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Short Communication

Pyrrolylbenzothiazole Derivatives as Aldose Reductase Inhibitors

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INTRODUCTION

Activation of the aldose reductase enzyme (AR, ALR2, E.C. 1.1.1.21) of the polyol pathway has suggested that it is implicated in a number of pathological conditions: a) in diabetic patients, for the development of the long term complications of the disease,[1] and b) in non diabetic individuals, for ischemic myocardial injury,[2] for abnormal proliferation of vascular smooth muscle cells[3] (which is an important feature of atherosclerosis, restenosis, and hypertension), and for bipolar and unipolar mood disorders.[4] Furthermore, about 29% of human liver cancers overexpress AR which might contribute to their resistance to chemotherapy.[5]

Although a considerable number of compounds have been synthesized and shown to be effective aldose reductase inhibitors (ARIs),[6] the only ARI available as a drug is Ono Pharmaceutical's epalrestat in Japan.[7] However, as the inhibition of AR is considered to be a quite promising therapeutic target,[8–11] the already marketed epalrestat[12,13] as well as new chemical entities[14,15] are being investigated in clinical trials.

In the present study, based on the above, (3-benzothiazol-2-yl-pyrrol-1-yl)acetic acid (**6**) and 4-(benzothiazol-2-yl-2-benzoylpyrrol-1-yl)acetic acid (**9**) (Scheme 1) were synthesized and tested for AR inhibitory activity. The design of structure **6** was based on the reported[16] AR inhibitory ability of (3-benzoylpyrrol-1-yl)acetic acid as well as the putative non-classical bioisosteric relationship between a carbonyl group and the thiazole ring.[17] Compound **9** combines structural features of **6** and of (2-benzoylpyrrol-1-yl)acetic acid. The latter is also an ARI[16] although comparatively weaker than its C-3 isomer.

MATERIALS AND METHODS

Melting points were uncorrected and determined in open glass capillaries using a Mel-Temp II apparatus. UV spectra were recorded with a Perkin–Elmer 554 spectrophotometer, IR spectra were recorded with a Perkin–Elmer 597 spectrophotometer and ^{1}H-NMR spectra with a Bruker AW-80 spectrometer with internal TMS standard. Elemental analyses were performed on a Perkin–Elmer 2400 CHN analyser. Flash chromatography was carried out using Merck 9385 silica gel. Petroleum ether refers to the fraction bp 40°–60°C.

1-Benzenesulfonyl-1*H*-pyrrol-3-carbonyl Chloride (2)

1-Benzenesulfonyl-1*H*-pyrrole-3-carboxylic acid (**1**)[18] (0.2 g, 0.8 mmol) was dissolved in THF (2 mL); DMF (4 μL) and oxalyl dichloride (0.29 g, 1.09 mmol) were added and the mixture was stirred under a N_2 atmosphere for 30 min. The volatile materials were

