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Short communication

Synthesis and aldose reductase inhibitory activity of *N*-(arylsulfonyl)- and *N*-(aroyl)-*N*-(arylmethyloxy)glycines*

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Summary — Some *N*-(arylsulfonyl)- **C** and *N*-(aroyl)-*N*-(arylmethyloxy)glycines **D** were synthesised and tested as aldose reductase inhibitors (ARIs). They are structurally related to the previously described ARIs of type **A** and **B**, from which they differ owing to the presence of a spacer, an OCH_2 group, between the amino-acid nitrogen and the aromatic ring. The inhibitory activity was evaluated on the bovine lens aldose reductase enzyme. Compounds of types **C** and **D** show an inhibitory activity which, in the case of compounds **D**, is very similar to that reported for the parent compounds **B**. Kinetic studies carried out on the most active compound (**8a**), reveal that it produces an inhibition which, depending on its concentration, may be either uncompetitive or noncompetitive with respect to the substrate and the cofactor.

aldose reductase inhibitor / *N*-(arylsulfonyl)-*N*-(arylmethyloxy)glycine / *N*-(aroyl)-*N*-(arylmethyloxy)glycine

Introduction

Diabetes frequently leads to long-term complications such as neuropathy, nephropathy, or retinopathy. These pathologies originate in tissues such as nerve, lens, retina, or kidney, which do not require insulin for glucose transport, and are therefore exposed to the ambient blood glucose level, which may increase dramatically, even in well-controlled diabetic patients.

Aldose reductase (AR) is the first enzyme in the polyol pathway (fig 1); it catalyses the reduction of glucose by NADPH to sorbitol, which can in turn be oxidised by the enzyme sorbitol dehydrogenase (SDH) and by NAD^+ to fructose [2–5].

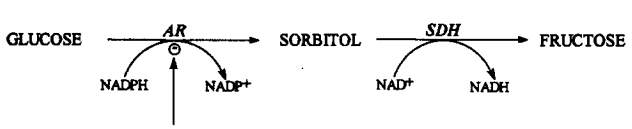


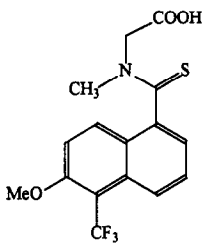
Fig 1. Aldose reductase and the polyol pathway.

In many tissues, AR must compete directly with hexokinase for the utilisation of glucose. The affinity of hexokinase for glucose, however, is greater than that of AR, with the result that, under normal physiological conditions, available glucose is preferentially phosphorylated by hexokinase. In these tissues, significant increases in sorbitol are only produced under non-physiological conditions, such as in diabetes, where hexokinase is saturated by elevated levels of glucose. Under these conditions, sorbitol is formed more rapidly than it is converted to fructose, resulting in a net accumulation of sorbitol.

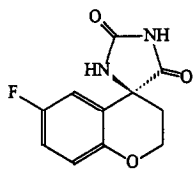
Sorbitol accumulation is also enhanced by the polarity of the polyol, which hinders penetration through membranes and subsequent removal from tissues through diffusion. The intracellular accumulation of polyols can thus produce a hyperosmotic effect that results in an influx of fluid, which ultimately produces the disruption of cell integrity and tissue degeneration [6].

One approach currently investigated to prevent or at least delay these complications associated with diabetes involves aldose reductase inhibitors (ARIs). ARIs are a family of compounds which are chemically not very homogeneous. Most of the important members of this class of drugs belong to the structural classes of carboxylic acids, such as tolrestat **1**, or hydantoins, like sorbinil **2** [7].

*A report of this work was presented at the *2nd Joint Meeting of the Society for Drug Research and the Società Chimica Italiana*, see reference [1].



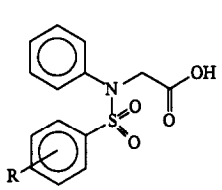
Tolrestat, 1



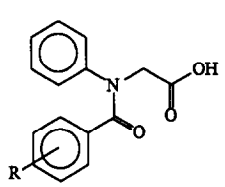
Sorbinil, 2

Some *N*-(arylsulfonyl)- **A** and *N*-(aroyl)-*N*-(phenyl)-glycines **B** have recently been reported to possess an AR inhibitory activity which, in the case of the *p*-nitro- and *p*-methoxy-substituted phenylsulfonyl and benzoyl derivatives, is only slightly lower than that of sorbinil **2** [8, 9].

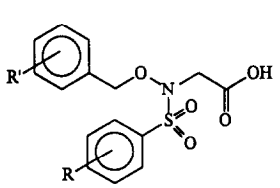
N-(Arylsulfonyl)- **C** and *N*-(aroyl)-*N*-(arylmethyloxy)glycines **D** are structurally related to compounds of types **A** and **B**, respectively, from which they differ owing to the presence of a spacer, the OCH_2 group, between the amino-acid nitrogen and the aromatic ring. In the field of adrenergic drugs, for example, the insertion of this group (OCH_2) between the aryl and the ethanolaminic portion of arylethanolaminic β-blocking drugs, leads to aryloxypropanolaminic compounds, which still possess good β-antagonistic properties [10].



