ELSEVIER

Il Farmaco 53 (1998) 369–373

IL FARMACO

Short Communication

Synthesis and aldose reductase inhibitory activity of new *N*-(benzyloxy)glycine derivatives

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Received 11 November 1997; accepted 4 March 1998

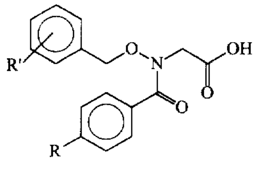
Abstract

This paper reports the synthesis and aldose reductase (AR) inhibitory properties of some *N*-(benzyloxy)glycine derivatives (compounds **2**–**6**), structurally related to the previously described *N*-(aroyl)-*N*-(arylmethyloxy)glycines **A** which had proved to possess an appreciable AR inhibitory activity. In compounds **2**–**5**, spacers of different lengths and degrees of rigidity were inserted between the phenyl ring and the carbonyl group of type **A** derivatives; compound **6** differs from the most active type **A** derivative (compound **1**) in the replacement of the methoxy moiety in the *para* position of the benzoyl side-chain with a group with different electronic characteristics, such as the trifluoromethyl moiety. Biological results indicated that among compounds **2**–**5** only derivative **3**, which presents a CH_2CH_2 spacer between the phenyl and the carbonyl moiety, proved to possess AR inhibitory properties analogous to those of **1**, while all the other compounds proved to be devoid of any significant activity. Furthermore, compound **6** showed an inhibitory activity about 3 times lower than that of **1**.

Keywords: Aldose reductase inhibitors; *N*-(benzyloxy)glycine derivatives

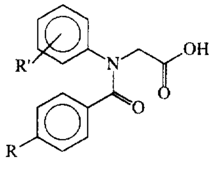
1. Introduction

Aldose reductase (AR) is the first enzyme in the polyol pathway and catalyses the NADPH-dependent reduction of D-glucose to D-sorbitol. This enzyme has a low affinity for glucose and, under normal conditions, it processes little substrate. However, in diabetes mellitus, the marked rise in intracellular glucose that occurs in some cells causes marked production of sorbitol. The increased flux and accumulation of sorbitol through this pathway is damaging and has been linked to the progression of a number of diabetic complications, such as neuropathies, nephropathies and retinopathies [1–4]. For this reason, aldose reductase inhibitors (ARIs), which are able to suppress the marked production of sorbitol, are considered to offer therapeutic possibilities in the treatment of these chronic complications associated with diabetes [1].



A

1, R = MeO, R' = H



B

In a previous paper [5], we reported that the *N*-(aroyl)-*N*-(arylmethyloxy)glycines **A**, which differ from the previously described type **B** ARIs [6] in the presence of an OCH_2 spacer between the amino-acid nitrogen and the aromatic ring, generally possess appreciable AR inhibitory properties very similar to those of the parent compounds **B**; results indicated that the introduction of this kind of spacer does not influence the ability of these types of compounds to interact with the AR enzyme binding site. Furthermore, among the new type **A** derivatives studied, the one in which R=MeO and R'=H (**1**) proved to possess the best inhibitory properties.

A further development of these studies was the insertion of a CH_2 spacer into the structure of type **A** compounds, provoking a shift either of the aryl moiety away from the

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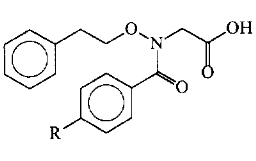
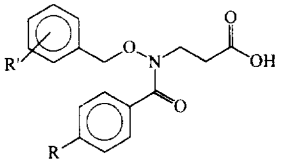
PII S0014-827X(98)00029-9

oxyamidic oxygen (compounds **C**), or of the oxyamidic nitrogen away from the carboxyl group (compounds **D**) [7]:



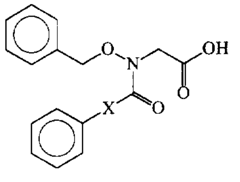
C



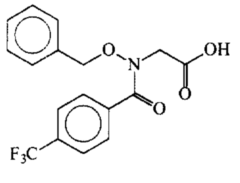
D

compounds **C** revealed a certain loss of inhibitory activity with respect to the corresponding type **A** compounds, while compounds **D** were found to be devoid of any appreciable inhibitory activity, thus showing that these types of modifications generally exert a more or less marked negative effect on the interaction of these types of compounds with the AR enzyme binding site.