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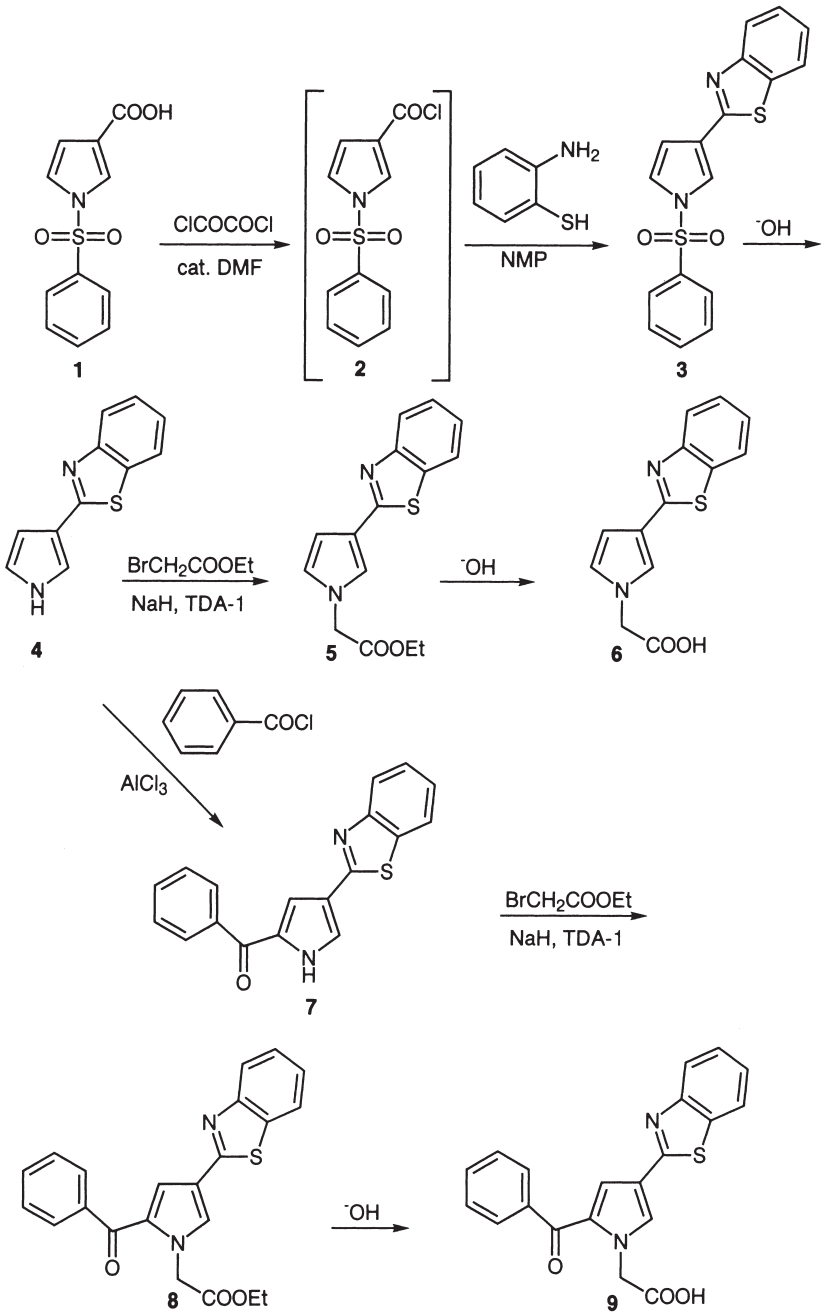

SCHEME 1 Synthesis of target acids **6** and **9**.

removed under reduced pressure, and the resulting solid was used without further purification in the subsequent step. IR (nujol) 1720 cm^{-1} (C = O).

2-(1-Benzenesulfonyl-1*H*-pyrrol-3-yl)benzothiazole (3)

1-Benzenesulfonyl-1*H*-pyrrol-3-carbonyl chloride (**2**) (0.32 g) was dissolved in 1-methylpyrrolidin-2-one (0.7 ml) under a N_2 atmosphere; then 2-aminobenzenthiol (0.1 mL, 0.94 mmol) was added at room temperature and the mixture was heated at 100°C for 1 h under a N_2 atmosphere. After cooling, the solution was poured into water and the pH of the mixture was adjusted to 8–9 by the addition of a 7N aqueous NH_4OH solution. The precipitate was filtered, washed with water, dried and flash chromatographed with ethyl acetate/petroleum ether (3:1) followed by recrystallization from isopropanol/petroleum ether to afford 0.192 g (60% yield) of a white solid, mp 170°–172°C. ^{1}H-NMR ($CDCl_3$/d_6-DMSO) δ 6.33–7.05 (m, 1H, pyrrole C-4-H), 7.30–8.30 (m, 11H, Ar-H). Found: C, 59.72; H, 3.62; N, 8.59. $C_{17}H_{12}N_2O_2S_2$, requires: C, 59.98; H, 3.55; N, 8.23%.

