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Synthesis and Aldose Reductase Inhibitory Activity of 5-Arylidene-2,4-thiazolidinediones†

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Abstract—Several (*Z*)-5-arylidene-2,4-thiazolidinediones were synthesized and tested as aldose reductase inhibitors (ARIs). The most active of the *N*-unsubstituted derivatives (**2**) exerted the same inhibitory activity of Sorbinil. The introduction of an acetic side chain on N-3 of the thiazolidinedione moiety led to a marked increase in lending inhibitory activity, conducting to the discovery of a very potent ARI (**4c**), whose activity level ($IC_{50} = 0.13$ μM) was in the same range of Tolrestat. Moreover, the corresponding methyl esters (**3**), devoid of any acidic functionality, showed appreciable inhibitory activity similar to that of the *N*-unsubstituted compounds. It was also found that the substitution pattern on the 5-benzylidene moiety markedly influenced the activity of *N*-unsubstituted 2,4-thiazolidinediones **2**, compounds with substituents at the *meta* position being generally more effective than the *para*-substituted ones; however, this SAR was not evidenced in acetates **3** and acids **4**.

Introduction

Aldose reductase (EC 1.1.1.21, ALR2), a member of the aldo-keto reductase superfamily, is the first enzyme of the polyol pathway: it catalyzes the NADPH-dependent reduction of glucose to sorbitol, which in turn is oxidized by sorbitol dehydrogenase to fructose. ALR2 has low affinity for glucose, so there is little flux of glucose through the polyol pathway under normal conditions. However, under conditions of hyperglycaemia such as in diabetes, there is a marked increase of glucose metabolized by the polyol pathway in tissues possessing insulin-independent glucose transport. A large amount of evidence has demonstrated a link between enhanced metabolism of glucose through the polyol pathway and the onset and progression of long-term diabetic complications (retinopathy, cataracts, neuropathy and nephropathy).[1–5] Therefore, ALR2 inhibition has received attention as an attractive strategy to prevent or delay the onset and to minimize the seriousness of chronic diabetic complications.

Over the last 20 years, numerous structurally different compounds have been reported to inhibit ALR2, but many of them have produced little clinical benefit in human studies, because of unfavourable pharmacokinetic properties, toxic side-effects or poor potency.[1,3,6–11] Two main classes of orally active aldose reductase inhibitors (ARIs) have been clinically tested: cyclic imides (mostly hydantoins, for example Sorbinil) and carboxylic acids (for example Tolrestat). The carboxylic acid derivatives in vitro are very active; however, in vivo they are generally less active than imides, probably owing to their lower pK_a values that can result in less favourable pharmacokinetics. Currently, Epalrestat (ONO-2235) is the only ARI available on the market.[8,12]

There is a great interest in 2,4-thiazolidinedione derivatives as ARIs,[3,8,13–15] since they can be viewed as hydantoin bioisosters potentially free of the hypersensitivity reactions which are linked to the presence of the hydantoin system. Moreover, they are useful both in the treatment of non insulin-dependent diabetes mellitus

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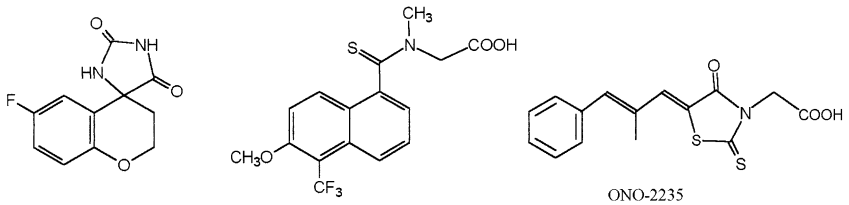


(NIDDM)[16–24] and in the prevention of its late complications. In fact, to date, several 2,4-thiazolidinediones have been patented as antihyperglycaemic and ALR2 inhibitory agents.[3,8]

On these considerations, in a search for new ARIs, we have synthesized and tested 5-arylidene-2,4-thiazolidinediones **2** and **4**. They appear to possess the essential structural requisites (an acidic proton, hydrogen-bond acceptor groups and an aromatic moiety) for ALR2 inhibitory effect, in accordance with known pharmacophoric requirements.[25–31] In particular it is known that the presence of an acidic functionality is an important requirement for all ARIs, since they interact, in their ionized form, with the active site of the enzyme.[3,26–28] Compounds of series **2** possess the acidic hydrogen of the thiazolidinedione imidic moiety, with pK_a higher than those of carboxylic acids.[18,32] Thus, the corresponding carboxylic acids **4** have been synthesized and their activity compared with that of compounds **2**. Compounds of structure **3**, which do not possess acidic protons, have also been tested.

