On the Synthesis of Bioisosters of *O*-Benzothiazolyl-oxybenzoic Acids and Evaluation as Aldose Reductase Inhibitors

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In continuation of our attempts to develop novel aldose reductase inhibitors (ARIs), a number of compounds characterized by bioisosteric replacement of pharmacophors were prepared. On the one hand, the acidic function was formally replaced by an oxime or a nitro group and on the other hand the lipophilic substituent was modified. The results of the biological evaluation of these derivatives enabled us to gain insight into structural features critical for the aldose reductase inhibition.

Keywords: Aldose reductase inhibitors; Enzyme inhibitors; Bioisosteric replacement; Diabetic complications

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Introduction

Aldose reductase (EC 1.1.1.21, ALR 2) is a member of the NADPH-dependent aldo-keto reductase family which represents a super family of monomeric oxidoreductases. ALR 2 is the first and rate-limiting enzyme in the polyol pathway and catalyzes the reduction of glucose to sorbitol with the associated oxidation of NADPH to $NADP^+$. A number of studies have suggested a correlation between the increased polyol pathway activity and the occurrence of chronic diabetic complications. Inhibiting aldose reductase and thus preventing the entry of glucose in the polyol pathway can decrease the damaging effects of late-onset diabetic complications such as neuropathy, nephropathy, retinopathy, and cataracts [1].

In several clinical studies the effects of aldose reductase inhibitors (ARIs), most notably Sorbinil, Tolrestat, Zopolrestat, and Zenarestat were demonstrated. However, these inhibitors were withdrawn from clinical trials due to lack of high efficacy or toxicity. To date, the only drug launched on the market is Epalrestat [2]. Another drug, AS-3201, has recently entered phase III trials to study safety and efficacy in the treatment of diabetic sensorimotor polyneuropathy [3].

ARIs primarily contain either a carboxylic acid or an ionisable hydantoin group suggesting that both can interact in a similar manner with the cationic site of the enzyme. Moreover, the potent inhibitors are characterized by a 5-trifluoromethylbenzothiazol-2-yl (*e.g.* Zopolrestat) or a 4-bromo-2-fluorobenzyl residue (*e.g.* AS-3201, Minalrestat, and Zenarestat, see Figure 1). The X-ray structure of aldose reductase in complex with various inhibitors has indicated the presence of a hydrophobic pocket ('specificity pocket') in the target enzyme particularly suited for the above mentioned substituents [4-8]. Furthermore, the latter subunit was found to be effective for selectivity (*i.e.* differentiation between ALR 2 and the closely related enzyme aldehyde reductase) [1].

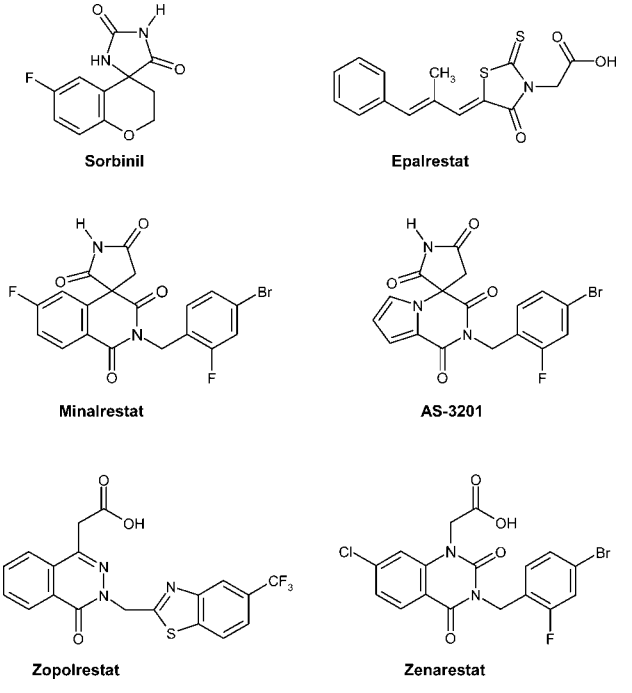

Figure 1. Aldose reductase inhibitors.