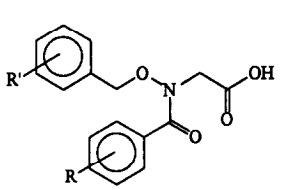
A



B



C



D

At first, on the basis of these considerations, *N*-(phenylsulfonyl)- **3** and *N*-(benzoyl)-*N*-(benzyloxy)-glycines **6** were prepared together with their *p*-nitro- **4**, **7a** and *p*-methoxy- **5**, **8a** substituted analogues.

As the *p*-nitro- **7a** and *p*-methoxybenzamido **8a** derivatives proved to possess the best AR inhibitory activity among these compounds (see tables I and II), the analogues of **7a** and **8a**, substituted on the 3 possible positions of their benzylic aromatic ring by substituents with different electronic characteristics such as the chlorine atom (**7b–d**, **8b–d**) and the methoxy group (**7e–g**, **8e–g**), were also subsequently synthesised. Furthermore, in order to allow a more homogeneous comparison of biochemical data, the previously reported *N*-(*p*-nitrophenylsulfonyl)- **9**, *N*-(*p*-methoxyphenylsulfonyl)- **10**, *N*-(*p*-nitrobenzoyl)- **11** and *N*-(*p*-methoxybenzoyl)-*N*-(phenyl)glycine **12** were also synthesised.

Chemistry

N-(Arylsulfonyl)- **3–5** and *N*-(aroyl)-*N*-(arylmethyloxy)glycines **6**, **7a–g**, **8a–g** were prepared as outlined in scheme 1. Treatment of the substituted benzyl chloride (**13b**, **d**, **f**, **g**) or bromide (**9c**), with *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide, in the presence of triethylamine, followed by hydrazinolysis of the intermediate crude *endo*-*N*-(arylmethyloxy)-5-norbornene-2,3-dicarboximides, yielded the *O*-(arylmethyl)hydroxylamines **14b–d**, **f**, **g**.

O-(*o*-Methoxybenzyl)hydroxylamine **14e** was obtained by condensation of *o*-methoxybenzyl alcohol **13e**, with *N*-hydroxyphthalimide, in the presence of triphenylphosphine and diethylazodicarboxylate, and subsequent hydrazinolysis of the intermediate crude *N*-(*o*-methoxybenzyloxy)phthalimide.

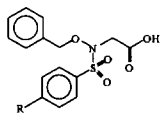
Treatment of **14b–g** and commercially available **14a** with glyoxylic acid, afforded only one of the possible *E*/*Z* isomers of the corresponding *N*-(arylmethyloxy)iminoacetic acids **15a–g**, for which no effort was made to establish the configuration. Reduction of **15a–g** with borane–triethylamine complex in hydroalcoholic solution in the presence of HCl yielded the corresponding *N*-(arylmethyloxy)-glycines **16a–g**. Reaction of **16a** with the appropriate arylsulfonylchlorides gave the *N*-(arylsulfonyl)-*N*-(benzyloxy)glycines **3–5**. Treatment of **16a–g** with KOH and then the appropriate aroylchlorides, yielded the *N*-(aroyl)-*N*-(arylmethyloxy)glycines **6**, **7a–g**, **8a–g**.

Biochemistry

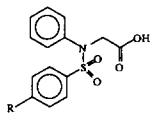
Bovine lens aldose reductase inhibition

The *N*-arylsulfonyl (**3–5** and **9**, **10**) and the *N*-aroyl (**6**, **7a–g**, **8a–g** and **11**, **12**) derivatives were tested for their ability to inhibit bovine lens aldose reductase (see tables I and II). The AR-inhibitory activity of **3–5**, **6**, **7a–g**, **8a–g** is expressed both as percentage inhibition and IC_{50}; for sorbinil **2**, taken as a reference compound, and the previously reported compounds **9–12**, only the IC_{50} values were determined.

Table I. Chemical and biochemical data of *N*-(arylsulfonyl)-*N*-(benzyloxy)glycines **3–5**, *N*-(*p*-nitrophenylsulfonyl)- **9** and *N*-(*p*-methoxyphenylsulfonyl)-*N*-(phenyl)glycine **10**.



3-5



9, 10

Compound	*R*	*Mp (°C)*	*Recrystallisation solvent*	*Yield (%)*[a]	*Formula*[b]	*Aldose reductase inhibitory activity*	
						% Inhibition at 50 μM (SEM)[c]	*IC_{50}, μM*[d] *(95% CL)*[e]
3	H	142–143	$(i\text{-Pr})_2O$/hexane	51	$C_{15}H_{15}NO_5S$	13 (3)	369.0 (331–407)
4	NO_2	144–145 dec	Et_2O	25	$C_{15}H_{14}N_2O_7S$	39 (2)	72.5 (62.6–82.4)
5	MeO	141–142	Benzene	56	$C_{16}H_{17}NO_6S$	30 (1)	91.4 (83.8–98.9)
9	NO_2	220–222[f]	EtOH/H_2O	68		ND[g]	10.5 (7.16–13.8)[h]
10	MeO	171–173[i]	EtOH	45		ND	28.2 (25.6–30.8)[l]
2, Sorbinil						ND	0.65 (0.62–0.69)

[a]For sulfonylation; no effort was made to optimise yields. [b]Anal C, H, N. [c]Percentage inhibition at an inhibitor concentration of 50 μM followed by the standard error of the mean (SEM). [d]IC_{50} values represent the concentration required to produce 50% enzyme inhibition. [e]95% confidence limits. [f]Lit [8] mp 221–223°C (EtOH/H_2O). [g]Not determined. [h]Reference [8]: IC_{50} 4.4 μM on rat lens aldose reductase enzyme. [i]Lit [8] mp 170–172°C (EtOH). [l]Reference [8]: IC_{50} 1.8 μM on rat lens aldose reductase enzyme.