Chemistry

5-Arylidene-2,4-thiazolidinediones **2** were synthesized by the condensation, according to Knoevenagel, of commercially available 2,4-thiazolidinedione **1** with various *meta*- and *para*-substituted benzaldehydes, using piperidine as base, in refluxing ethanol, according to a known procedure.[19]

The treatment of **2** with methyl bromoacetate provided esters **3** which in turn were hydrolyzed in acidic medium providing acids **4** in high yields (Table 1, Scheme 1).

The structures of all synthesized compounds were assigned on the basis of elemental analyses, IR, ^{1}H and ^{13}C NMR data (Tables 2–5). In particular, the *Z* configuration of the exocyclic double bond was unambigously determined on the basis of X-ray diffractometric analysis of compound **2e** (Fig. 1), which confirmed what we had presumed by means of ^{1}H NMR experiments: in fact, in the spectra of **2** recorded in the presence of Eu(fod)$_3$, a significant downfield shift of the methylidene proton resonance had been observed, without any effect on the aromatic protons signals. (*E*)-**2** isomers were never obtained, also under different experimental conditions (i.e., performing the condensation at room temperature, or in acetic acid and in the presence of sodium acetate,[33] or in toluene and in presence of piperidine[34]).

The ^{1}H NMR spectra of compounds **2** show only one kind of 5-methylidene proton in the range 7.72–7.97 ppm (Table 2). In the ^{13}C NMR spectra, the signals of 5-methylidene carbon (at 130.1–132.8 ppm) and thiazolidinedione C_5 (in the range 122.0–126.7 ppm), as well as the absence of the 5-CH_2 resonance of thiazolidinedione **1**, are diagnostic for the structural assigment of **2** (Table 4).

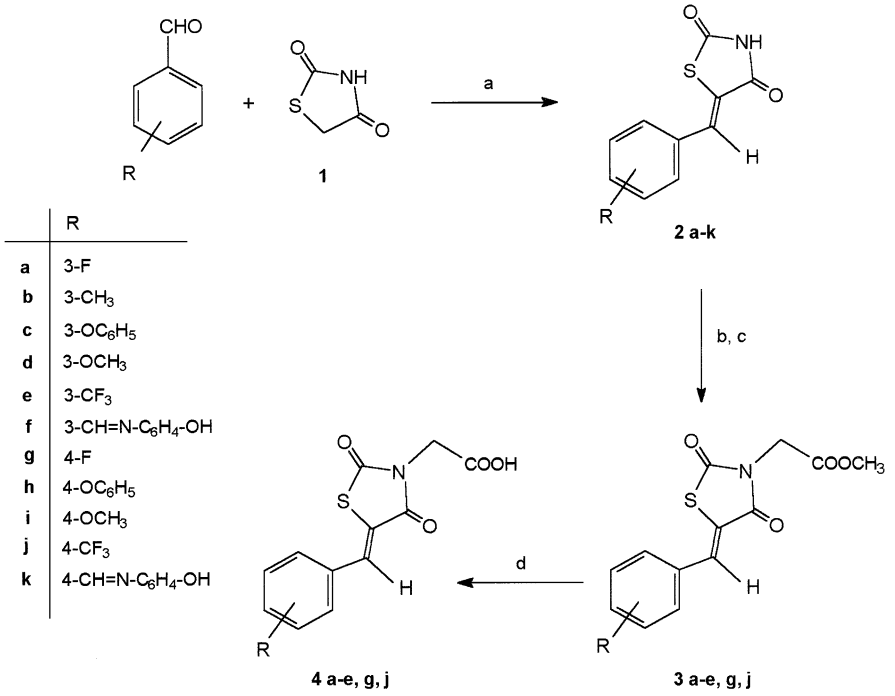
Compounds (*Z*)-**2** appear to be thermodynamically stable. Attempts to isomerize them by irradiation with a high pressure mercury-vapour lamp in acetonitrile for 24 h at room temperature failed. ^{1}H NMR monitoring of the experiment did not reveal any change, contrary to what has been reported for some analogous compounds.[19,35,36]

^{1}H and ^{13}C NMR spectra of compounds **3** and **4** show a single signal due to the resonance of 5-methylidene group (the proton in the range 7.75–8.01 ppm and the carbon at 130.1–134.9 ppm) and a singlet attributable to N–CH_2 resonance (the proton at 4.22–4.56 ppm and the carbon at 40.9–42.5 ppm) (Tables 3 and 5).

