off; then the solvent was evaporated under reduced pressure and the solid residue was recrystallized from ethanol providing pure 2-butenoate **5**.

5.5.1. 4-[2,4-Dioxo-5-(3-phenoxybenzylidene)thiazolidin-3-yl]-2-butenoic acid methyl ester (5a). Yield 75%; mp 107–110 °C; ^{1}H NMR ($CDCl_3$): δ 3.74 (s, 3H, CH_3); 4.48 (dd, J = 6.0 and 1.5 Hz, 2H, CH_2); 5.98 (dt, J = 15.0 and 1.5 Hz, 1H, CH); 6.85 (dt, J = 15.0 and 6.0 Hz, 1H, CH); 7.05–7.12 (m, 3H, arom); 7.23–7.31 (m, 3H, arom); 7.41–7.48 (m, 3H, arom); 7.86 (s, 1H, CH methylidene); ^{13}C NMR ($CDCl_3$): δ 41.8 (CH_2); 51.8 (CH_3); 119.2, 119.6, 120.6, 124.2, 124.6, 130.0, 134.0 (CH arom); 121.7 (5-C); 123.6, 130.6 (CH=CH); 139.3 (CH methylidene); 134.6, 156.0, 158.3 (Cq arom); 165.4, 165.8, 166.5 (CO); Anal. ($C_{21}H_{17}NO_5S$) C, H, N.

5.5.2. 4-[2,4-Dioxo-5-(4-phenoxybenzylidene)thiazolidin-3-yl]-2-butenoic acid methyl ester (5b). Yield 72%; mp 103–105 °C; ^{1}H NMR ($CDCl_3$): δ 3.74 (s, 3H, CH_3); 4.50 (dd, J = 6.0 and 1.5 Hz, 2H, CH_2); 5.91 (dt, J = 15.0 and 1.5 Hz, 1H, CH); 6.81 (dt, J = 15.0 and 6.0 Hz, 1H, CH); 7.05–7.11 (m, 3H, arom); 7.22–7.26 (m, 3H, arom); 7.42–7.50 (m, 3H, arom); 7.90 (s, 1H, CH methylidene); ^{13}C NMR ($CDCl_3$): δ 41.7 (CH_2); 51.7 (CH_3); 118.2, 120.1, 124.6, 130.0, 134.0 (CH arom); 118.9 (5-C); 123.5, 132.2 (CH=CH); 139.4 (CH methylidene); 127.3, 155.3, 160.0 (Cq arom); 165.6, 165.8, 167.2 (CO); Anal. ($C_{21}H_{17}NO_5S$) C, H, N.

5.5.3. 4-[5-(4-Benzyloxybenzylidene)-2,4-dioxothiazolidin-3-yl]-2-butenoic acid methyl ester (5c). Yield 98%; mp 120–122 °C; ^{1}H NMR (DMSO-d_6): δ 3.44 (s, 3H, CH_3); 4.41 (dd, J = 6.0 and 3.0 Hz, 2H, CH_2); 5.15 (s, 2H, CH_2O); 5.87 (dt, J = 15.0 and 3.0 Hz, 1H, CH); 6.82 (dt, J = 15.0 and 6.0 Hz, 1H, CH); 7.15 (m, 2H, arom); 7.34–7.44 (m, 5H, arom); 7.57 (m, 2H, arom); 7.85 (s, 1H, CH methylidene); ^{13}C NMR (DMSO-d_6): δ 42.2 (CH_2); 51.8 (CH_3); 69.7 (CH_2O); 116.0, 126.0, 128.0, 128.3, 128.7 (CH arom); 118.5 (5-C); 121.8, 133.1 (CH=CH); 132.5 (CH methylidene); 125.8, 136.3, 160.8 (Cq arom); 166.2, 167.5, 168.5 (CO); Anal. ($C_{22}H_{19}NO_5S$) C, H, N.

5.5.4. 4-(5-Biphenyl-4-ylmethylene-2,4-dioxothiazolidin-3-yl)-2-butenoic acid methyl ester (5d). Yield 90%; mp 158–160 °C; ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 3H, CH_3); 4.52 (dd, J = 5.7 and 2.1 Hz, 2H, CH_2); 6.00 (dt, J = 16.2 and 2.1 Hz, 1H, CH); 6.88 (dt, J = 16.2 and 5.7 Hz, 1H, CH); 7.42–7.52 (m, 3H, arom); 7.60–7.66 (m, 4H, arom); 7.75 (m, 2H, arom); 7.98 (s, 1H, CH

methylidene); ^{13}C NMR ($CDCl_3$): δ 41.8 (CH_2); 51.8 (CH_3); 120.4 (5-C); 127.1, 127.8, 128.2, 129.0, 134.2 (CH arom); 123.6, 130.8 (CH=CH); 139.4 (CH methylidene); 128.9, 131.8, 143.4 (Cq arom); 165.6, 166.9, 167.3 (CO); Anal. ($C_{21}H_{17}NO_4S$) C, H, N.