The *N*-arylsulfonyl derivatives **3–5** (table I) were found to be relatively weak inhibitors of aldose reductase, with percent inhibition at 50 μM and IC_{50} values ranging from 13 to 39 and from 72.5 to 369 μM, respectively. The previously described *p*-nitro- **9** and *p*-methoxy- **10** benzensulfonamido derivatives showed IC_{50} values of 10.5 and 28.2 μM, respectively.

On the other hand, the *N*-aroyl derivatives **6**, **7a–g** and **8a–g** revealed an appreciable inhibitory activity (see table II). In this series, the unsubstituted compound **6** showed an IC_{50} value of 13.5 μM, while the corresponding *p*-nitro- **7a** and *p*-methoxy- **8a** benzoyl derivatives exhibited IC_{50} values of 7.4 and 3.5 μM, respectively. The analogues of **7a** substituted on the benzylic ring, *ie* **7b–g**, proved to possess an inhibitory activity with IC_{50} values ranging from 8.8 to 13.5 μM, in the case of the chloro-substituted compounds **7b–d**, and from 10.6 to 19 μM in the case of the methoxy-substituted derivatives **7e–g**. The analogues of **8a**, substituted on the benzylic ring, *ie* **8b–g**, showed an inhibitory activity with IC_{50} values ranging from 6.3 to 13.5 μM, in the case of the chloro-substituted compounds **8b–d**, and from 12.4 to 19.7 μM, in the case of the methoxy-substituted derivatives **8e–g**.

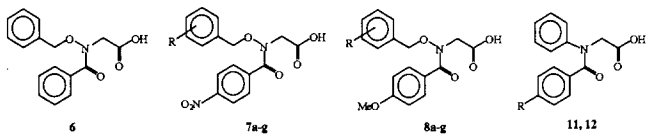
For both the *p*-nitro- **7b–g** and *p*-methoxybenzamido derivatives **8b–g** substituted on the benzylic moiety, IC_{50} values were higher than those of the corresponding unsubstituted compounds **7a** and **8a**, respectively.

All *N*-aroyl derivatives **6**, **7a–g**, **8a–g**, showed percentage inhibition values at 50 μM higher than 50%. The previously described *p*-nitro- **11** and *p*-methoxy- **12** benzamido derivatives showed IC_{50} values of 2.6 and 5 μM, respectively.

Kinetic analyses

The kinetics of the inhibition of the most potent derivative **8a** was studied as a function of inhibitor concentration relative to both substrate (DL-glyceraldehyde) and cofactor (NADPH). Kinetic analyses were conducted using 3 concentrations of the inhibitor (0.5, 3.5 and 7 μM).

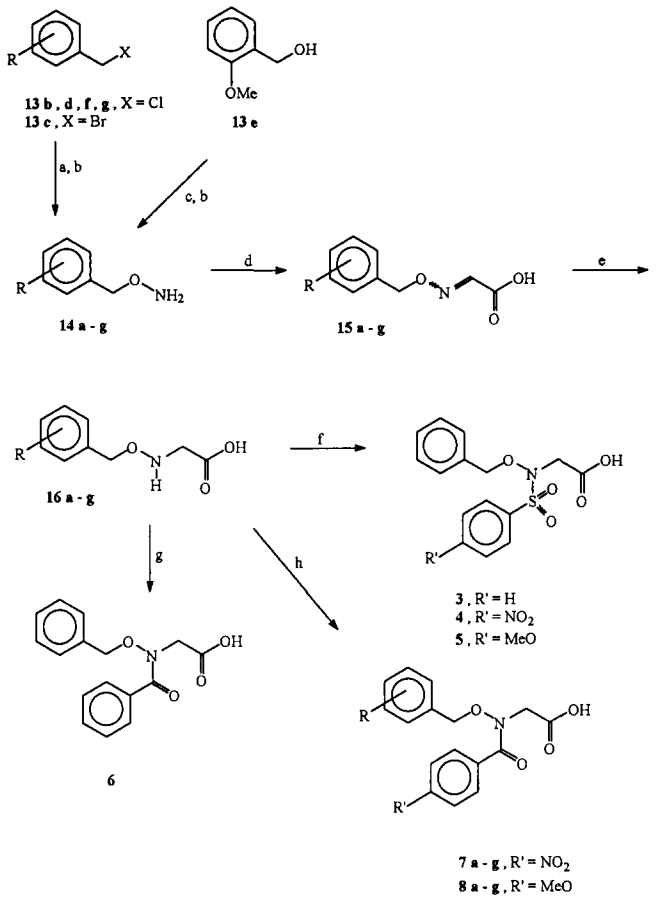
Table II. Chemical and biochemical data of *N*-(aroyl)-*N*-(arylmethyloxy)glycines **6**, **7a–g**, **8a–g**, *N*-(*p*-nitrobenzoyl)- **11** and *N*-(*p*-methoxybenzoyl)-*N*-(phenyl)glycine **12**.