Table 1. Chemical-physical data of 2,4-thiazolidinediones **2**–**4**

Compd	Mp (°C)[a]	Yield (%)	Formula
2a	169–172	52	$C_{10}H_6FNO_2S$
2b	178–180	56	$C_{11}H_9NO_2S$
2c	146–148	65	$C_{16}H_{11}NO_3S$
2d	187–190	52	$C_{11}H_9NO_3S$
2e	180–182	57	$C_{11}H_6F_3NO_2S$
2f	263–265	88	$C_{17}H_{12}N_2O_3S$
2g	210–212	65	$C_{10}H_6FNO_2S$
2h	183–186	55	$C_{16}H_{11}NO_3S$
2i	216–218	70	$C_{11}H_9NO_3S$
2j	206–208	54	$C_{11}H_6F_3NO_2S$
2k	288–289	90	$C_{17}H_{12}N_2O_3S$
3a	112–115	96	$C_{13}H_{10}FNO_4S$
3b	92–95	78	$C_{14}H_{13}NO_4S$
3c	117–120	76	$C_{19}H_{15}NO_5S$
3d	48–50	98	$C_{14}H_{13}NO_5S$
3e	170–173	88	$C_{14}H_{10}F_3NO_4S$
3g	221–223	78	$C_{13}H_{10}FNO_4S$
3j	192–195	89	$C_{14}H_{10}F_3NO_4S$
4a	215–216	96	$C_{12}H_8FNO_4S$
4b	230–231	98	$C_{13}H_{11}NO_4S$
4c	216–217	94	$C_{18}H_{13}NO_5S$
4d	153–155	98	$C_{13}H_{11}NO_5S$
4e	213–215	98	$C_{13}H_8F_3NO_4S$
4g	240–245 (dec.)	96	$C_{12}H_8FNO_4S$
4j	251–252	97	$C_{13}H_8F_3NO_4S$

[a]Recrystallization solvent: MeOH for compounds **2** (EtOH/H_2O for **2f**); EtOH/H_2O for **3**; EtOH for **4**.



	R
a	3-F
b	3-CH_3
c	3-OC_6H_5
d	3-OCH_3
e	3-CF_3
f	3-CH=N-C_6H_4-OH
g	4-F
h	4-OC_6H_5
i	4-OCH_3
j	4-CF_3
k	4-CH=N-C_6H_4-OH

Scheme 1. (a) $C_5H_{11}N$, C_2H_5OH, reflux; then, *p*-aminophenol for **2f** and **2k**; (b) NaH, DMF, 80°C; (c) $BrCH_2COOCH_3$, 80°C; (d) AcOH, HCl, reflux.

Table 2. ^{1}H NMR data of 5-arylidene-2,4-thiazolidinediones **2**[a]

Compd	CH[b] arylidene	Aromatic	NH[c]	Others
2a	7.78	7.30–7.35 7.40–7.45 7.54–7.61	12.71	—
2b	7.72	7.27–7.44	12.62	2.35 (s, 3H, CH_3)
2c	7.76	7.07–7.23 7.34–7.56	12.64	—
2d	7.78	7.05-7.16 7.43–7.49	12.63	3.81 (s, 3H, OCH_3)
2e	7.97	7.76–7.91	12.36	—
2f	7.83	6.81, 7.26, 7.68, 7.72, 7.96, 8.07	12.20	8.67 (s, 1H, CH=N), 9.56 (s, 1H, OH, exchangeable with D_2O)
2g	7.80	7.39 (dd, J = 10.1, 8.6) 7.68 (dd, J = 7.8, 5.5)	12.62	—
2h	7.77	7.08–7.25 7.43, 7.61	12.56	—
2i	7.75	7.09, 7.55	12.44	3.82 (s, 3H, OCH_3)
2j	7.86	7.80, 7.88	12.68	—
2k	7.84	6.84, 7.29, 7.72, 8.03	12.28	8.69 (s, 1H, CH=N), 9.63 (s, 1H, OH, exchangeable with D_2O)

J are expressed in Hz.
[a]DMSO-d_6 solution.
[b]Singlet.
[c]Broad signal (exchangeable with D_2O).