As a continuation of these studies, we thought it conceivable to verify the effects of structural modifications to the benzoyl side-chain of type **A** compounds: we here report on the synthesis and AR inhibitory properties of derivatives **2**–**5**, in which the phenyl ring is shifted away from the carbonyl group by the insertion of spacers of different lengths and degrees of rigidity, such as the CH_2 of **2**, the CH_2CH_2 of **3**, the NH of **4** and the CH_2NH of **5**; we also prepared compound **6**, which differs from **1** in the replacement of the methoxy moiety in the *para* position of the benzoyl side-chain with a group with different electronic characteristics, such as the trifluoromethyl moiety.



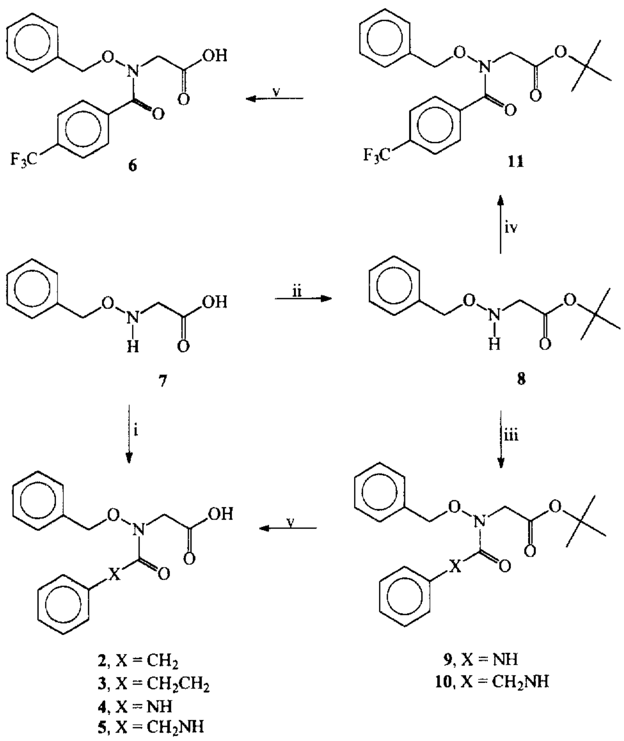
2, X = CH_2
3, X = CH_2CH_2
4, X = NH
5, X = CH_2NH



6

2. Chemistry

Compounds **2**, **3** (Scheme 1) were synthesized by treatment of the *N*-(benzyloxy)glycine **7** with KOH, followed by phenylacetyl chloride (for **2**) or 3-phenylpropionyl chloride (for **3**). As regards the preparation of compounds **4**–**6** (Scheme 1), treatment of the *N*-(benzyloxy)glycine **7** with isobutylene in the presence of concentrated H_2SO_4 afforded the *t*-butyl ester **8**. After reaction with phenyl isocyanate or benzyl isocyanate in the presence of triethylamine and a catalytic amount of dimethylaminopyridine (DMAP), or with 4-(trifluoromethyl)benzoyl chloride and triethylamine, the *t*-butyl ester **8** gave the intermediates **9**, **10** and **11**, respectively. Hydrolysis of **9**–**11** with trifluoroacetic acid and anisole yielded the final compounds **4**–**6**.



Scheme 1. Reagents and conditions. (i) Phenylacetyl chloride or 3-phenylpropionyl chloride, dioxane, H_2O, KOH, r.t., 12 h; (ii) isobutylene, H_2SO_4, dioxane, r.t., 3 h; (iii) phenylisocyanate or benzylisocyanate, ET_3N, DMAP, THF, r.t., 12 h; (iv) 4-(trifluoromethyl)benzoyl chloride, ET_3N, THF, r.t., 12 h; (v) TFA, anisole, CH_2Cl_2, 0°C, 12 h.