5.6. General method for the synthesis of 4-(5-arylidene-2,4-dioxothiazolidin-3-yl)-2-butenoic acids 6a, b, d, g

A mixture of methyl ester **5** (10 mmol), glacial AcOH (40 mL) and HCl 12 N (10 mL) was refluxed for 2 h. After evaporation to dryness in vacuo, the crude solid was washed with H_2O and recrystallized from methanol providing pure carboxylic acid **6**.

5.6.1. 4-[2,4-Dioxo-5-(3-phenoxybenzylidene)thiazolidin-3-yl]-2-butenoic acid (6a). Yield 92%; mp 155–157 °C; ^{1}H NMR (DMSO-d_6): δ 4.40 (dd, J = 5.1 and 2.0 Hz, 2H, CH_2); 5.81 (dt, J = 15.6 and 2.0 Hz, 1H, CH); 6.75 (dt, J = 15.6 and 5.1 Hz, 1H, CH); 7.05–7.21 (m, 3H, arom); 7.35–7.44 (m, 4H, arom); 7.50–7.55 (m, 2H, arom); 7.90 (s, 1H, CH methylidene); ^{13}C NMR (DMSO-d_6): δ 41.8 (CH_2); 118.9, 119.2, 120.3, 124.1, 124.8, 130.2, 140.4 (CH arom); 122.3 (5-C); 123.0, 132.4 (CH=CH); 131.0 (CH methylidene); 134.8, 155.7, 157.6 (Cq arom); 165.0, 166.3, 166.7 (CO); Anal. ($C_{20}H_{15}NO_5S$) C, H, N.

5.6.2. 4-[2,4-Dioxo-5-(4-phenoxybenzylidene)thiazolidin-3-yl]-2-butenoic acid (6b). Yield 50%; mp 190–192 °C; ^{1}H NMR ($CDCl_3$): δ 4.52 (dd, J = 5.1 and 2.0 Hz, 2H, CH_2); 5.94 (dt, J = 15.9 and 2.0 Hz, 1H, CH); 6.98 (dt, J = 15.9 and 5.1 Hz, 1H, CH); 7.00–7.05 (m, 3H, arom); 7.15 (m, 2H, arom); 7.29–7.33 (m, 4H, arom); 7.90 (s, 1H, CH methylidene); ^{13}C NMR ($CDCl_3$): δ 41.3 (CH_2); 117.8, 119.6, 124.2, 129.6, 133.4 (CH arom); 118.5 (5-C); 124.0, 131.8 (CH=CH); 138.5 (CH methylidene); 126.9, 154.8, 159.5 (Cq arom); 165.0, 165.1, 166.6 (CO); Anal. ($C_{20}H_{15}NO_5S$) C, H, N.

5.6.3. 4-(5-Biphenyl-4-ylmethylene-2,4-dioxothiazolidin-3-yl)-2-butenoic acid (6d). Yield 45%; mp 248–250 °C; ^{1}H NMR (DMSO-d_6): δ 4.45 (dd, J = 6.0 and 2.0 Hz, 2H, CH_2); 5.85 (dt, J = 15.0 and 2.0 Hz, 1H, CH); 6.80 (dt, J = 15.0 and 6.0 Hz, 1H, CH); 7.46–7.56 (m, 5H, arom); 7.76 (m, 2H, arom); 7.88 (m, 2H, arom); 8.01 (s, 1H, CH methylidene); ^{13}C NMR (DMSO-d_6): δ 41.9 (CH_2); 121.0 (5-C); 126.8, 127.5, 128.3, 129.1, 132.7 (CH arom); 123.0, 130.9 (CH=CH); 140.7 (CH methylidene); 132.0, 138.7, 142.0 (Cq arom); 165.2, 166.4, 167.0 (CO); Anal. ($C_{20}H_{15}NO_4S$) C, H, N.

5.6.4. 4-[5-(4-Hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl]-2-butenoic acid (6g). This was synthesized as above described, starting from 4-[5-(4-benzyloxybenzylidene)-2,4-dioxothiazolidin-3-yl]-2-butenoic acid methyl ester (**5c**). Yield 40%; mp 292–295 °C; ^{1}H NMR (DMSO-d_6): δ 4.42 (dd, J = 5.8 and 1.2 Hz, 2H, CH_2); 5.80 (dt, J = 15.9 and 1.2 Hz, 1H, CH); 6.79 (dt, J = 15.9 and 5.8 Hz, 1H, CH); 6.93 (m, 2H, arom); 7.51 (m, 2H, arom); 7.87 (s, 1H, CH methylidene); 10.40 (br s, 1H, OH); 12.40 (br s, 1H, COOH); ^{13}C NMR (DMSO-d_6): δ 42.4 (CH_2); 117.2, 133.4 (CH arom); 123.6, 134.5 (CH=CH); 124.6 (5-C); 140.4 (CH methylidene); 117.3, 160.9 (Cq arom); 166.1, 167.0, 167.9 (CO); Anal. ($C_{14}H_{11}NO_5S$) C, H, N.