The structure of acids **4** was also assigned on the basis of their IR spectra, which show a very broad band at 3400–2450 cm^{-1} attributable to the stretching of carboxylic OH. In addition, the treatment of **4** with methanol and catalytic amount of H_2SO_4 provides parent esters **3**.

Results and Discussion

The ALR2 inhibitory activity of compounds **2**–**4** was evaluated in vitro by measuring their ability to inhibit the reduction of D,L-glyceraldehyde, using partially purified ALR2 from bovine lenses (Tables 6 and 7).

Table 3. ^{1}H NMR data of methyl 5-arylidene-2,4-thiazolidinedione-3-acetates **3**[a] and 5-arylidene-2,4-thiazolidinedione-3-acetic acids **4**[b]

Compd	CH[c] arylidene	Aromatic	CH_2[d]	Others
3a	7.93	7.34–7.66	4.53	3.72 (s, 3H, $COOCH_3$)
3b	7.88	7.21–7.40	4.48	2.40 (s, 3H, CH_3), 3.77 (s, 3H, $COOCH_3$)
3c	7.90	7.08–7.43	4.51	3.81 (s, 3H, $COOCH_3$)
3d	7.91	6.98–7.42	4.50	3.79 (s, 3H, $COOCH_3$), 3.86 (s, 3H, OCH_3)
3e	7.96	7.60–7.77	4.51	3.80 (s, 3H, $COOCH_3$)
3g	7.91	7.19, 7.52	4.50	3.80 (s, 3H, $COOCH_3$)
3j	7.95	7.63, 7.74	4.51	3.80 (s, 3H, $COOCH_3$)
4a	7.90	7.14–7.48	4.56	—
4b	7.75	7.04–7.21	4.23	2.21 (s, 3H, CH_3)
4c	7.82	7.05–7.46	4.35	—
4d	7.93	6.99–7.41	4.56	3.87 (s, 3H, OCH_3)
4e	7.95	7.77–7.92	4.41	—
4g	7.80	7.01, 7.32	4.22	—
4j	8.01	7.84–7.92	4.41	—

J are expressed in Hz.
[a]$CDCl_3$ solution.
[b]DMSO-d_6 solution.
[c,d]Singlets.

Table 4. ^{13}C NMR data of 5-arylidene-2,4-thiazolidinediones **2**[a]

Compd	CH	Aromatic	C_5	C=O	Others
2a	130.4	116.7 (d, $J_{CF}=22.2$), 117.2 (d, $J_{CF}=21.1$), 125.5 (d, $J_{CF}=2.25$), 131.3 (d, $J_{CF}=8.55$), 135.4 (d, $J_{CF}=7.95$), 162.3 (d, $J_{CF}=243.6$)	125.2	167.2, 167.6	—
2b	130.5	126.4, 128.6, 130.0, 131.2, 132.4, 138.1	122.7	166.7, 167.3	20.3 (CH_3)
2c	131.1	118.8, 119.4, 120.1, 124.3, 124.9, 130.3, 135.0, 155.9, 157.6	124.6	167.3, 167.8	—
2d	131.7	115.3, 116.2, 121.9, 130.4, 134.3, 159.5	123.8	167.2, 167.8	55.3 (OCH_3)
2e	132.8	126.5 (q, $J_{CF}=3.75$), 126.8 (q, $J_{CF}=3.75$), 130.0 (q, $J_{CF}=32.2$), 130.2, 130.4, 134.2	125.8	167.1, 167.4	127.4 (q, $J_{CF}=270.0$, CF_3)
2f	130.5	116.5, 123.5, 128.9, 131.8, 133.0, 134.3, 138.1, 142.8, 157.3	125.3	168.1, 168.6	156.8 (CH=N)
2g	130.9	116.5 (d, $J_{CF}=21.75$),129.8 (d, $J_{CF}=3.0$),132.6 (d, $J_{CF}=9$), 163.0 (d, $J_{CF}=255$)	123.4	167.5, 168.0	—
2h	131.3	118.4, 119.8, 124.6, 130.4, 132.2, 127.8, 155.5, 158.9	122.0	167.5, 168.1	—
2i	131.9	114.9, 120.3,132.2, 161.0	125.5	167.5, 168.1	55.5 (OCH_3)
2j	130.1	126.2, 129.6 (q, $J_{CF}=32.2$), 129.7, 137.1	126.7	167.3, 167.8	124.0 (q, $J_{CF}=270.0$, CF_3)
2k	130.4	115.8, 122.9, 128.9, 130.9, 135.1, 137.8, 142.2, 156.8	124.7	167.5, 167.9	155.9 (CH=N)