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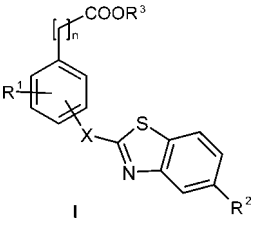


I

Figure 2. General structure of compounds of type **I.**

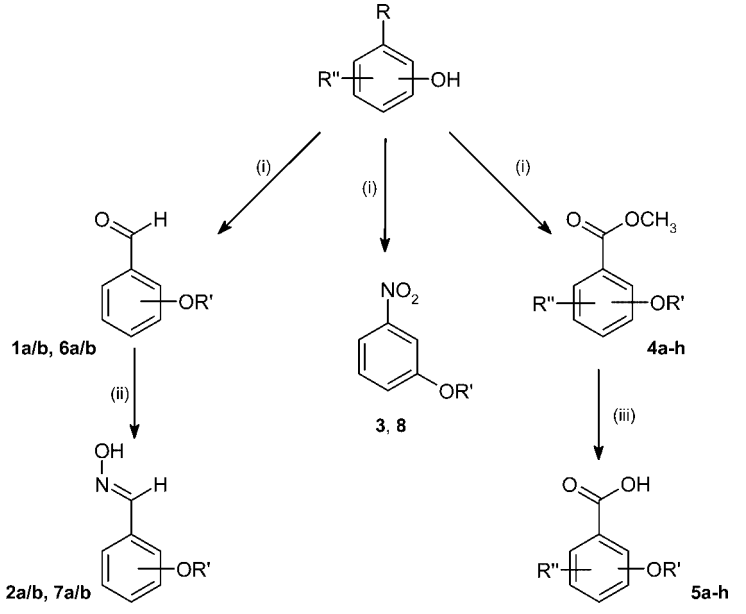
Recently, we have reported the synthesis and aldose-reductase inhibition of a variety of benzothiazolyloxy substituted benzoic acid derivatives (*i.e.* compounds of type **I** with n = 0, see Figure 2). In this series, we have found that an acidic moiety is necessary for enzyme inhibition. However, no significant influence could be observed concerning the position of the benzothiazolyloxy moiety at the benzoic acid core. Furthermore, neither an additional substituent in the benzene ring (*e.g.* hydroxy, methoxy, or carboxylic acid) nor in the benzothiazolyl ring (*e.*g. 5-trifluoromethyl) showed any effect [9].

In the course of our ongoing studies devoted to the development of novel aldose reductase inhibitors, we now focused our attention on derivatives characterized by bioisosteric replacement of the carboxylic acid function by an oxime group. In order to investigate this structural modification on enzyme inhibition, only selected examples were prepared since in the series of benzoic acid derivatives the position of the benzothiazolyloxy moiety exhibited no influence on biological activity.

Results and discussion

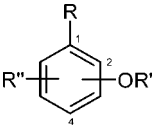
The target oximes **2a/b** were prepared starting from the appropriate hydroxybenzaldehyde by heteroarylation followed by treatment with hydroxylamine hydrochloride in the presence of sodium acetate (Scheme 1). According to TLC and ^{1}H-NMR spectroscopy, in both cases only one product was isolated which could be determined as the *E*-isomer by means of NOE difference spectroscopy. Inhibitory activities of these compounds were evaluated in a spectrometric assay with D,L-glyceraldehyde as the substrate and NADPH as the cofactor.

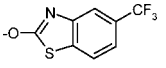
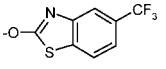
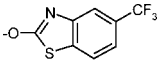
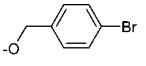
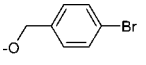
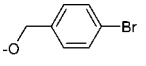
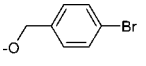
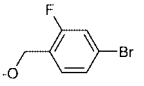
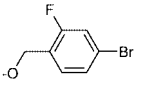
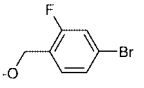
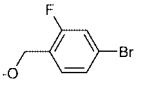
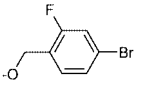
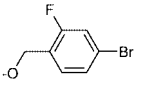
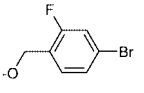
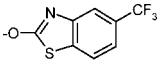
According to the results obtained (Table 1), compounds **2a/b** can be considered as aldose reductase inhibitors (IC_{50} = 44.9 μM and 38% at 50 μM, respectively). In contrast to the findings for the substituted benzoic acid derivatives [9], in this class the substitution pattern possess an influence on the biological activity. Moreover, considering the bioisosteric potential, the results reveal that formal replacement of the carboxylic acid group by an oxime function potentiates the aldose reductase inhibition from 36% at 117 μM for 3-[(5′-trifluoromethylbenzothiazol-2′-yl)-oxy]benzoic acid **9** [9] to an IC_{50} value of 44.9 μM for **2a**. Whereas it is well demonstrated that an acidic moiety is essential for interaction of the inhibitor with the aldose reductase, these results surprisingly demonstrate that there is no correlation between the enzyme inhibition and the strength of acidity. Thus, we assume that the enhancement of the biological activity results from steric effects. This explanation is supported by results obtained from compounds of type **I** with n > 0 and R^3 = H which will be presented in a subsequent paper.