3. Biological results

Compounds **2**–**6** along with compound **1**, i.e. the most active of the previously described type **A** derivatives, and Sorbinil, taken as the reference drug, were studied in vitro for their ability to inhibit purified bovine lens aldose reductase.

Among the new compounds studied, compound **3**, which presents a CH_2CH_2 spacer between the phenyl and the carbonyl moieties, showed the highest inhibitory activity, with an IC_{50} (14 μM versus 2 μM for Sorbinil) similar to that of the most active type **A** compound (**1**) (15 μM)[1]. Compound **6**, i.e. the *p*-(trifluoromethyl)benzoyl substituted one, proved to possess an inhibitory activity about 3 times lower than that of compound **1**, with an IC_{50} value of 40 μM. As regards the activity of the other new compounds (**2**, **4**, **5**), they were found to be devoid of any appreciable inhibitory activity, with inhibition percentages at a concentration of 50 μM varying from 39% to 5%.

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[1] Compound **1**, when assayed using bovine lens extracts containing unpurified AR enzyme, showed an IC_{50} value of 3.5 μM vs 0.65 μM for Sorbinil [5].

4. Theoretical calculations

A conformational analysis study was carried out on compounds **1–5** by means of theoretical calculations with the aim of understanding how the different spacers modify the spatial relationships between the molecular moieties of **1–5** and how this fact can influence their biological behaviour.

The preferred conformation of **1–5** was determined using the molecular mechanics program Discover (version 2.9.5, Biosym, San Diego, CA). The starting geometries were constructed using the molecular modelling program Insight II (version 2.3, Biosym, San Diego, CA), which employs standard bond angles and lengths. Calculations were made using the consistent valence force field (CVFF) and a value of 4 (distance dependent) for the dielectric constant. Fig. 1 shows compounds **2–5** each superimposed on **1**, in such a manner as to optimize the overlay of the CH_2COOH portions: all compounds **2–5** and **1** were in their preferred conformations.

It may be pointed out that, in the case of **2** (Fig. 1(a)), both the phenyl rings of **2** have a spatial arrangement different from those of **1**.

As regards compound **3** (Fig. 1(b)), we found that its benzyloxy moiety is nicely superimposable on the same portion of **1**; furthermore, its 3-phenylpropionyl moiety is almost coplanar with the *p*-methoxybenzoyl portion of **1**, with the spacer CH_2CH_2 shifting the phenyl ring to the region occupied by the methoxy group of **1**.

Also in the case of compounds **4** and **5** in their preferred conformations (Fig. 1(c) and (d), respectively), we found a good superimposition with **1** as regards the common benzyloxy moiety; however, the phenylaminocarbonyl portion of **4** as well as the benzylaminocarbonyl portion of **5** proved to possess a different spatial arrangement which was not coplanar with the *p*-methoxybenzoyl moiety of **1**. Furthermore, the preferred conformations of compounds **4** and **5** were found to be favoured by more than 5 kcal/mol with respect to those in which the phenylaminocarbonyl portion of **4** and the benzylaminocarbonyl portion of **5** are coplanar with the *p*-methoxybenzoyl moiety of **1**.

5. Discussion and conclusions

Compounds **2–5** were prepared in order to verify the effects on the AR inhibitory properties of the shift of the phenyl ring of **1** away from the carbonyl group due to the insertion of spacers with different lengths and degrees of rigidity; biological results indicated that only compound **3**, which presents a CH_2CH_2 spacer between the phenyl and the carbonyl moiety, proved to possess inhibitory properties analogous to those of **1**, whereas all the other compounds proved to be devoid of any significant activity. Furthermore, theoretical calculations indicated that only compound **3** was nicely superimposable on **1**: the 3-phenylpropionyl moiety of **3** proved to be almost coplanar with the *p*-methoxybenzoyl portion of **1**, with the spacer CH_2CH_2 shifting the phenyl ring to the region occupied by the methoxy group of **1**. On the basis of these results, it may be hypothesized that sterically similar, albeit electronically different, portions, such as the 3-phenylpropionyl portion of **3** and the *p*-methoxybenzoyl portion of **1**, might be able to dock with the corresponding enzyme site in an analogous manner.