2-(1*H*-pyrrol-3-yl)benzothiazole (4)

2-(1-Benzenesulfonyl-1*H*-pyrrol-3-yl)benzothiazole (**3**) (1.36 g, 3.98 mmol) was dissolved in dioxane (85 mL) and to this an aqueous solution of NaOH (5N, 85 mL) was added. The reaction mixture was vigorously stirred at room temperature for 48 h. The organic layer was collected and the aqueous phase was extracted with ethyl acetate (2 × 100 mL). The combined organic layer and extracts were washed with saturated aqueous NaCl solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was flash chromatographed with ethyl acetate/petroleum ether (3:1) followed by recrystallization from toluene/petroleum ether to afford 0.51 g (64% yield) of a white solid, mp 92°–95°C. IR (nujol) 3200 cm^{-1} (NH). ^{1}H-NMR ($CDCl_3$/d_6-DMSO) δ 6.53–7.00 (m, 2H, pyrrole C-4-H and C-5-H), 7.10–7.70 (m, 3H, pyrrole C-2-H, benzothiazole C-5-H and C-6-H), 7.70–8.13 (m, 2H, benzothiazole C-4-H and C-7-H), 11.20 (br s, 1H, NH). Found: C, 65.73; H, 3.68; N, 13.63. $C_{11}H_8N_2S$ requires: C, 65.98; H, 4.03; N, 13.99%.

(4-Benzothiazol-2-yl-1*H*-pyrrol-2-yl)phenylmethanone (7)

A solution of benzoyl choride (0.065 g, 0.56 mmol) in CH_2Cl_2 (1 mL) was slowly added to a stirred suspension of anhydrous $AlCl_3$ (0.1 g, 1.2 mmol) in CH_2Cl_2 (1 mL), at room temperature and under a N_2 atmosphere. After 10 min, a solution of 2-(1*H*-pyrrol-3-yl)benzothiazole (**4**) (0.1 g, 0.5 mmol) in CH_2Cl_2 (2 mL) was added dropwise at room temperature, and the resulting mixture was stirred for 30 min. The reaction was quenched with a mixture of ice and H_2O and the product was extracted into CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed with saturated aqueous NaCl solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was flash chromatographed with ethyl acetate/petroleum ether (5:1) followed by recrystallization from CH_2Cl_2/petroleum ether to afford 0.11 g (66% yield) of a white solid, mp 235°–237°C. IR (nujol) 3275 (NH), 1610 cm^{-1} (CO). ^{1}H-NMR ($CDCl_3$/d_6-DMSO) δ 7.08–7.68 (m, 6H, pyrrole C-4-H, benzothiazole C-5-H and C-6-H, phenyl-H), 7.68–8.20 (m, 5H, pyrrole C-2-H, benzothiazole C-4-H and C-7-H, phenyl-H). Found: C, 70.49; H, 3.77; N, 9.65. $C_{18}H_{12}N_2OS.0.01CH_2Cl_2$ requires: C, 70.87; H, 3.97; N, 9.18%. (For the previous calculation, the co-crystallization of 0.01 mol CH_2Cl_2, which was used for the recrystallization of this compound and its presence was also evident in the ^{1}H-NMR spectrum, was taken into account).

General Procedure for the Preparation of Compounds (5) and (8)

To a cold (ice bath), stirred and under a N_2 atmosphere mixture of either compound **4** or compound **7** (1 mmol) and TDA-1 (0.05 mL, 0.16 mmol) in toluene (15 mL), was added bromoacetic acid ethyl ester (0.2 mL, 1.8 mmol) and NaH (50% dispersion in mineral oil) (0.06 g, 1.5 mmol) and the resulting mixture stirred at room temperature for 24 h. After this period, it was poured into a stirred, ice cold, mixture of Et_2O (30 mL) and 5% aqueous HCl solution (30 mL), the two phases separated and the aqueous phase extracted with Et_2O (2 × 10 mL). The combined organic phase and extracts were washed with 10% aqueous $NaHCO_3$ solution (2 × 10 mL), saturated aqueous NaCl solution and dried (anhydrous K_2CO_3). The solvents were evaporated under reduced pressure and the residue was flash chromatographed with ethyl acetate/petroleum ether (5/1) followed by recrystallization from CH_2Cl_2/petroleum ether.