Scheme 1. Synthesis of the target compounds.
(i): 1) K_2CO_3 in dry DMF, 2) 2-chloro-5-trifluoromethylbenzothiazole, rt. or: 1) K_2CO_3 in dry DMF, 2) (substituted) 4-bromobenzylbromide, rt.; (ii): $NH_2OH{\cdot}HCl$, CH_3COONa in dry EtOH, rt.; (iii): 1) 2N NaOH in EtOH, rt.; 2) HCl.

Table 1. Biological data.



Compound	R	OR′	Position	R″	Enzyme inhibition at concentration *or* IC_{50} value (95% CL)
2a	CH(=NOH)		3	H	44.9 μM (33.5–60.2)
2b	CH(=NOH)		4	H	38% at 50 μM
3	NO_2		3	H	26% at 50 μM
5a	COOH		2	H	0% at 100 μM
5b	COOH		3	H	7% at 100 μM
5c	COOH		4	3-OCH_3	0% at 100 μM
5d	COOH		3	5-COOH	30% at 100 μM
5e	COOH		2	H	0% at 100 μM
5f	COOH		3	H	3% at 100 μM
5g	COOH		4	3-OCH_3	26% at 100 μM
5h	COOH		3	5-COOH	26% at 100 μM
7a	CH(=NOH)		3	H	40% at 50 μM
7b	CH(=NOH)		4	H	46% at 50 μM
8	NO_2		3	H	31.9 μM (28.8–35.2)
9 [9]	COOH		3	H	36% at 117 μM
	Sorbinil (used as the reference)				1.2 μM (0.8–1.6)

In order to get further insight into structural features critical for aldose reductase inhibition, compound **3** became an object of interest, too. This target compound is characterized by bioisosteric replacement of the carboxylate anion of the deprotonated **9** by a nitro function. Despite the lack of an acidic function, such a compound should interact directly with the cationic site of the enzyme. This consideration may be supported by molecular docking experiments

recently published by Rastelli et al. [10]. The desired **3** was prepared by reaction of 3-nitrophenol with 2-chloro-5-trifluoromethylbenzothiazole in the presence of potassium carbonate in dry *N*,*N*-dimethylformamide (Scheme 1). Quantitatively, this structural modification does not result in a substantial enhancement of enzyme inhibition (26% at 50 μM *versus* 36% at 117 μM for **9**).

It is well known from the literature that introduction of a (substituted) benzothiazolyl or a (substituted) benzyl moiety leads to increased aldose reductase inhibition [1, 2]. Besides, we became interested in formal replacement of the lipophilic residue. This structural modification was planed for the oximes **2a/b**, the nitro analogue **3**, and for some of the recently published benzoic acid derivatives [9].

The derivatives with substituted benzyloxy subunit became accessible by reaction of the appropriate phenols (methyl hydroxybenzoates, hydroxybenzaldehydes, or 3-nitrophenol, respectively) with 4-bromobenzylbromide or 4-bromo-2-fluorobenzylbromide in the presence of base. Subsequently, alkaline hydrolysis of the benzoic acid derivatives **4a**−**h** or treatment of the aldehydes **6a/b** with hydroxylamine led to our desired compounds **5a**−**h** and **7a/b**, respectively (Scheme 1). The structures of these novel compounds were confirmed by elemental analyses, IR, and NMR spectroscopy as well as MS data.

In the class of benzoic acids, the formal exchange of the benzothiazolyl substituent turned out not to be beneficial. Almost no change of enzyme inhibition was found for the derivatives with isophthalic acid subunit (**5d** and **5h**) as well as for **5g**, however, (nearly) complete loss of the aldose reductase inhibitory activity (at 100 μM) resulted in all other cases. Moreover, in the case of the oximes, this modification was found to be detrimental (3-substituted) or did not lead to a significant change in activity (4-substituted). On the other hand, starting from 3-(5-trifluoromethylbenzothiazol-2-yloxy)nitrobenzene **3** formal replacement of the heteroaryl subunit by 4-bromo-2-fluorobenzyl resulted in a remarkable increase in activity (26% at 50 μM *versus* IC_{50} value of 31.9 μM for **8**).

Conclusion

In continuation of our attempts to develop aldose reductase inhibitors, a number of bioisosters of recently reported benzothiazolyloxy-substituted benzoic acids were synthesized and tested for their biological activity. The findings described above allowed us to gain knowledge of structural features critical for the enzyme inhibition. Based on these results, we intend to expand the modifications within this class of compounds.