6 7a-g 8a-g 11, 12

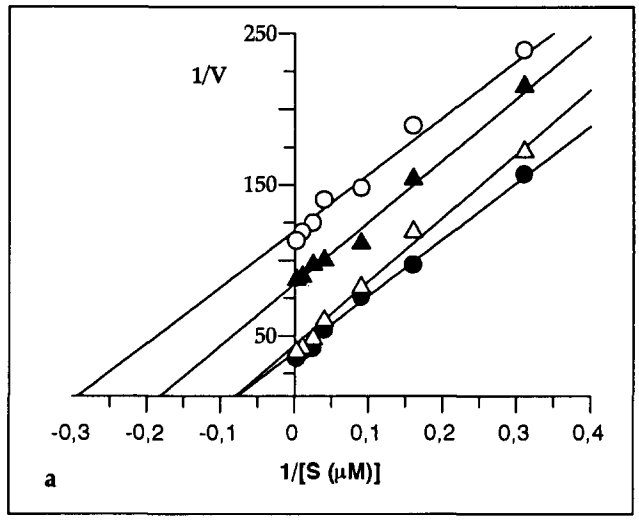
Compound	*R*	*Mp (°C)*	*Recrystallisation solvent*	*Yield (%)*[a]	*Formula*[b]	*Aldose reductase inhibitory activity*	
						% Inhibition at 50 μM (SEM)[c]	*IC_{50}, μM*[d] *(95% CL)*[e]
6		107–108	$(i\text{-Pr})_2O$	63	$C_{16}H_{15}NO_4$	85(2)	13.5 (11.4–15.6)
7a	H	139–140	Benzene	51	$C_{16}H_{14}N_2O_6$	87 (2)	7.4 (6.5–8.3)
7b	*o*-Cl	145–146	$(i\text{-Pr})_2O$	50	$C_{16}H_{13}N_2O_6Cl$	85 (3)	8.8 (7.7–9.8)
7c	*m*-Cl	172–173	AcOEt/MeOH	51	$C_{16}H_{13}N_2O_6Cl{\cdot}C_{12}H_{23}N$	82 (1)	13.5 (12.3–14.7)
7d	*p*-Cl	165–166	Benzene/AcOEt	59	$C_{16}H_{13}N_2O_6Cl$	72 (1)	9.2 (7.8–10.5)
7e	*o*-MeO	154–155	$EtOH/H_2O$	49	$C_{17}H_{16}N_2O_7$	57 (2)	19.0 (16.2–21.7)
7f	*m*-MeO	141–142	$Et_2O/(i\text{-Pr})_2O$	41	$C_{17}H_{16}N_2O_7$	68 (2)	18.0 (15.7–20.2)
7g	*p*-MeO	133–134	Benzene	47	$C_{17}H_{16}N_2O_7$	53 (1)	10.6 (8.7–12.5)
8a	H	134–135	Benzene/Et_2O	72	$C_{17}H_{17}NO_5{\cdot}C_{12}H_{23}N$	89 (3)	3.5 (3.0–3.9)
8b	*o*-Cl	150–151	Et_2O/CH_2Cl_2	57	$C_{17}H_{16}NO_5Cl{\cdot}C_{12}H_{23}N$	70 (2)	12.0 (10.5–13.4)
8c	*m*-Cl	124–125	Benzene	56	$C_{17}H_{16}NO_5Cl$	78 (2)	13.5 (12.1–14.8)
8d	*p*-Cl	99–100	$(i\text{-Pr})_2O$	62	$C_{17}H_{16}NO_5Cl$	89 (5)	6.3 (5.3–7.3)
8e	*o*-MeO	113–114	AcOEt/hexane	55	$C_{18}H_{19}NO_6{\cdot}C_{12}H_{23}N$	66 (3)	19.7 (17.3–22.1)
8f	*m*-MeO	89–90	Benzene/hexane	49	$C_{18}H_{19}NO_6$	63 (2)	12.4 (10.9–13.8)
8g	*p*-MeO	153–155	Benzene/Et_2O	47	$C_{18}H_{19}NO_6{\cdot}C_{12}H_{23}N$	62 (1)	19.7 (17.2–22.2)
11	NO_2	171–173[f]	$EtOH/H_2O$	29		ND[g]	2.6 (1.8–3.2)[h]
12	MeO	161–163[i]	$EtOH/H_2O$	25		ND	5.0 (4.1–5.8)[l]
2, Sorbinil						ND	0.65 (0.62–0.69)

[a]For acylation; no effort was made to optimise yields. [b]Anal C, H, N. [c]Percentage inhibition at an inhibitor concentration of 50 μM followed by the standard error of the mean (SEM). [d]IC_{50} values represent the concentration required to produce 50% enzyme inhibition. [e]95% confidence limits. [f]Lit [9] mp 172–175°C ($EtOH/H_2O$). [g]Not determined. [h]Reference [9]: IC_{50} 0.56 μM on rat lens aldose reductase enzyme. [i]Lit [9] mp 158–163°C ($EtOH/H_2O$). [l]Reference [9]: IC_{50} 3.2 μM on rat lens aldose reductase enzyme.



