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N-Substituted tetrahydro-2,4-dioxoquinazolin-1-yl acetic acids as aldose reductase inhibitors

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Novel *N*-substituted tetrahydro-2,4-dioxoquinazolin-1-yl acetic acids characterized by formal replacement of the substituted benzyl moiety by cyclohexylmethyl and *n*-heptyl residues, respectively, were synthesized and evaluated as aldose reductase inhibitors.

Reduction of glucose by the key enzyme of the polyol pathway, aldose reductase (EC 1.1.1.21, ALR 2) and subsequent reactions have been linked to the development of secondary diabetic complications such as nephropathy, neuropathy, retinopathy, and cataract. Aldose reductase inhibitors (ARIs) therefore offer a possibility of safely preventing or arresting the progression of such long-term complications without disturbing the blood glucose level. Most of the so far identified aldose reductase inhibitors are characterized by the presence of both, a lipophilic part (especially 4-bromo-2-fluorobenzyl or 5-trifluoromethylbenzothiazol-2-yl) and a polar group (e.g. carboxylate) connected by a linking structure (Miyamoto 2002; Costantino et al. 1997, 2000). However, to date none is currently marketed for worldwide use due to lack of high efficacy and selectivity or due to toxicity.

In the course of our ongoing studies aimed towards the development of novel ARIs (Rakowitz et al. 2006a, 2006b as well as references cited therein), we have recently investigated the enzyme inhibitory potency of *O*-substituted hydroxyphenyl acetic acid derivatives. Here, we have found that within these series, formal replacement of the (substituted) benzyloxy substructure by cyclohexylmethyloxy as well as by its ring-opened residues (especially *n*-heptyloxy) leads to derivatives with comparable aldose reductase inhibitory activity. To our knowledge, this is the first time that in the class of aldose reductase inhibitors an alkyl moiety has been successfully employed as the lipophilic part instead of a substituted benzyl. Based on these results, we intended to investigate if cyclohexylmethyl or *n*-heptyl, respectively, may also act as a bioisoster for the 4-bromo-2-fluorobenzyl group in highly potent ARIs like those with a quinazolin-2,4-dione core (e.g. zenarestat and analogues) (Hashimoto et al. 1987).

Table: Aldose reductase inhibition of compounds 5a–c

Compd.	R	IC_{50} (nM)	Confidence limit
5a	4-Bromo-2-fluorobenzyl	51	27–97
5b	Cyclohexylmethyl	430	120–1490
5c	*n*-Heptyl	830	640–1070

The target compounds **5a**–**c** were prepared in analogy to a previously described procedure (Hashimoto et al. 1987) starting from anthranilamide (**1**) as depicted in the Scheme. Then the inhibitory activities were evaluated in a spectrometric assay with D,L-glyceraldehyde as the substrate and NADPH as the cofactor.

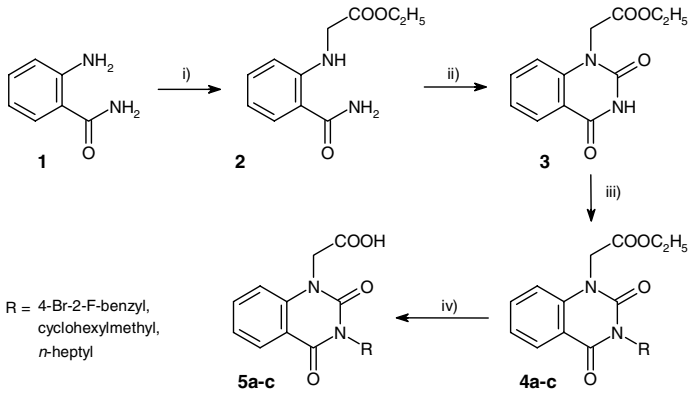
From the biological results listed in the Table it can be seen that the known *N*-(4-bromo-2-fluorobenzylated) derivative **5a** (Hashimoto et al. 1987) as well as its analogues with cyclohexylmethyl (**5b**) or *n*-heptyl (**5c**) moiety represent aldose reductase inhibitors with IC_{50} values in the nanomolar range. However, in contrast to our previous findings (Rakowitz et al. 2006a, 2006b), in these series formal replacement of the 4-bromo-2-fluorobenzyl group by the above mentioned alkyl residues does not lead to compounds with comparable enzyme inhibitory potency.

Experimental

1. General procedure for the N-substitution of compound 3

Ethyl (1,2,3,4-tetrahydro-2,4-dioxoquinazolin-1-yl)acetate (**3**) (0.60 mmol) was added to an ice-cooled suspension of sodium hydride (0.69 mmol, 60% in mineral oil) in 3 mL of dry *N*,*N*-dimethylformamide under nitrogen atmosphere. After stirring for 15 min, a solution of the appropriate (ar)alkylating

Scheme Reagents and conditions: i) $ICH_2COOC_2H_5$, K_2CO_3, DMF; ii) CDI; iii) R-X, NaH, DMF; iv) 1. NaOH, 2. HCl



agent (0.69 mmol) in 1 mL of dry *N*,*N*-dimethylformamide was added and stirring was continued at room temperature until TLC indicated no further conversion. Then, the mixture was poured into cold water and was acidified with 2 N HCl. In the case of **4a** and **4c**, the crystals thus obtained were collected, washed with water and light petroleum and recrystallised from diisopropylether/ethyl acetate. For **4b**, the product was extracted exhaustively with ethyl acetate and the organic layer was washed with 2 N NaOH, water, and brine, dried over anhydrous sodium sulphate and evaporated to dryness. The residue thus obtained was collected and recrystallized from diisopropylether/ethyl acetate to give the *N*-substituted products as colourless crystals.