Experimental

Chemistry

Melting points were determined with a Kofler hot-stage microscope (Reichert, Vienna; Austria) and are uncorrected. Infrared spectra (KBr pellets) were recorded on a Mattson Galaxy Series FTIR 3000 spectrophotometer (Mattson, Instruments, Inc., Madison, WI, USA). Mass spectra were obtained on a Finnigan MAT SSQ 7000 spectrometer (EI, 70 eV or CI, 200 eV, reactant gas: methane) (Thermo Electron. Corporation, Bremen, Germany). All NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ solution in 5 mm tubes at 30 °C on a Varian Gemini 200 spectrometer (199.98 MHz for ^1H; Varian Inc., Palo Alto, CA, USA) with the deuterium signal of the solvent as the lock and TMS as internal standard. Chemical shifts are expressed in parts per million. Reactions were monitored by TLC using Polygram® SIL G/UV_{254} (Macherey-Nagel, Düren, Germany) plastic-backed plates (0.25 mm layer thickness). The yields given are not optimized. Light petroleum refers to the fraction of bp. 40−60 °C. Elemental analyses were performed by Mag. J. Theiner, 'Mikroanalytisches Laboratorium', Faculty of Chemistry, University of Vienna, Austria.

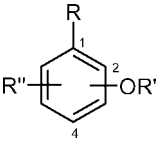
2-Chloro-5-trifluoromethylbenzothiazol was readily available by reaction of 2-chloro-5-trifluoromethylaniline with carbon disulfide in the presence of sodium hydride to give 5-trifluoromethyl-2-mercaptobenzothiazol [11], which was subsequently chlorinated with sulfuryl chloride in analogy to literature [12]. 4-Bromo-2-fluorobenzylbromide was synthesized by radical bromination of 4-bromo-

Table 2. General procedure data for *O*-substitution (compounds 1, 3, 4, 6, and 8).

R	R′	Equivalents				
		batch size	reaction condition	hydroxy derivative	R′-X	base
CHO	5-CF_3-benzothiazol-2-yl 4-Br-2-F-benzyl	8.19 mmol 2.05 mmol	50 °C rt.	1.1	1.0	2.2
NO_2	5-CF_3-benzothiazol-2-yl 4-Br-2-F-benzyl	1.80 mmol 0.72 mmol	rt. rt.	1.1	1.0	2.2
$COOCH_3$	4-Br-benzyl 4-Br-2-F-benzyl	4.00 mmol 1.87 mmol	rt. rt.	2.0	1.0	4.0

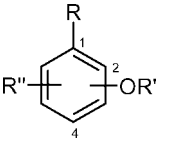
rt.; room temperature

Table 3. Data of compounds **1**–**8**.