J are expressed in Hz.
[a]DMSO-d_6 solution.

5-Arylidene-2,4-thiazolidinediones **2** with various substitution patterns on the benzylidene moiety were tested and displayed IC_{50} values ranging from 1.86 to 40.83 μM (Table 6). The most active of them (**2f**) exerted inhibitory activity ($IC_{50}=1.86$ μM) lying in the same range of Sorbinil, followed by **2c** and **2g** with $IC_{50}=6.14$ and 8.21 μM, respectively.[37] *Meta*-substitution patterns on the 5-benzylidene moiety generally improved activity, independently of the nature of the substituent group.

The insertion on N-3 of an acetic chain led to 2,4-thiazolidinedione-3-acetic acids **4**, which were significantly more potent than *N*-unsubstituted analogues **2** (Table 7). The increase in the inhibitory activity varied from about 10 times (**4g**) to almost 100 times (**4j**). The 3-phenoxy substituted derivative (**4c**), almost 50 times as effective as its counterpart **2c**, displayed the most significant inhibitory properties ($IC_{50}=0.13$ μM), nearly reaching those of Tolrestat. All acids **4** showed to be at least threefold (from 3- to 23-fold) more active than Sorbinil.

On the other hand, esters **3**, devoid of any acidic proton, in general proved to have ALR2 inhibitory properties similar to those of 2,4-thiazolidinediones **2** (Table 7), with the exception of **3c** and **3j** which were 5 and 10 times, respectively, more effective than their counterparts **2c** and **2j**. In particular, **3c** proved to be the most active among these acetates ($IC_{50}=1.32$ μM) and also twice as active as Sorbinil, confirming the positive influence exerted by *meta*-phenoxy substitution on activity. Thus, in this series of compounds, the presence

Table 5. ^{13}C NMR data of methyl 5-arylidene-2,4-thiazolidinedione-3-acetates **3**[a] and 5-arylidene-2,4- thiazolidinedione-3-acetic acids **4**[b]