(3-Benzothiazol-2-yl-pyrrol-1-yl)acetic acid ethyl ester, **5**: 0.195 g (68% yield) as a white solid, mp 102°–104°C. IR (nujol) 1740 cm^{-1} (COOEt). ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 3H, CH_3), 4.24 (q, 2H, OCH_2), 4.70 (s, 2H, CH_2), 6.60–6.96 (m, 2H, pyrrole C-4-H and C-5-H), 7.12–7.68 (m, 3H, pyrrole C-2-H, benzothiazole C-5-H and C-6-H), 7.68–8.16 (m, 2H, benzothiazole C-4-H and C-7-H). Found: C, 62.40; H, 4.54; N, 10.16. $C_{15}H_{14}N_2O_2S.0.01CH_2Cl_2$ requires: C, 62.77; H, 4.92; N, 9.76%.

(4-Benzothiazol-2-yl-2-benzoylpyrrol-1-yl)acetic acid ethyl ester, **8**: 0.195 g (50% yield) as a white solid, mp 95–97°C. IR (nujol): 1720 (COOEt), 1610 cm^{-1} (CO). ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 3H, CH_3), 4.24 (q, 2H, OCH_2), 4.70 (s, 2H, CH_2), 7.16–7.70 (m, 7H, pyrrole C-2-H and C-4-H, benzothiazole C-5-H and C-6-H, phenyl-H), 7.80–8.20 (m, 4H, benzothiazole C-4-H and C-7-H, phenyl-H). Found: C, 67.56; H, 4.31; N, 7.20. $C_{22}H_{18}N_2O_3S$ requires: C, 67.68; H, 4.65; N, 7.17%.

General Procedure for the Preparation of Compounds 6 and 9

A mixture of either compound **5** or compound **8** (2.48 mmol), dioxane (10 mL) and aqueous NaOH solution (5%, 10 mL) was stirred at room temperature for 1 h. After this period, it was concentrated to half of its volume, H_2O (10 mL) added and the mixture cooled (ice bath) and acidified with concentrated aqueous HCl solution. The precipitate formed was collected by filtration and the filtrate was extracted with CH_2Cl_2 (2 × 20 mL). The organic phase was washed with saturated aqueous NaCl solution and evaporated under reduced pressure.

TABLE I Aldose reductase inhibitory activity

Inhibitor	Concentration % Inhibition (SEM)[a]			IC_{50}^{d}
	10^{-6} M	10^{-7} M	10^{-8} M	10^{-8} M
6	80% (4.0)	63% (0.8)	40% (2.0)	4.9 (0.4)
9	94% (2.0)	53% (3.2)	36% (1.0)	4.5 (0.5)
I[b]	27% (2.0)			250[e]
II[c]	36% (2.0) at: 10^{-5} M			2500[e]
sorbinil		48% (0.5) at: 2.5×10^{-7} M		25[e]

[a]n = 3. [b](3-Benzoylpyrrol-1-yl)acetic acid. [c](2-Benzoylpyrrol-1-yl)acetic acid. [d]Mean (standard error from 3 determinations). [e]The data are from Reference 16.

The residue was combined with the precipitate and recrystallized from isopropanol/petroleum ether.

(3-Benzothiazol-2-yl-pyrrol-1-yl)acetic acid, **6**: 0.615 g (96% yield) as a white solid, mp 241.5°–242°C. IR (nujol): 3300–2500 (OH), 1690 cm^{-1} (CO). ^{1}H-NMR ($CDCl_3/d_6$-DMSO) δ 4.50 (s, 2H, CH_2), 6.50–6.90 (m, 2H, pyrrole C-4-H and C-5-H), 7.10–7.50 (m, 3H, pyrrole C-2-H, benzothiazole C-5-H and C-6-H), 7.70–8.00 (m, 2H, benzothiazole C-4-H and C-7-H). Found: C, 60.13; H, 3.46; N, 11.25. $C_{13}H_{10}N_2O_2S$ requires: C, 60.45; H, 3.90; N, 10.85%.