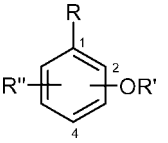
Compound	R	R′[†]	Position of OR′	R″	Solvent[‡] Yield Mp.	Formula[§] MS	Spectroscopic Data[$]
1a	CHO	A	3	H	DIPE 62% 92–94 °C	$C_{15}H_8F_3NO_2S$ (*m/z*) 323 (M^+)	IR 1697 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 10.06 (s, 1H, CHO), 8.00–7.99 (m, 1H, ArH), 7.95–7.93 (m, 1H, ArH), 7.88–7.81 (m, 2H, ArH), 7.71–7.65 (m, 2H, ArH), 7.58–7.53 (m, 1H, ArH)
1b	CHO	A	4	H	DIPE 56% 93–95 °C	$C_{15}H_8F_3NO_2S$ (*m/z*) 323 (M^+)	IR 1695 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 10.05 (s, 1H, CHO), 8.05–7.98 (m, 3H, ArH), 7.85 (d, J = 8.4 Hz, 1H, ArH), 7.63–7.55 (m, 3H, ArH)
2a	CH(=NOH)	A	3	H	Et_2O/LP 24% 153–155 °C	$C_{15}H_9F_3N_2O_2S$ (*m/z*) 339 ($M+1^+$)	IR 3178 cm^{-1} (OH), 1616 cm^{-1} (C=N) ^{1}H-NMR ($CDCl_3$) δ 8.15 (s, 1H, CH), 8.00 (s, 1H, OH), 7.80 (d, J = 8.4 Hz, 1H, ArH), 7.65 ("d", J = 1.6 Hz, 1H, ArH), 7.55–7.37 (m, 5H, ArH)
2b	CH(=NOH)	A	4	H	DIPE 41% 142–144 °C	$C_{15}H_9F_3N_2O_2S$· 0.1 DIPE (*m/z*) 339 ($M+1^+$)	IR 3266 cm^{-1} (OH), 1614 cm^{-1} (C=N) ^{1}H-NMR ($CDCl_3$) δ 8.16 (s, 1H, CH), 8.00 (br s, 1H, OH), 7.81 (d, J = 8.4 Hz, 1H, ArH), 7.73–7.66 (m, 2H, ArH), 7.54 (dd, J = 1.8 Hz, J = 8.4 Hz, 1H, ArH), 7.45–7.38 (m, 2H, ArH), 7.36 (s, 1H, ArH)
3	NO_2	A	3	H	DIPE/LP 47% 142–144 °C	$C_{14}H_7F_3N_2O_3S$ (*m/z*) 340 (M^+)	IR 1523 cm^{-1} (C-NO_2) ^{1}H-NMR ($CDCl_3$) δ 8.35–8.33 (m, 1H, ArH), 8.24–8.18 (m, 1H, ArH), 8.01–7.99 (m, 1H, ArH), 7.86 (d, J = 8.4 Hz, 1H, ArH), 7.82–7.76 (m, 1H, ArH), 7.71–7.63 (m, 1H, ArH), 7.58 (dd, J = 1.8 Hz, J = 8.4 Hz, 1H, ArH)
4a	$COOCH_3$	B	2	H	DIPE 59% 70–71 °C	$C_{15}H_{13}BrO_3$ (*m/z*) 320 (M^+)	IR 1718 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 7.83 (dd, J = 1.8 Hz, J = 7.6 Hz, 1H, ArH), 7.54-7.36 (m, 5H, ArH), 7.05–6.96 (m, 2H, ArH), 5.13 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3)
4b	$COOCH_3$	B	3	H	DIPE 95% 75–78 °C	$C_{15}H_{13}BrO_3$ (*m/z*) 320 (M^+)	IR 1716 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 7.68–7.62 (m, 2H, ArH), 7.55–7.49 (m, 2H, ArH), 7.39–7.29 (m, 3H, ArH), 7.17–7.11 (m, 1H, ArH), 5.06 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3)
4c	$COOCH_3$	B	4	3-OCH_3	DIPE/EA 95% 113–115 °C	$C_{16}H_{15}BrO_4$ (*m/z*) 350 (M^+)	IR 1700 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 7.61 (dd, J = 2.0 Hz, J = 8.3 Hz, 1H, ArH), 7.57 (d, J = 2.0 Hz, 1H, ArH), 7.52–7.44 (m, 2H, ArH), 7.33–7.26 (m, 2H, ArH), 6.86 (d, J = 8.3 Hz, 1H, ArH), 5.15 (s, 2H, CH_2), 3.93 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3)
4d	$COOCH_3$	B	3	5-$COOCH_3$	DIPE/EA 88% 124–128 °C	$C_{17}H_{15}BrO_5$ (*m/z*) 378 (M^+)	IR 1725 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 8.31–8.29 (m, 1H, ArH), 7.81 (d, J = 1.6 Hz, 2H, ArH), 7.55–7.51 (m, 2H, ArH), 7.34–7.30 (m, 2H, ArH), 5.10 (s, 2H, CH_2), 3.94 (s, 6H, 2 × OCH_3)