Compd	CH	Aromatic	C_5	CH_2	C=O	Others
3a	133.0	116.5 (d, $J_{CF}=22.5$), 117.5 (d, $J_{CF}=21.3$), 125.8 (d, $J_{CF}=2.85$), 130.8 (d, $J_{CF}=8.25$), 135.0 (d, $J_{CF}=7.65$), 162.8 (d, $J_{CF}=247$)	122.5	41.9	165.0, 166.5, 166.6	52.8 ($COO\underline{C}H_3$)
3b	134.9	127.3, 129.1, 130.9, 131.6, 132.9, 139.0	120.5	41.8	164.2, 166.2	36.5 (CH_3), 52.8 ($COO\underline{C}H_3$)
3c	133.8	119.0, 119.3, 120.4, 124.0, 124.5, 129.8, 130.4, 134.4, 155.9, 158.1	121.6	41.7	165.1, 166.5, 168.1	52.6 ($COO\underline{C}H_3$)
3d	130.2	116.7, 117.1, 119.2, 122.6, 134.2, 159.9	120.6	41.8	164.4, 166.7	52.8 ($COO\underline{C}H_3$), 55.3 ($O\underline{C}H_3$)
3e	132.6	126.9, 127.0, 127.2, 129.9, 131.9 (q, $J_{CF}=37.5$), 133.8	121.7	42.0	166.5, 166.7	52.9 ($COO\underline{C}H_3$), 123.5 (q, $J_{CF}=\overline{2}70.0$, CF_3)
3g	133.5	116.6 (d, $J_{CF}=22.2$), 129.3 (d, $J_{CF}=3.45$), 132.3 (d, $J_{CF}=8.85$), 163.7 (d, $J_{CF}=253.3$)	120.6	41.9	164.1, 165.5, 166.6	52.9 ($COO\underline{C}H_3$)
3j	132.6	126.1, 130.2, 131.5 (q, $J_{CF}=34.5$), 136.3	121.7	42.1	164.1, 166.5, 166.6	53.0 ($COO\underline{C}H_3$), 123.5 (q, $J_{CF}=\overline{2}77.5$, CF_3)
4a	132.1	115.9 (d, $J_{CF}=22.2$), 117.0 (d, $J_{CF}=21.1$), 125.3 (d, $J_{CF}=2.85$), 130.4 (d, $J_{CF}=9.3$), 134.5 (d, $J_{CF}=7.65$), 162.2 (d, $J_{CF}=253.5$)	122.2	41.7	163.8, 166.3, 167.4	—
4b	130.4	126.7, 128.7, 131.0, 134.0, 132.5, 138.5	120.3	41.6	166.5, 167.6	20.3 (CH_3)
4c	132.1	117.8, 118.1, 119.1, 122.9, 123.5, 128.7, 129.4, 133.3, 154.7, 156.7	120.6	40.9	163.8, 165.6, 166.6	—
4d	130.1	114.6, 116.0, 122.1, 133.7, 159.4	120.9	41.6	167.3, 167.5	54.8 (OCH_3)
4e	132.8	127.0 (q, $J_{CF}=4.5$), 127.3 (q, $J_{CF}=4.5$), 130.0 (q, $J_{CF}=31.9$), 130.6, 132.3, 133.9	122.9	42.4	164.8, 166.5, 167.9	127.7 (q, $J_{CF}=271.5$, CF_3)
4g	132.5	116.0 (d, $J_{CF}=21.9$), 128.9, 131.8 (d, $J_{CF}=8.85$), 160.0 (d, $J_{CF}=248.2$)	123.0	41.6	165.1, 167.3, 167.5	—
4j	130.8	126.3, 130.2 (q, $J_{CF}=31.5$),132.2, 136.8	123.8	42.5	164.9, 166.7, 168.1	123.9 (q, $J_{CF}=270.75$, CF_3)

J are expressed in Hz.
[a]$CDCl_3$ solution.
[b]DMSO-d_6 solution.

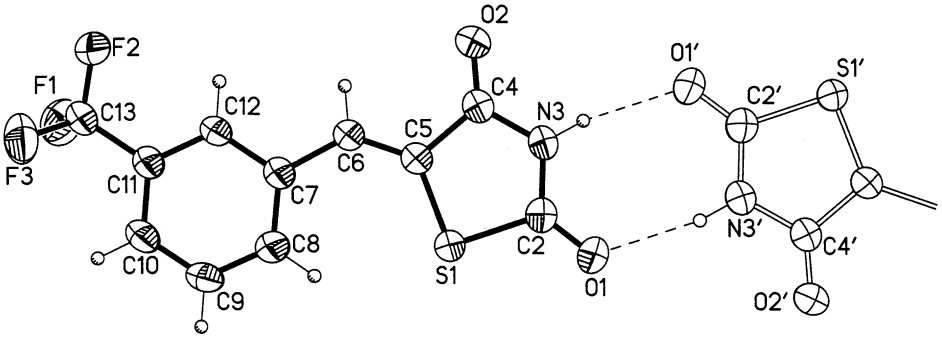


Figure 1. View of **2e** on the molecule mean plane showing the atom numbering scheme. Empty atoms are of the centrosymmetric molecule at (1-x, 1-y, -1-z) evidencing the strong H-bonds of the dimer. Thermal ellipsoids are drawn at 50% of probability, while H size is arbitrary.

of an acidic proton did not appear to be an essential structural requisite. Instead, it could be hypothesized that the N-3 acetate chain was able to get and bind (through H-bonds) the polar positively charged recognition region of the ALR2 active site formed by Tyr48, His110, Trp111 residues and the nicotinamide ring of $NADP^+$.[3,27,28]

In any case, acids **4** proved to be more efficacious inhibitors than esters **3**, clearly due to the presence of the carboxylic anionic head of the N-3 acetic chain.