5.7. Molecular modelling

All calculations and graphic manipulations were performed on a SGI Octane double processor R10K workstation by means of the Catalyst 4.7 software package[30] for pharmacophore model building and the Cerius^2 4.9 software package[29] for docking. All the compounds used in this study were built using the 2D–3D sketcher of the program Catalyst. A representative family of conformations was generated for each molecule using the poling algorithm and the 'best quality conformational analysis' method. The parameter set was employed to perform all the conformational calculations derived from the CHARMm force field. Conformational diversity was emphasized by selection of the conformers that fell within 20 kcal/mol range above the lowest energy conformation found.

The docking approach was carried out with the tool LigandFit implemented in the Cerius^2 software package.[29] Calculations were performed using the force field cff 1.01. The site definition was derived from the bound ligand including the unoccupied grid points within 2.5 Å from heavy atoms and within 2 Å from hydrogen atoms of the ligand. The compounds were rigidly docked keeping all parameters of interaction energy minimization, rigid body minimization and site partition at their default values. A final energy minimization of the ligands in the protein followed the fitting process. Prioritization of the docked ligand orientations was achieved employing all seven scoring functions in Cerius^2 (Ligscore1, Ligscore2, PLP1, PLP2, JAIN, PMF and LUDI) using the predefined settings. Finally the scores resulting from these functions were combined through the computation of a consensus score value. The investigation of the best 100 poses (according to their consensus score) resulted in 19 different orientations of the selected ARIs according to the degree of superimposition onto Idd384.

5.8. Enzyme section

NADPH, DL-glyceraldehyde and dithiothreitol (DDT) were purchased from Sigma Chemical Co. DEAE-cellulose (DE-52) was obtained from Whatman. Sorbinil was a gift from Prof. 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.[31] 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 catalyzed 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 nonenzymatic 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.[32]

Appendix A. Experimental analyses

Compd		C (%)	H (%)	N (%)
2c	Calcd	65.58	4.21	4.50
	Found	65.55	4.48	4.17
2d	Calcd	68.31	3.94	4.98
	Found	68.13	4.14	4.69
2e	Calcd	65.87	3.55	5.49
	Found	65.54	3.71	5.29
2f	Calcd	54.29	3.19	6.33
	Found	54.02	3.36	6.12
2g	Calcd	54.29	3.19	6.33
	Found	54.46	3.11	6.07
2h	Calcd	54.53	3.66	12.72
	Found	54.28	3.91	12.45
2i	Calcd	54.53	3.66	12.72
	Found	54.39	3.87	12.51
2j	Calcd	48.00	2.42	11.19
	Found	47.78	2.65	11.00
2k	Calcd	48.00	2.42	11.19
	Found	48.34	2.58	10.95
3b	Calcd	61.78	4.09	3.79
	Found	61.47	4.19	3.63
3c	Calcd	62.65	4.47	3.65
	Found	62.98	4.61	3.46
3d	Calcd	64.58	4.28	3.96
	Found	64.25	4.54	3.72
3e	Calcd	62.37	4.00	4.28
	Found	62.21	3.83	4.35
3f	Calcd	53.24	3.78	4.78
	Found	53.14	3.97	4.54
3g	Calcd	53.24	3.78	4.78
	Found	53.00	4.05	4.58
3h	Calcd	53.42	4.14	9.58
	Found	53.29	4.28	9.27
3i	Calcd	53.42	4.14	9.58
	Found	53.48	4.16	9.36
3j	Calcd	48.45	3.13	8.69
	Found	48.16	3.32	8.46
3k	Calcd	48.45	3.13	8.69
	Found	48.17	3.28	8.57
4b	Calcd	60.84	3.69	3.94
	Found	60.58	3.82	3.71
4c	Calcd	61.78	4.09	3.79
	Found	61.58	4.33	3.68
4d	Calcd	63.71	3.86	4.13
	Found	63.49	3.94	4.02
4e	Calcd	61.33	3.54	4.47
	Found	61.00	3.78	4.28
4f	Calcd	51.61	3.25	5.02
	Found	51.39	3.58	4.86
4g	Calcd	51.61	3.25	5.02
	Found	51.80	3.01	5.22
4j	Calcd	46.76	2.62	9.09
	Found	46.91	2.84	8.90
4k	Calcd	46.76	2.62	9.09
	Found	46.58	2.90	8.98
5a	Calcd	63.79	4.33	3.54
	Found	63.51	4.47	3.32
5b	Calcd	63.79	4.33	3.54
	Found	63.42	4.51	3.39
5c	Calcd	64.53	4.68	3.42
	Found	64.36	4.88	3.21
5d	Calcd	66.48	4.52	3.69
	Found	66.09	4.39	3.88
6a	Calcd	62.98	3.96	3.67
	Found	63.11	3.90	3.75
6b	Calcd	62.98	3.96	3.67
	Found	62.76	4.01	3.42
6d	Calcd	65.74	4.14	3.83
	Found	65.95	4.00	3.68
6g	Calcd	55.08	3.63	4.59
	Found	54.69	3.75	4.32

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.02.026.

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