Table 3. *(continued).*



Compound	R	R′[†]	Position of OR′	R″	Solvent[‡] Yield Mp.	Formula[§] MS	Spectroscopic Data[$]
4e	$COOCH_3$	C	2	H	DIPE 95% 72–74 °C	$C_{15}H_{12}BrFO_3$ (*m/z*) 338 (M^+)	IR 1720 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 7.84 (dd, J = 1.7 Hz, J = 7.9 Hz, 1H, ArH), 7.66–7.24 (m, 4H, ArH), 7.07–7.00 (m, 2H, ArH), 5.18 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3)
4f	$COOCH_3$	C	3	H	DIPE 99% 102–104 °C	$C_{15}H_{12}BrFO_3$ (*m/z*) 338 (M^+)	IR 1716 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 7.69–7.64 (m, 2H, ArH), 7.44–7.26 (m, 4H, ArH), 7.18–7.11 (m, 1H, ArH), 5.12 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3)
4g	$COOCH_3$	C	4	3-OCH_3	DIPE 96% 108–115 °C	$C_{16}H_{14}BrFO_4$ (*m/z*) 368 (M^+)	IR 1716 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 7.63 (dd, J = 1.7 Hz, J = 8.4 Hz, 1H, ArH), 7.57 (d, J = 1.7 Hz, 1H, ArH), 7.44–7.26 (m, 3H, ArH), 6.90 (d, J = 8.4 Hz, 1H, ArH), 5.20 (s, 2H, CH_2), 3.93 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3)
4h	$COOCH_3$	C	3	5-$COOCH_3$	DIPE/EA 95% 125–129 °C	$C_{17}H_{14}BrFO_5$ (*m/z*) 396 (M^+)	IR 1725 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 8.32–8.31 (m, 1H, ArH), 7.83 (d, J = 1.4 Hz, 2H, ArH), 7.44–7.28 (m, 3H, ArH), 5.15 (s, 2H, CH_2), 3.94 (s, 6H, 2 × OCH_3)
5a	COOH	B	2	H	DIPE 94% 116–117 °C	$C_{14}H_{11}BrO_3$ (*m/z*) 306 (M^+)	IR 1700 cm^{-1} (C=O) ^{1}H-NMR (DMSO-d_6) δ 7.64 (dd, J = 1.8 Hz, J = 7.6 Hz, 1H, ArH), 7.60–7.55 (m, 2H, ArH), 7.51–7.43 (m, 3H, ArH), 7.16 (d, J = 7.6 Hz, 1H, ArH), 7.04–6.96 (m, 1H, ArH), 5.17 (s, 2H, CH_2)
5b	COOH	B	3	H	DIPE/EA 96% 182–183 °C	$C_{14}H_{11}BrO_3$ (*m/z*) 306 (M^+)	IR 1685 cm^{-1} (C=O) ^{1}H-NMR (DMSO-d_6) δ 7.61–7.37 (m, 7H, ArH), 7.27–7.22 (m, 1H, ArH), 5.14 (s, 2H, CH_2)
5c	COOH	B	4	3-OCH_3	THF/EA 94% 224–225 °C	$C_{15}H_{13}BrO_4$ (*m/z*) 336 (M^+)	IR 1684 cm^{-1} (C=O) ^{1}H-NMR (DMSO-d_6) δ 7.61–7.38 (m, 6H, ArH), 7.10 (d, J = 8.4 Hz, 1H, ArH), 5.14 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3)
5d	COOH	B	3	5-COOH	THF/EA 97% 114–116 °C	$C_{15}H_{11}BrO_5$ (*m/z*) 350 (M^+)	IR 1685 cm^{-1} (C=O) ^{1}H-NMR (DMSO-d_6) δ 8.09–8.07 (m, 1H, ArH), 7.72 (d, J = 1.2 Hz, 2H, ArH), 7.61–7.57 (m, 2H, ArH), 7.45–7.41 (m, 2H, ArH), 5.22 (s, 2H, CH_2)
5e	COOH	C	2	H	DIPE 89% 135–137 °C	$C_{14}H_{10}BrFO_3$ (*m/z*) 324 (M^+)	IR 1702 cm^{-1} (C=O) ^{1}H-NMR (DMSO-d_6) δ 7.68–7.43 (m, 5H, ArH), 7.21 (d, J = 8.0 Hz, 1H, ArH), 7.07–7.00 (m, 1H, ArH), 5.19 (s, 2H, CH_2)
5f	COOH	C	3	H	DIPE 90% 155–157 °C	$C_{14}H_{10}BrFO_3$ (*m/z*) 324 (M^+)	IR 1685 cm^{-1} (C=O) ^{1}H-NMR (DMSO-d_6) δ 7.63–7.38 (m, 6H, ArH), 7.29–7.23 (m, 1H, ArH), 5.17 (s, 2H, CH_2)
5g	COOH	C	4	3-OCH_3	DIPE/EA 98% 194–196 °C	$C_{15}H_{12}BrFO_4$ (*m/z*) 354 (M^+)	IR 1685 cm^{-1} (C=O) ^{1}H-NMR (DMSO-d_6) δ 7.63–7.44 (m, 5H, ArH), 7.16 (d, J = 8.4 Hz, 1H, ArH), 5.16 (s, 2H, CH_2), 3.79 (s, 3H, OCH_3)