(4-Benzothiazol-2-yl-2-benzoylpyrrol-1-yl)acetic acid, **9**: 0.728 g (79%) as a white solid, mp 228°–229°C. IR (nujol): 3300–2500 (OH), 1710 (COOH), 1610 cm^{-1} (CO). ^{1}H-NMR ($CDCl_3/d_6$-DMSO) δ 5.20 (s, 2H, CH_2), 7.09–8.26 (m, 11H, ArH). Found: C, 64.72; H, 4.24; N, 7.67. $C_{20}H_{14}N_2O_3S.0.5H_2O$ requires: C, 64.67; H, 4.07; N, 7.54%.

In Vitro Aldose Reductase Enzyme Assay

The test compounds **6**, **9**, (3-benzoylpyrrol-1-yl)acetic acid, (2-benzoylpyrrol-1-yl)acetic acid, and sorbinil (reference) were dissolved in 0.2 M $NaHCO_3$. Lenses were quickly removed from Fischer-344 rats of both sexes following euthanasia, and enzyme preparation and assay were performed as previously described[16,19] with few modifications. Specifically, the total volume of the reaction mixture was 1.1 mL, and the added volume of the solution of the test compounds at the desired concentration was 34 μL. To generate IC_{50} values, compounds **6** and **9** were tested at five concentrations. Log-dose-response curves were then constructed from the inhibitory data and IC_{50} values calculated by least-squares analysis of the linear portions of log dose-response curves ($0.933 < r^2 < 0.977$). All experiments were performed in triplicate. Results are shown in Table I.

RESULTS AND DISCUSSION

The steps for the synthesis of **6** and **9** are shown in Scheme 1. Main points of the preparation were the use of a catalytic amount of DMF for the conversion of acid **1** to its corresponding chloride,[20] the employment as solvent in the formation of the benzothiazole ring the weakly basic 1-methylpyrrolidin-2-one (NMP),[21] and the utilization of the phase transfer catalyst tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) for the introduction of the acetate moiety into the pyrrole ring.[16]

The synthesized target compounds **6** and **9** were tested *in vitro* for their ability to inhibit rat lens AR. It has been shown that there is an approximately 85% sequence similarity between rat lens and human AR, while the proposed active sites of both enzymes are identical.[22] The performed assay was based on the spectrophotometric monitoring of NADPH oxidation, which has proved to be a quite reliable method.[23]

It was found (Table I) that both compounds **6** and **9** inhibit AR with potencies comparable, or moderately lower, to those of known inhibitors. This is supported by literature IC_{50} values, cited in parentheses, for some well studied inhibitors such as sorbinil (7^{24}, 25^{16}, 23^{19}, $90^{25} \times 10^{-8}$ M), tolrestat (3.5×10^{-8} M[26]), epalrestat (1×10^{-8} M[27]), zopolrestat (0.31×10^{-8} M[28]) and fidarestat (3.5×10^{-8} M[25]). Furthermore, compounds **6** and **9** exhibit improved potency on the lead compounds on which their design is based, (3-benzoylpyrrol-1-yl)acetic acid and (2-benzoylpyrrol-1-yl)acetic acid (Table I). Finally, it was noted that the benzoyl substituent in compound **9** did not confer an increase in inhibitory potency. This may indicate the importance of the benzothiazole ring in the enzyme–inhibitor interaction. The benzothiazole ring is a common structural feature in diverse potent ARIs, and it has been proposed[28] that there is a binding site on AR enzyme with strong affinity for benzothiazoles at some distance from a site that binds to acidic groups. This proposal is also supported from the structure of the human aldose reductase complexed with the potent inhibitor zopolrestat,[29] where residue Trp-111, which stacks against the A face of the benzothiazole ring, plays a dominant role by making 38 contacts. Furthermore, the side chain of Leu-300 apposes the B face of the benzothiazole.

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