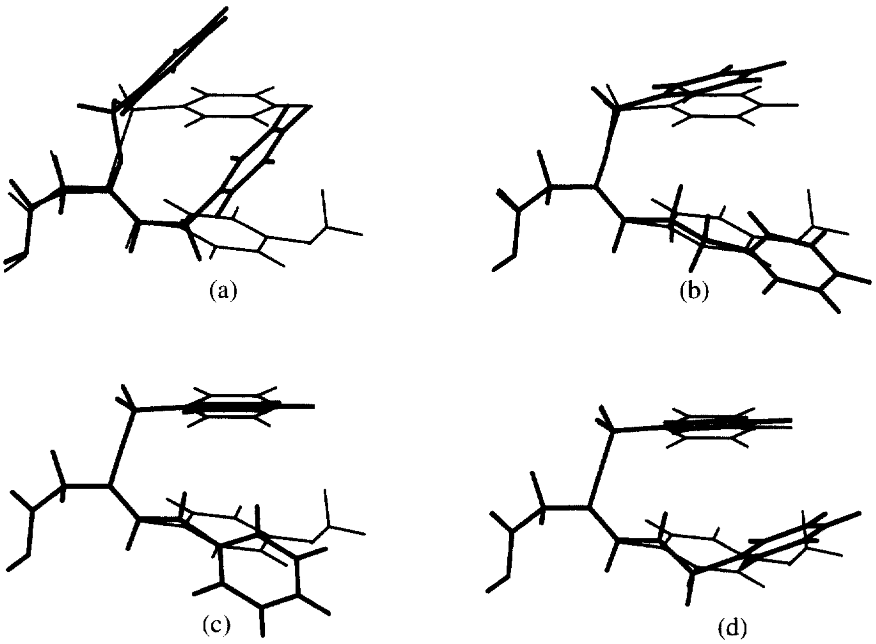

Fig. 1. Superimposition of compound **1** (thinner line) on (a) compound **2**, (b) compound **3**, (c) compound **4**, (d) compound **5**, all in their preferred conformations.

6. Experimental

6.1. Chemistry

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 film on a Mattson 1000 FT-IR spectrometer. ^{1}H NMR spectra of all compounds were obtained with a Varian CFT-20 instrument operating at 80 MHz in a ca. 2% solution of $CDCl_3$ or DMSO-d_6, using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$, respectively, as the internal standard. The proton magnetic resonance assignments were established on the basis of the expected chemical shifts and the multiplicity of the signals. For ^{1}H NMR spectra, only the most significant details are reported. Analytical thin layer chromatography (TLC) was carried out on 0.25 mm layer silica gel plates containing a fluorescent indicator; spots were detected under UV light (254 nm). Column chromatographs were performed using 230–400 mesh silica gel (Macherey-Nagel silica gel 60, Art. Nr. 815381). Magnesium sulfate was always used as the drying agent. Evaporations were made in vacuo (rotating evaporator). Elemental analyses were carried out by our analytical laboratory and were consistent with theoretical values to within ±0.4%.