In conclusion, the in vitro results here reported indicated 5-arylidene-2,4-thiazolidinediones **2**–**4** as potentially promising ARIs: among them, 3-acetic acids **4**, showing IC_{50} values lower than 1 μM, proved to be the most active ones. Their ALR2 inhibitory potency is largely independent of the position of substitutents on the benzylidene moiety, whereas in series **2** substituents in *meta* position are generally more favourable than *para*-substitution. Since the most active compounds in all series **2**–**4** bear an additional aromatic ring (see compounds **c** and **f**), it can be hypothesized that a larger lipophilic moiety increases the enzyme–inhibitor complex stability through interactions with the hydrophobic region of the ALR2 active site. A lot of observations were reported about the hydrophobic nature of this active site which greatly favours the binding with lipophilic compounds.[3,27,28] In addition, in **2f** the *p*-aminophenol moiety could be involved in further interactions through H-bonds.

The importance of the carboxylic function on N-3 was stressed by the finding that all esters **3** were less active than acids **4**. However, this activity order (**4** > **3**) might be reversed in vivo, since the ester function could improve pharmacokinetics, unless it undergoes fast hydrolysis. Analogously, *N*-unsubstituted derivatives **2**, possessing pK_a values higher than carboxylic acids, might display more favourable pharmacokinetic profiles, without the adverse effects related to the hydantoin moiety of Sorbinil and analogues.

Experimental

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). 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 MHz spectrometer; chemical shifts are given in δ units (ppm) relative to internal standard Me_4Si and refer to $CDCl_3$ or DMSO-d_6 solutions. ^{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. Europium tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate), Eu(fod)$_3$, was used as lanthanide shift reagent. Mass spectra were recorded on a Shimadzu GC17A/MSQP5050 spectrometer. Elemental analyses (C, H, N) were performed at Redox Labs, Cologno Monzese, Milan (Italy). Irradiation of compounds **2** were carried out by using a high pressure mercury-vapour lamp (250 W) in acetonitrile for 24 h at room temperature. Crystals suitable for X-ray analysis were obtained by recrystallization of compound **2e** from methanol solution. Diffraction data were collected at room temperature from a colorless 0.92×0.75×0.36 mm^3 prismatic crystal sample by using a Siemens P4 automated four-circle single-crystal diffractometer with graphite-monochromated MoK_α radiation (λ = 0.71073 Å). Crystallographic data (excluding structure factors) for compound **2e** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 167055. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 lEZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

General method for the synthesis of 5-arylidene-2,4-thiazolidinediones (2)

A mixture of 2,4-thiazolidinedione **1** (2.4 g, 20 mmol), aldehyde (20 mmol), piperidine (1.4 g, 16 mmol) and EtOH (150 mL) was refluxed for 16–24 h. The reaction mixture was poured into H_2O and acidified with AcOH to give **2a**–**e**,**g**–**j** as solids which were recrystallized from methanol.

Synthesis of 5-[3-(4-hydroxyphenylaminomethylidene)-phenyl]-methylidene-2,4-thiazolidinedione (2f) and 5-[4-(4-hydroxyphenylaminomethylidene)phenyl]-methylidene-2,4-thiazolidinedione (2k). A mixture of 2,4-thiazolidinedione **1** (2.4 g, 20 mmol), piperidine (1.4 g, 16 mmol) and EtOH (120 mL) was added dropwise to a solution of isophthalaldehyde or terephthalaldehyde (2.68 g, 20 mmol) in EtOH. The reaction mixture was refluxed for 24 h and then poured into H_2O and acidified with AcOH to give 5-(3-formylphenyl)-methylidene-2,4-thiazolidinedione **5** and 5-(4-formylphenyl)-methylidene-2,4-thiazolidinedione **6**. The solid products were purified by column chromatography on silica gel, using $CHCl_3$ (for **5**) or diethyl ether/light petroleum 40–60 °C = 7:3 (for **6**) as eluant.

A solution of *p*-aminophenol (1.1 g, 10 mmol) in EtOH was added to a solution of **5** or **6** (2.8 g, 12 mmol) in EtOH and the reaction mixture was stirred at room temperature for 24 h. The solid product (**2f** or **2k**) was isolated by filtration and recrystallized from methanol (**2k**) or EtOH/H_2O (**2f**).