Scheme 1. **a** R = H; **b** R = *o*-Cl; **c** R = *m*-Cl; **d** R = *p*-Cl; **e** R = *o*-MeO; **f** R = *m*-MeO; **g** R = *p*-MeO. Reagents/conditions: (a) *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide, Et_3N, DMF, room temperature, 12 h; (b) hydrazine, EtOH, reflux, 3 h; (c) *N*-hydroxyphthalimide, $P(Ph)_3$, *N*,*N*-diethylazodicarboxylate, THF, room temperature, 12 h; (d) glyoxylic acid, CH_3CN, room temperature, 12 h; (e) borane–triethylamine complex, EtOH, HCl 10%, room temperature, 2 h; (f) arylsulfonylchloride, THF, pyridine, room temperature, 3 h; (g) benzoyl chloride, dioxane, H_2O, KOH, room temperature, 4 h; (h) aroylchloride, dioxane, H_2O, KOH, room temperature, 4 h.

The Lineweaver–Burk plots obtained from analyses of **8a** *versus* the substrate DL-glyceraldehyde (see fig 2a) showed that this compound produces non-competitive inhibition at the lowest concentration (0.5 μM), as indicated by the fact that the straight line (Δ) meets the control one (●) on the abscissa. At higher concentrations (3.5–7 μM), the inhibition is uncompetitive, as shown by the straight lines (▲, ○) which are parallel to the control one (●).



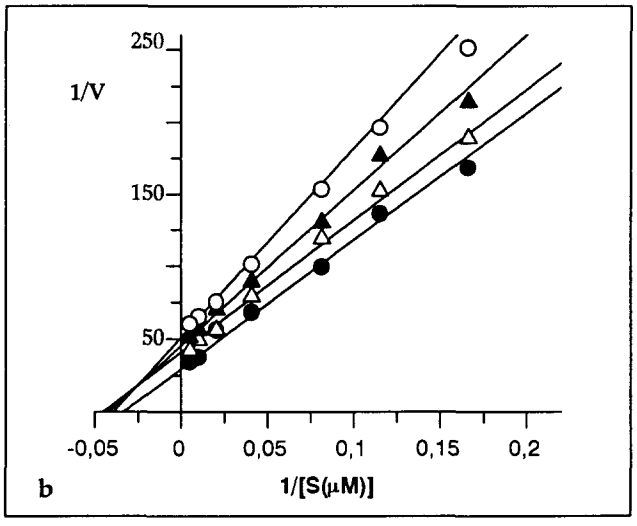


Fig 2. a. Double-reciprocal plot of initial enzyme velocity *versus* concentration of substrate (DL-glyceraldehyde) with and without inhibitor **8a** (no inhibitor (●); in the presence of 0.5 μM (Δ), 3.5 μM (▲) and 7 μM (○) inhibitor). **b.** Double-reciprocal plot of initial enzyme velocity *versus* concentration of cofactor (NADPH) with and without inhibitor **8a** (no inhibitor (●); in the presence of 0.5 μM (Δ), 3.5 μM (▲) and 7 μM (○) inhibitor).

Enzyme kinetic studies of **8a** relative to the cofactor NADPH (see fig 2b), revealed that the inhibition is uncompetitive at the lowest concentration (0.5 μM), as indicated by the fact that the straight line (Δ) and the control one (●) are parallel. At higher concentrations (3.5–7 μM), **8a** produces a mixed-type inhibition, as shown by the fact that the straight lines (▲, ○) if prolonged, would meet the control one (●) under the abscissa.

Discussion and conclusions

An examination of the inhibitory data shown in tables I and II reveals that the *N*-arylsulfonyl derivatives **3–5** are only weak inhibitors of the aldose reductase enzyme, while the *N*-aroyl derivatives **6**, **7a–g** and **8a–g** show more appreciable inhibitory properties.

In the series of the *N*-aroyl derivatives **6**, **7a–g** and **8a–g**, the introduction of either the nitro or the methoxy group into the *para* position of the benzoyl portion of the unsubstituted compound **6** leads to compounds **7a** and **8a**, respectively, which exhibit an increase in inhibitory activity which is higher in the case of the *p*-methoxy-substituted compound **8a** with respect to its *p*-nitro-substituted analogue **7a**.

Furthermore, among the scarcely active *N*-arylsulfonylic compounds **3–5**, the inhibitory activity indices appear to improve slightly on passing from the unsubstituted compound **3** to its analogues **4** and **5** substituted on the sulfonamidic phenyl portion.

The introduction of a chlorine and a methoxy group on the aromatic ring of the benzyloxy portion of **7a** and **8a** generally leads to a slight decrease in the inhibitory activity, which is less evident in the case of the *o*- and *p*-chloro-substituted analogues of **7a** (**7b** and **7d**) and in the case of the *p*-chloro substituted analogue of **8a** (**8d**), for which the IC_{50} values are fairly similar to those of the corresponding unsubstituted compounds **7a** and **8a**.