*6.1.1. Synthesis of N-(benzyloxy)glycine derivatives **2**, **3***

A solution of phenylacetyl chloride or 3-phenylpropionyl chloride (2.75 mmol) in dioxane (12 ml) was added portionwise over a 15–30 min period to a cold (0°C) solution of *N*-(benzyloxy)glycine [5] **7** (0.600 g, 3.3 mmol) and KOH (0.340 g, 6.05 mmol) in 18 ml 1:1 dioxane/water solution. After the addition had been completed, the reaction mixture was stirred for 12 h at room temperature. After evaporation of dioxane and addition of water (8 ml), the resulting mixture was acidified to pH 3 at 0°C with aqueous 10% HCl and extracted with $CHCl_3$ (2×30 ml). The organic phase was extracted with a saturated $NaHCO_3$ solution (2×30 ml). The aqueous phase was then acidified to pH 3 at 0°C with 10% HCl and extracted with $CHCl_3$ (2×30 ml). The organic phase was dried and evaporated to give the appropriate glycine derivatives **2**, **3**, which were further purified respectively by crystallization from H_2O (compound **3**) or by conversion to its dicyclohexylammonium salt (compound **2**): an equimolar amount of dicyclohexylamine was added dropwise to a cooled (0°C) solution of **2** in anhydrous Et_2O and the resulting mixture was stirred for 1 h. The resulting precipitate was filtered and crystallized from H_2O to yield the pure dicyclohexylammonium salt of **2**.

2·$(C_6H_{11})_2NH$ (37%): m.p. 126–128°C. ^{1}H NMR ($CDCl_3$): δ 1.55 (m, 22H, $2\times C_6H_{11}$), 3.73 (s, 2H, CH_2CO), 4.17 (s, 2H, NCH_2), 4.81 (s, 2H, CH_2O), 7.25 (m, 10H, $2\times C_6H_5$). *Anal.* $C_{29}H_{40}N_2O_4$ (C, H, N).

3 (25%): m.p. 94–96°C. ^{1}H NMR ($CDCl_3$): δ 2.85 (m, 4H, CH_2CH_2CO), 4.28 (s, 2H, NCH_2), 4.76 (s, 2H, CH_2O), 7.20 (m, 10H, $2\times C_6H_5$). *Anal.* $C_{18}H_{19}NO_4$ (C, H, N).

*6.1.2. Synthesis of t-butyl ester of N-(benzyloxy)glycine **8***

Isobutylene (14 ml) was added to a cooled (−10°C) solution of *N*-(benzyloxy)glycine **7** (2.02 g, 11 mmol) and concentrated H_2SO_4 (3 ml) in anhydrous dioxane (25 ml). The resulting mixture was stirred at room temperature for 3 h and then poured into an excess of ice-cold saturated $NaHCO_3$. Extraction with EtOAc (3×50 ml) and evaporation of the washed (H_2O) organic extract gave **8** (61%) as an oil, which was used in the subsequent transformations without any further purification. ^{1}H NMR ($CDCl_3$): δ 1.48 (s, 9H, $C(CH_3)_3$), 3.53 (s, 2H, NCH_2), 4.77 (s, 2H, CH_2O), 7.40 (m, 5H, C_6H_5).

*6.1.3. Synthesis of **9–10***

A THF (50 ml) solution of **8** (0.618 g, 3.7 mmol), Et_3N (0.51 ml, 3.7 mmol) and a catalytic amount of DMAP (10 mg) was treated dropwise, under stirring, with a THF solution (2 ml) of phenylisocyanate (0.652 ml, 6 mmol) (for **9**) or benzylisocyanate (0.741 ml, 6 mmol) (for **10**). The reaction mixture was stirred at room temperature for 12 h. The organic phase was evaporated and the residue was purified by column chromatography, eluting with a 5:1 hexane/EtOAc mixture, to yield pure derivatives **9–10** as white solids.

9 (41%): m.p. 80–81°C. ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H, $C(CH_3)_3$), 4.17 (s, 2H, NCH_2), 4.88 (s, 2H, CH_2O), 7.27 (m, 10H, $2\times C_6H_5$). *Anal.* $C_{20}H_{24}N_2O_4$ (C, H, N).