4a (94%): m.p. 147–150 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.09 (dd, J = 7.8 Hz and 1.4 Hz, 1 H, phenyl H), 7.82–7.74 (m, 1 H, phenyl H), 7.53 (dd, J = 10.0 Hz and 1.8 Hz, 1 H, phenyl H), 7.49–7.31 (m, 3 H, phenyl H), 7.13 (t, J = 8.2 Hz, 1 H, phenyl H), 5.15 (s, 2 H, CH_2), 4.97 (s, 2 H, CH_2CO), 4.15 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 1.18 (t, J = 7.2 Hz, 3 H, OCH_2CH_3). MS (CI, m/z): 435/437 ($M + 1^+$).

4b (48%): m.p. 94–96 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.07 (dd, J = 8.0 Hz and 1.6 Hz, 1 H, phenyl H), 7.78–7.70 (m, 1 H, phenyl H), 7.41–7.27 (m, 2 H, phenyl H), 4.95 (s, 2 H, CH_2CO), 4.15 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 3.82 (t, J = 7.0 Hz, 2 H, NCH_2), 1.64–1.54 (m, 6 H, 3× CH_2), 1.23–0.87 (m, 8 H, 2× CH_2, CH, OCH_2CH_3). MS (EI, m/z): 344 (M^+).

4c (58%): m.p. 78–79 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.25 (dd, J = 8.0 Hz and 1.6 Hz, 1 H, phenyl H), 7.68–7.59 (m, 1 H, phenyl H), 7.30–7.23 (m, 1 H, phenyl H), 6.95 (d, J = 8.4 Hz, 1 H, phenyl H), 4.90 (s, 2 H, CH_2CO), 4.25 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 4.08 (t, J = 6.9 Hz, 2 H, NCH_2), 1.71–1.61 (m, 2 H, CH_2), 1.36–1.24 (m, 11 H, 4× CH_2, OCH_2CH_3), 0.87 (t, J = 6.9 Hz, 3 H, CH_3). MS (EI, m/z) 346 (M^+).

2. Synthesis of the target compounds **5a–c**

A solution of the appropriate ester (**4a**: 0.45 mmol, **4b**: 0.61 mmol, **4c**: 0.28 mmol) in 5 mL of ethanol was treated with 2 N NaOH (1.2 equivalents) and stirred at room temperature for 3 h. The solvent was then evaporated, the residue treated with a small portion of water, and the pH adjusted to 5 with 2 N HCl. The mixture was extracted with ethyl acetate, the organic layer was washed with water and brine, dried over anhydrous sodium sulphate and evaporated to dryness. The products thus obtained were recrystallized to afford colorless analytically-pure crystals.

5a (92% from diisopropylether): m.p. 212–214 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.09 (dd, J = 8.1 Hz and 1.5 Hz, 1 H, phenyl H), 7.82–7.73 (m, 1 H, phenyl H), 7.53 (dd, J = 10.0 Hz and 1.8 Hz, 1 H, phenyl H), 7.43–7.30 (m, 3 H, phenyl H), 7.13 (t, J = 8.3 Hz, 1 H, phenyl H), 5.15 (s, 2 H, CH_2), 4.88 (s, 2 H, CH_2CO). MS (CI, m/z): 407 ($M + 1^+$).

5b (78% from diisopropylether/light petroleum): m.p. 151–153 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.07 (d, J = 6.6 Hz, 1 H, phenyl H), 7.74 (d, J = 7.2 Hz, 1 H, phenyl H), 7.38–7.26 (m, 2 H, phenyl H), 4.86 (s, 2 H, CH_2CO), 3.64 (t, J = 7.0 Hz, 2 H, NCH_2), 1.74–1.46 (m, 5 H, 2× CH_2, CH), 1.24–0.84 (m, 6 H, 3× CH_2). MS (CI, m/z): 317 (M^+). $C_{17}H_{20}N_2O_4 \times 0.2\,H_2O$.

5c (90% from diisopropylether): m.p. 115–117 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.07 (dd, J = 7.8 Hz and 1.6 Hz, 1 H, phenyl H), 7.73 (dd, J = 7.9 Hz and 1.6 Hz, 1 H, phenyl H), 7.38–7.26 (m, 2 H, phenyl H), 4.86 (s, 2 H, CH_2CO), 3.93 (t, J = 7.3 Hz, 2 H, NCH_2), 1.60–1.50 (m, 2 H, CH_2), 1.27–1.24 (m, 8 H, 4× CH_2), 0.84 (t, J = 6.6 Hz, 3 H, CH_3). MS (CI, m/z): 319 (M^+). $C_{17}H_{22}N_2O_4$.

3. Enzyme inhibition

Isolation of ALR 2 from calf lenses and purification of this enzyme have been described previously (Costantino et al. 1996). IC_{50} values were determined from least squares analyses of the linear portion of the log dose-inhibition curves by using CalcuSyn software (Chou and Hayball 1996). Each curve was generated using at least three concentrations of the test compounds (added as a solution in DMSO; final concentration of DMSO in the incubation mixture was 1%) causing an inhibition between 20% and 80%, with two replicates at each concentration. For details concerning the assay procedure see (Rakowitz et al. 2006a/b).

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