The ARI activity profile found for these new compounds of types **C** and **D** indicates that the insertion of a spacer OCH_2 between the amino-acid nitrogen and the aromatic ring of the previously described compounds of types **A** and **B** generally leads to either the preservation of the activity, in the case of type **D** compounds, or a marked drop in the case of type **C** ones.

In compounds of type **D**, the introduction of the spacer OCH_2, which generates the shift of the aromatic ring away from the amino-acid nitrogen (which in type **B** compounds are directly linked) does not substantially influence their capacity to interact with the enzyme binding site.

The fact that, as already seen for compounds of type **B**, the most active compound of type **D**, *ie* **8a**, does not produce competitive inhibition, indicates that **8a**, and presumably its type **D** analogues, might bind to a common site on the enzyme, which is distinct from the substrate and NADPH binding sites. This common site of interaction has been termed as 'the inhibitor binding site' [8, 9, 11].

Experimental protocols

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken as paraffin oil mulls or as liquid films on a Mattson 1000 Series FTIR spectrometer. ^{1}H-NMR spectra of all compounds were obtained with a Varian CFT-20 instrument operating at 80 MHz in *ca* 2% solution of $CDCl_3$ or D_2O (for **14b–g**), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the internal standard, respectively. The proton magnetic resonance assignments were established on the basis of the expected chemical shifts and the multiplicity of the signals.

Analytical TLCs were carried out on 0.25-mm silica-gel plates (Merck F_{254}) containing a fluorescent indicator; spots were detected under UV light (254 nm). Evaporations were made *in vacuo* (rotating evaporator); $MgSO_4$ was always used as the drying agent. Elemental analyses were performed in our analytical laboratory and agreed with the theoretical values to within ± 0.4%.

Chemistry

*Synthesis of O-(arylmethyl)hydroxylamine hydrochlorides **14b–d, f, g***

A solution of *endo-N*-hydroxy-5-norbornene-2,3-dicarboximide (28.7 g, 0.16 mol) and triethylamine (44.6 ml, 0.32 mol) in anhydrous DMF (120 ml) was treated with the appropriate substituted benzyl chloride (**13b, d, f, g**) or bromide (**13c**) (0.16 mol), and the resulting mixture was stirred for 12 h at room temperature. After addition of water (100 ml), the solution was extracted with $CHCl_3$ and the organic phase was dried and evaporated to give the appropriate crude intermediate *endo-N*-(arylmethyloxy)-5-norbornene-2,3-dicarboximide, whose ^{1}H-NMR spectrum shows a singlet attributable to the benzylic CH_2 protons with chemical shift ranging from 4.9 to 5.1 ppm. This crude material was dissolved in absolute EtOH (170 ml) and, after addition of hydrazine monohydrate (7.76 ml, 0.16 mol), was refluxed for 3 h. The mixture was then concentrated to a volume of *ca* 40 ml to give a white precipitate which was separated by filtration. The resulting solution, after addition of $CHCl_3$ (20 ml), was acidified to pH 3 at 0°C by addition of a saturated Et_2O/HCl solution to give a white precipitate of the appropriate **14b–d, f, g** which was purified by crystallisation. **14b** (83%): mp 142–144°C (Et_2O/MeOH) (lit [12] mp 143–145°C). **14c** (73%): mp 209–210°C (EtOH) (lit [13] mp 210–211°C). **14d** (99%): mp 241–243°C (EtOH) (lit [13] mp 243–244°C). **14f** (70%): mp 124–125°C (Et_2O/EtOH) (lit [12] mp 75°C as free base). Anal for $C_8H_{11}NO_2$·HCl (C, H, N). **14g** (77%): mp 213–214°C dec (Et_2O/MeOH) (lit [14] mp 216°C dec).

*Synthesis of O-(o-methoxy)benzylhydroxylamine **14e***

A solution of *N*-hydroxyphthalimide (27.1 g, 0.166 mol), triphenylphosphine (43.6 g, 0.166 mol) and *N,N*-diethylazodicarboxylate (28.8 ml, 0.183 mol) in anhydrous THF (680 ml) was treated with *o*-methoxybenzyl alcohol **13e** (22.1 ml 0.166 mol) and the resulting mixture was stirred for 12 h at room temperature. The solvent was evaporated and the residue was filtered through a silica-gel column using CH_2Cl_2 as the eluent. Evaporation of the organic solvent gave crude *N*-(*o*-methoxybenzyloxy)phthalimide (^{1}H-NMR δ 5.3 (s, 2H, CH_2O), 7.6 (m, 4H, Ft)) which, without any further purification, was treated as above for intermediates *endo-N*-(arylmethyloxy)-5-norbornene-2,3-dicarboximide of **14b–d, f, g**, to give **14e** which was then purified by crystallisation. **14e** (84%) had mp 108–109°C (Et_2O/EtOH); ^{1}H-NMR δ 3.9 (s, 3H, CH_3O), 5.4 (s, 2H, CH_2O), 7.0–7.6 (m, 4H, C_6H_4). Anal for $C_8H_{11}NO_2$·HCl (C, H, N).