10 (65%): m.p. 82–83°C. ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, $C(CH_3)_3$), 4.11 (s, 2H, NCH_2), 4.32 (d, 2H, $J=5.6$ Hz, CH_2NHCO), 4.77 (s, 2H, CH_2O), 7.22 (m, 10H, $2\times C_6H_5$). *Anal.* $C_{21}H_{26}N_2O_4$ (C, H, N).

*6.1.4. Synthesis of **11***

A tetrahydrofuran (THF) (50 ml) solution of **8** (0.618 g, 3.7 mmol) and Et_3N (1.48 ml, 10.6 mmol) was treated dropwise, under stirring, with a THF solution (2 ml) of 4-(trifluoromethyl)benzoyl chloride (0.66 ml, 4.44 mmol). The reaction mixture was stirred at room temperature for 12 h. The organic phase was evaporated and the residue was purified by column chromatography, eluting with a 5:1 hexane/EtOAc mixture, to yield pure **11** (96%) as a white solid. **11** had m.p. 83–85°C.

^{1}H NMR ($CDCl_3$): δ 1.50 (s, 9H, $C(CH_3)_3$), 4.33 (s, 2H, NCH_2), 4.65 (s, 2H, CH_2O), 7.32 (m, 9H, $C_6H_4+C_6H_5$). *Anal.* $C_{21}H_{22}NO_4F_3$ (C, H, N).

*6.1.5. Synthesis of N-(benzyloxy)glycine derivatives **4–6***

Trifluoroacetic acid (TFA) (1.65 ml, 21 mmol) was added dropwise to a stirred solution of the appropriate *t*-butyl ester **9–11** (1.71 mmol) in anisole (0.38 ml, 3.5 mmol) and anhydrous CH_2Cl_2 (2 ml) cooled at 0°C. The mixture was stirred for 12 h at the same temperature, concentrated at reduced pressure, diluted with EtOAc (20 ml) and extracted with saturated $NaHCO_3$ solution (2×10 ml). The aqueous phase was cooled to 0°C and acidified with 10% aqueous HCl at pH 2.5–2.7 and extracted with EtOAc (2×10 ml). Evaporation of the washed (H_2O) organic extract gave a white solid which was crystallized from the appropriate solvent.

4 (50%): m.p. 100–101°C. ^{1}H NMR ($CDCl_3$): δ 4.29 (s, 2H, NCH_2), 4.89 (s, 2H, CH_2O), 7.28 (m, 10H, $2\times C_6H_5$). *Anal.* for $C_{16}H_{16}N_2O_4$ (C, H, N).

5 (76%): m.p. 122–125°C. ^{1}H NMR ($CDCl_3$): δ 4.19 (s, 2H, NCH_2), 4.33 (d, 2H, $J=5.6$ Hz, CH_2NHCO), 4.79 (s, 2H, CH_2O), 7.24 (m, 10H, $2\times C_6H_5$). *Anal.* $C_{17}H_{18}N_2O_4$ (C, H, N).

6 (45%): m.p. 157–159°C. ^{1}H NMR (CDl_3): δ 4.49 (s, 2H, NCH_2), 4.66 (s, 2H, CH_2O), 7.36 (m, 9H, $C_6H_5+C_6H_4$). *Anal.* $C_{17}H_{14}NO_4F_3$ (C, H, N).

6.2. Enzyme assays

Bovine lens aldose reductase was purified as previously described [8]. The assay for enzyme activity was performed at 37°C, using 4.7 mM D,L-glyceraldehyde as the substrate in 0.25 M sodium phosphate buffer pH 6.8 containing 0.38 M ammonium sulfate and 0.11 mM NADPH. One unit of enzyme activity is the amount of the enzyme which catalyses the oxidation of 1 μmol of NADPH per min. Inhibitors were dissolved in DMSO at suitable concentrations and 5 μl of the different solutions were added to the assay mixture.

The IC_{50} (inhibitor concentration producing 50% inhibition of reaction rate) was computed by linear regression analysis of the linear portion of the dose–response curve.

Acknowledgements

This work was supported by a grant from the Consiglio Nazionale delle Ricerche and the Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

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