Table 3. *(continued).*



Compound	R	R′[†]	Position of OR′	R″	Solvent[‡] Yield Mp.	Formula[§] MS	Spectroscopic Data[$]
5h	COOH	C	3	5-COOH	DIPE/EA 98% 292−300 °C	$C_{15}H_{10}BrFO_5$ (*m/z*) 368 (M^+)	IR 1698 cm^{-1} (C=O) ^{1}H-NMR (DMSO-d_6) δ 8.11−8.09 (m, 1H, ArH), 7.73 (d, J = 1.2 Hz, 2H, ArH), 7.64−7.44 (m, 3H, ArH), 5.25 (s, 2H, CH_2)
6a	CHO	C	3	H	DIPE 43% 72−74 °C	$C_{14}H_{10}BrFO_2$ (*m/z*) 308 (M^+)	IR 1702 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 9.99 (s, 1H, CHO), 7.53−7.21 (m, 7H, ArH), 5.14 (s, 2H, CH_2)
6b	CHO	C	4	H	DIPE 73−77 °C	$C_{14}H_{10}BrFO_2$ (*m/z*) 308 (M^+)	IR 1689 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ 9.90 (s, 1H, CHO), 7.89−7.82 (m, 2H, ArH), 7.42−7.29 (m, 3H, ArH), 7.11−7.04 (m, 2H, ArH), 5.16 (s, 2H, CH_2)
7a	CH(=NOH)	C	3	H	DIPE/LP 45% 107−110 °C	$C_{14}H_{11}BrFNO_2$ (*m/z*) 324 ($M+1^+$)	IR 3201 cm^{-1} (OH), 1604 cm^{-1} (C=N) ^{1}H-NMR ($CDCl_3$) δ 8.10 (s, 1H, CH), 7.44−7.22 (m, 6H, ArH, OH), 7.16 (d, J = 7.8 Hz, 1H, ArH), 7.02−6.96 (m, 1H, ArH), 5.10 (s, 2H, CH_2)
7b	CH(=NOH)	C	4	H	DIPE/LP 36% 93−95 °C	$C_{14}H_{11}BrFNO_2$ (*m/z*) 324 ($M+1^+$)	IR 3251 cm^{-1} (OH), 1604 cm^{-1} (C=N) ^{1}H-NMR ($CDCl_3$) δ 8.08 (s, 1H, CH), 7.56−7.49 (m, *2*H, ArH), 7.42−7.26 (m, 4H, ArH, OH), 7.00−6.93 (m, 2H, ArH), 5.10 (s, 2H, CH_2)
8	NO_2	C	3	H	DIPE 21% 69−70 °C	$C_{13}H_9BrFNO_3$ (*m/z*) 325 (M^+)	IR 1533 cm^{-1} (C-NO_2) ^{1}H-NMR ($CDCl_3$) δ 7.90−7.82 (m, 2H, ArH), 7.50−7.30 (m, 5H, ArH), 5.15 (s, 2H, CH_2)

[†] The following abbreviations are used: A: 5-trifluoromethylbenzothiazol-2-yl; B: 4-bromobenzyl; C: 4-bromo-2-fluorobenzyl.
[‡] DIPE: diisopropyl ether; LP: light petroleum; EA: ethyl acetate; THF: tetrahydrofurane.
[§] All compounds tested and the carboxylic esters of **4** were analyzed for C, H, N. Analytical results obtained for these elements were within ± 0.4% of the theoretical values.
[$] In the NMR spectra of compounds **5** (in DMSO-d_6) no signal could be detected for the carboxylic acid proton(s).

2-fluorotoluene with *N*-bromosuccinimide and a catalytic amount of azobisisobutyronitrile in CCl_4 as described in the literature [13].

*General procedure for the O-substitution to prepare compounds of type **1**, **3**, **4**, **6**, and **8***

Powdered potassium carbonate was added to a solution of the hydroxy derivative in dry *N,N*-dimethylformamide under an atmosphere of nitrogen. After stirring for 30 minutes at room temperature, the appropriate ar(alk)yl halide (2-chloro-5-trifluoromethylbenzothiazole, 4-bromobenzylbromide, or 4-bromo-2-fluorobenzylbromide) was added and stirring was continued until TLC indicated no further conversion (further information, Table 2). Then, the mixture was poured into cold 2N HCl and the product was extracted exhaustively with diethyl ether. The organic layer was washed with 2N NaOH, water, and brine, dried over anhydrous sodium sulfate and evaporated to dryness. The residue thus obtained was purified by recrystallization (Table 3).

*General procedure for the synthesis of the oximes **2a/b** and **7a/b***

A solution of one equivalent of the appropriate aldehyde derivative (**1a/b**: 3.71 mmol, **6a/b**: 0.81 mmol) in dry ethanol was treated with three equivalents of hydroxylamine hydrochloride and four equivalents of sodium acetate and the reaction mixture was stirred at room temperature until TLC indicated no further conversion. Then, the solvent was removed *in vacuo* and the residue was treated with a small amount of water. After neutralisation, the aqueous phase was extracted exhaustively with ethyl acetate and the organic layer was then washed with water and brine, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The resulting product was purified by recrystallization (Table 3).