*Synthesis of N-(arylmethyloxy)iminoacetic acids **15a–g***

A suspension of the appropriate *O*-(arylmethyl)hydroxylamine hydrochloride **14a–g** (0.041 mol) in acetonitrile (130 ml) was

treated with glyoxylic acid monohydrate (4.5 g, 0.049 mol) and the resulting mixture was stirred for 12 h at room temperature. The solvent was evaporated and the residue was dissolved in CH_2Cl_2 (100 ml) and washed with water. The organic layer was then extracted with saturated aqueous $NaHCO_3$ solution (200 ml). The aqueous phase was acidified to pH 3 at 0°C with 10% aqueous HCl, and extracted with CH_2Cl_2. The organic phase was dried and evaporated to give the corresponding acid **15a–g** in only 1 of the 2 possible *E/Z* isomers (as shown by only one singlet attributable to the CH=N proton), which was then purified by crystallisation from $CHCl_3$/hexane. **15a** (97%): mp 79–81°C (lit [15] mp 77–80°C). **15b** (89%): mp 114–115°C. Anal for $C_9H_8NO_3Cl$ (C, H, N). **15c** (59%) mp 65–67°C. Anal for $C_9H_8NO_3Cl$ (C, H, N). **15d** (68%): mp 122–124°C. Anal for $C_9H_8NO_3Cl$ (C, H, N). **15e** (80%): mp 74–75°C. Anal for $C_{10}H_{11}NO_4$ (C, H, N). **15f** (95%): mp 59–60°C. Anal for $C_{10}H_{11}NO_4$ (C, H, N). **15g** (73%): mp 101–102°C. Anal for $C_{10}H_{11}NO_4$ (C, H, N).

In the ^{1}H-NMR spectra ($CDCl_3$) of **15a–g**, there are 2 singlets attributable to the CH_2O and CH=N protons, with chemical shifts varying from 5.2 to 5.4 ppm and from 7.5 to 7.6 ppm, respectively.

*Synthesis of N-(arylmethyloxy)glycines **16a–g***

A cooled (0°C) solution of the appropriate **15a–g** (0.014 mol) and borane–triethylamine complex (9.8 ml, 0.066 mol) in EtOH (26 ml) was treated dropwise under stirring with aqueous 10% HCl (47 ml). After stirring for 2 h at room temperature, the resulting mixture was washed with $CHCl_3$, and then treated at 0°C with a solution of 1 N aqueous NaOH until a white precipitate was formed (pH ~ 3–4). The white precipitate was then extracted with CH_2Cl_2. The organic phase was dried and evaporated to give the corresponding acid as a white solid in the case of **16a–d, f, g** and as a gum in the case of **16e**. **16a–d, f, g** were purified by crystallisation, while **16e** was used for the following reaction without any further purification. An analytical sample of **16e** was obtained by its conversion into dicyclohexylammonium salt: a solution of dicyclohexylamine (172 mg, 0.95 mmol) in hexane (3 ml) was added dropwise to a cooled (0°C) solution of **16e** (200 mg, 0.95 mmol) in AcOEt (2 ml) and the resulting mixture was stirred at room temperature for 5 h. After evaporation of the solvent, the residue was triturated with Et_2O to yield **16e**•$C_{12}H_{23}N$ which was then purified by crystallisation. **16a** (59%): mp 115–116°C (H_2O) (lit [16] mp 116–117°C). **16b** (43%): mp 114–115°C (toluene). Anal for $C_9H_{10}NO_3Cl$ (C, H, N). **16c** (47%): mp 104–105°C (H_2O). Anal for $C_9H_{10}NO_3Cl$ (C, H, N). **16d** (53%): mp 122– 123°C (H_2O/EtOH). Anal for $C_9H_{10}NO_3Cl$ (C, H, N). **16e**•$C_{12}H_{23}N$ (70%): mp 148–150°C dec (benzene/AcOEt). Anal for $C_{10}H_{13}NO_4$•$C_{12}H_{23}N$ (C, H, N). **16f** (62%) mp 94–95°C (H_2O). Anal for $C_{10}H_{13}NO_4$ (C, H, N). **16g** (69%): mp 111–112°C (H_2O). Anal for $C_{10}H_{13}NO_4$ (C, H, N).

In the ^{1}H-NMR spectra ($CDCl_3$) of **16a–g**, there are 2 singlets attributable to the CH_2N and to the CH_2O protons, with chemical shifts varying from 3.5 to 3.6 ppm and from 4.6 to 4.8 ppm, respectively.

*Synthesis of N-(arylsulfonyl)-N-(benzyloxy)glycines **3–5***

A solution of the appropriate arylsulfonylchloride (1.10 mmol), in anhydrous THF (2 ml), was added dropwise to a cooled (0°C) and stirred solution of **16a** (200 mg, 1.10 mmol) in anhydrous pyridine (3 ml) and the resulting mixture was stirred at room temperature for 3 h. After evaporation of the solvents, the residue was dissolved in $CHCl_3$ (6 ml) and washed at 0°C with aqueous 5% HCl. The organic layer was then extracted with a saturated aqueous $NaHCO_3$ solution (20 ml). The aqueous phase was acidified at 0°C (pH 3) with aqueous 10% HCl, and extracted with CH_2Cl_2. The organic phase was dried and evaporated to give the appropriate acids **3–5**, which were then purified by crystallisation. (For chemical and analytical data, see table I.)