General method for the synthesis of methyl 5-arylidene-2,4-thiazolidinedione-3-acetates (3)

Sodium hydride (0.576 g, 24 mmol) was added portionwise to a solution of 5-arylidene-2,4-thiazolidinedione **2** (20 mmol) in dry DMF and the mixture was stirred at 80 °C for 1.5 h. The mixture was cooled to room temperature and a solution of methyl bromoacetate (3.7 g, 24 mmol) in dry DMF was added dropwise. After being stirred at 80 °C for 15–20 h, the reaction

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

Compd	IC_{50}[a]
2a	9.10 (8.60—9.63)
2b	9.23 (8.58—9.93)
2c	6.14 (5.54—6.81)
2d	13.28 (11.16–15.81)
2e	12.81 (10.41–15.76)
2f	1.86 (1.46—2.37)
2g	8.21 (7.44—9.06)
2h	41% (37 μM)
2i	40.83 (45.92–41.86)
2j	31.49 (29.18–33.99)
2k	22.93 (19.20–27.38)
Sorbinil	3.04 (2.91–3.52)
Tolrestat	0.074 (0.064–0.084)

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

Table 7. In vitro bovine lenses ALR2 inhibitory activity of methyl 5-arylidene-2,4-thiazolidinedione-3-acetates **3** and 5-arylidene-2,4-thiazolidinedione-3-acetic acids **4**

Compd	IC_{50}[a]	Compd	IC_{50}[a]
3a	40% (76 μM)	**4a**	0.74 (0.61–0.90)
3b	10.10 (8.70–11.73)	**4b**	0.65 (0.50–0.84)
3c	1.32 (1.00–1.74)	**4c**	0.13 (0.11–0.15)
3d	8.87 (6.28–12.52)	**4d**	0.48 (0.36–0.63)
3e	28.67 (24.63–33.37)	**4e**	0.47 (0.41–0.54)
3g	12.90 (11.50–14.47)	**4g**	1.14 (0.93–1.40)
3j	3.44 (2.93–4.04)	**4j**	0.46 (0.41–0.52)
Sorbinil	3.04 (2.91–3.52)		
Tolrestat	0.074 (0.064–0.084)		

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

mixture was poured into H_2O and the solid product filtered and recrystallized from EtOH/H_2O.

General method for the synthesis of 5-arylidene-2,4-thiazolidinedione-3-acetic acids (4)

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 EtOH providing pure carboxylic acid **4**.

Enzyme section

Sorbinil was a gift from Pfizer. Tolrestat was synthesized according to a published procedure.[38]

Calf lenses for the purification of ALR2 were obtained locally from freshly slaughtered animals. The capsule was incized and the frozen lenses were suspended in sodium potassium phosphate buffer pH 7 containing 5 mM DTT (1 g tissue/3.5 mL) and stirred in an ice-cold bath for 1 h. The suspension was then centrifuged at 22,000*g* at 4 °C for 40 min and the supernatant was subjected to ion exchange chromatography on DE52. Enzyme activity was measured by monitoring the change in absorbance at 340 nm which accompanies the oxidation of β-NADPH catalyzed by ALR2. The assay was performed at 37 °C as previously described,[39] using 4.7 mM D,L-glyceraldehyde as substrate in 0.25 M sodium phosphate buffer pH 6.8 containing 0.38 M ammonium sulphate and 0.11 mM NADPH. The sensitivity of ALR2 to inhibition by different ARIs was tested in the above assay conditions by including the inhibitors dissolved in DMSO at the desired concentrations in the reaction mixture. The DMSO in the assay mixture was kept at constant concentration of 1%. A reference blank containing all the above reagents except the substrate was used to correct for the non-enzymatic oxidation of NADPH. IC_{50} values (the concentration of the inhibitor required to produce 50% inhibition of the enzyme catalyzed reaction) were determined from least squares analyses of the linear portion of the log dose–inhibition curves. Each curve was generated using at least three concentrations of inhibitor causing an inhibition between 20 and 80% with two replicates at each concentration. The 95% confidence limits (95% CL) were calculated from T values for n-2, where n is the total number of determinations.[40]

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