General procedure for the synthesis of the carboxylic acids of type ***5***

A solution of the appropriate ester **4** (0.76−1.56 mmol) in ethanol was treated with 2N NaOH (1.1 equivalents) and stirred overnight at room temperature. The solvent was then evaporated, the residue treated with a small amount of water, and the pH adjusted to 5 with 2N HCl. The reaction mixture was extracted with ethyl acetate, the organic layer washed with water and brine, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The crystals thus obtained were purified by recrystallization (Table 3).

Aldose reductase inhibitory assay

NADPH, D,L glyceraldehyde, and dithiothreitol (DTT) were purchased from Sigma Chemical Co. (Sigma, Vienna, Austria) DEAE-cellulose (DE-52) was obtained from Whatman (Whatman International, Ltd., Maidstone, UK). Sorbinil was a gift from Prof. Dr. Luca Costantino, University of Modena (Italy) and was used as standard [IC_{50} = 1.2 (± 0.4) μM]. All other chemicals were commercial samples of good grade. Calf lenses for the isolation of ALR 2 were obtained locally from freshly slaughtered animals. The enzyme was purified by a chromatographic procedure as previously described [14]. Briefly, ALR 2 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 two hours. The suspension was centrifuged at 4000 rpm at 4 °C for 30 minutes and the supernatant was subjected to ion exchange chromatography on DE-52. Enzyme activity was assayed spectrophotometrically on a Cecil Super Aurius CE 3041 spectrophotometer (Cecil Instruments, Inc., Cambridge, UK) by measuring the decrease in absorption of NADPH at 340 nm which accompanies the oxidation of NADPH catalyzed by ALR 2. The assay was performed at 37 °C in a reaction mixture containing 0.25 M potassium phosphate buffer, pH 6.8, 0.38 M ammonium sulfate, 0.11 mM NADPH, and 4.7 mM D,L-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 the 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 [15] for dose effect analysis.

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References

[1] L. Costantino, G. Rastelli, G. Cignarella, P. Vianello, D. Barlocco, *Exp. Opin. Ther. Patents* **1997**, *7*, 843−858.

[2] S. Miyamoto, *Exp. Opin. Ther. Patents* **2002**, *12*, 621−631.

[3] National Institutes of Health, Study ID Number: AS-3201-253, ClinicalTrials.gov Identifier: NCT00101426; http://clinicaltrials.gov/show/NCT00101426.

[4] D. K. Wilson, I. Tarle, J. M. Petrash, F. A. Quiocho, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9847−9851.

[5] A. Urzhumtsev, F. Tete-Favier, A. Mitschler, J. Barbanton, P. Barth, L. Urzhumtseva, J.-F. Biellmann, A. D. Podjarny, D. Moras, *Structure (London)* **1997**, *5*, 601−612.

[6] T. Kinoshita, H. Miyake, T. Fujii, S. Takakura, T. Goto, *Acta Cryst.* **2002**, D*58*, 622−626.

[7] E. I. Howard, R. Sanishvili, R. E. Cachau, A. Mitschler, B. Chevrier, P. Barth, V. Lamour, M. Van Zandt, E. Sibley, C. Bon, D. Moras, T. R. Schneider, A. Joachimiak, A. Podjarny, *Proteins* **2004**, *55*, 792−804.

[8] A. Podjarny, R. E. Cachau, T. Schneider, M. Van Zandt, A. Joachimiak, *Cell. Mol. Life Sci.* **2004**, *61*, 763−773.

[9] D. Rakowitz, B. Hennig, M. Nagano, S. Steger, L. Costantino, B. Matuszczak, *Arch. Pharm. Chem. Life Sci.*, in press.

[10] G. Rastelli, A. M. Ferrari, L. Costantino, M. C. Gamberini, *Bioorg. Med. Chem.* **2002**, *10*, 1437−1450.

[11] J. Easmon, G. Heinisch, J. Hofmann, T. Langer, H. H. Grunicke, J. Fink, G. Pürstinger, *Eur. J. Med. Chem.* **1997**, *32*, 397−408.

[12] N. S. Moon (Eastman Kodak Co.), US 2469697. 1949 [*Chem. Abstr.* **1949**, 43, 6670c].

[13] M. L. Quan, A. T. Chiu, C. D. Ellis, P. C. Wong, R. R. Wexler, P. B. M. W. M. Timmermans, *J. Med. Chem.* **1995**, *38*, 2938−2945.

[14] L. Costantino, G. Rastelli, K. Vescovini, G. Cignarella, P. Vianello, A. Del Corso, M. Cappiello, U. Mura, D. Barlocco, *J. Med. Chem.* **1996**, *39*, 4396−4405.

[15] T.-C. Chou, M. P. Hayball, CalcuSyn software version 1.1.1., Biosoft, Cambridge, UK, 1996.