In the ^{1}H-NMR spectra ($CDCl_3$) of **3–5**, there are 2 singlets attributable to the CH_2N and CH_2O protons, with chemical shifts varying from 3.7 to 3.8 ppm and from 5.2 to 5.3 ppm, respectively.

*Synthesis of N-(aroyl)-N-(arylmethyloxy)glycines **6, 7a–g, 8a–g***

A solution of the appropriate aroylchloride (0.8 mmol) in dioxane (2.5 ml) was added portionwise over a 15–30 min period to a cold (0°C) solution of the appropriate **16a–g** (1.0 mmol) and KOH (1.8 mmol) in 1:1 dioxane water solution (2.5 ml each). Once the addition was complete, the reaction mixture was stirred for 4 h at room temperature. After evaporation of dioxane and addition of water (2.5 ml), the resulting mixture was acidified to pH 3 at 0°C with aqueous 10% HCl and extracted with $CHCl_3$ (2 x 5 ml). The organic phase was extracted with a saturated aqueous $NaHCO_3$ solution (2 x 15 ml). The aqueous phase was then acidified to pH 3 at 0°C with aqueous 10% HCl and extracted with $CHCl_3$ (2 x 10 ml). The organic phase was dried and evaporated to give **6, 7a, 7b, 7d–g, 8f** as a solid and **7c, 8a–e, 8g** as a gum. Compounds **6, 7a, 7b, 7d–g, 8f** were purified by crystallisation. Compounds **8c** and **8d** were triturated in hexane to give a solid which was purified by crystallisation. Compounds **7c, 8a, 8b, 8e** and **8g** were purified by conversion into their corresponding dicyclohexylammonium salt, following the method reported for the preparation of **16e**•$C_{12}H_{23}N$ (see above). (For analytical and chemical data, see table II.)

In the ^{1}H-NMR spectra ($CDCl_3$) of **6, 7a–g, 8a–g**, there are 2 singlets attributable to the CH_2N and CH_2O protons, with chemical shifts varying from 4.2 to 4.6 ppm and from 4.6 to 5.0 ppm, respectively.

*Synthesis of N-(p-nitrophenylsulfonyl)- **9** and N-(p-methoxy-phenylsulfonyl)-N-(phenyl)glycine **10***

Compounds **9** and **10** were obtained following the synthetic route previously described [8]. (For chemical data, see table I.)

*Synthesis of N-(p-nitrobenzoyl)- **11** and N-(p-methoxy-benzoyl)-N-(phenyl)glycine **12***

Compounds **11** and **12** were obtained following the synthetic route previously described [9]. (For chemical data, see table II.)

Enzyme assays

Bovine eyes were obtained from freshly slaughtered animals at the local slaughterhouse; the lenses were removed and kept frozen until used for the preparation of lens extract.

Crude enzyme supernatant was prepared as follows: frozen bovine lenses were suspended (1 lens / 5 ml) in 10 mM sodium phosphate buffer, pH 7 supplemented with 2 mM dithiotreitol and then stirred at 0°C for 40 min; the suspension was centrifuged at 40 000 *g* at 0°C for 30 min. The supernatant obtained is referred to as the 'lens extract'. The estimation of proteins was based on Lowry's method using bovine serum albumin as the standard [17].

Aldose reductase activity of the freshly prepared lens extract was assayed spectrophotometrically at 37°C by following the decrease in absorbance at 340 nm which parallels coenzyme oxidation. The control reaction mixture contained: 0.25 M sodium phosphate buffer, pH 6.8; 0.5 M $(NH_4)_2SO_4$; 0.1 mM NADPH; 4.2 mM DL-glyceraldehyde; 0.1 ml of the lens extract

in a total volume of 0.55 ml. A reference blank containing all the above reagents except the substrate glyceraldehyde was used to correct for oxidation of NADPH not associated with the reduction of the substrate.

The reactions were initiated by addition of glyceraldehyde and were monitored for 4 min after a 10 s incubation period: 0.1 ml of supernatant gave an average reaction rate of 0.02 ± 0.002 absorbance units per minute.

The inhibitory activity of the compounds was determined by including the inhibitor solution at the desired concentrations in the reaction mixture.

For IC_{50} determinations, each inhibitor was tested at no fewer than 5 concentrations with a minimum of 2 determinations at each concentration. The compounds were dissolved in DMSO (DMSO < 0.4% in the enzyme assay mixture) and the same solvent concentration was present in blank experiments.

The percentage of inhibition for each compound was calculated at all the concentrations by comparing the rate of reactions containing the inhibitor to that of the control reaction with no inhibitor; IC_{50} values were then obtained by log probit analysis.

Kinetic studies

Kinetic analysis were conducted using 3 concentrations of the inhibitor. For substrate kinetics, the concentrations of DL-glyceraldehyde ranged from 3 to 200 μM and the concentration of the cofactor was held constant at 100 μM. For cofactor kinetics, the concentrations of NADPH varied from 3 to 200 μM and the substrate concentration was held constant at 4.2 mM. For each value shown in figure 2, 4 different determinations were carried out.

The nature of the inhibition produced by each concentration of the inhibitor was determined by analysis of double reciprocal plots of enzyme velocity *versus* DL-glyceraldehyde or NADPH concentration, as generated by the linearisation fit of the data using the program GraFit [18].

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