ORIGINAL ARTICLES

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Synthesis of [5-(4-pyrrol-1-yl-benzoyl)-1*H*-pyrrol-2-yl)]-acetic acid and *in vitro* study of its inhibitory activity on aldose reductase enzyme and on protein glycation

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[5-(4-Pyrrol-1-yl-benzoyl)-1*H*-pyrrol-2-yl)]-acetic acid was synthesized in a Vilsmeier-Haack process and by other methods. The compound was found to inhibit the enzyme aldose reductase as well as the glycation process of proteins and could therefore be useful for the treatment of various pathological conditions.

1. Introduction

It has been suggested that activation of the aldose reductase enzyme (AR, ALR2, E.C. 1.1.1.21) of the polyol pathway is implicated in a number of pathological conditions.

One of these is the development of long term complications of the disease in diabetic patients [1]. However, it should be noted that the existing aldose reductase inhibitors (ARIs), although they have been proven to be effective when given prophylactically to experimental animals, they are ineffective in humans when long term complications have already occurred.

Other examples in non-diabetic individuals are ischemic myocardial injury [2], abnormal proliferation of vascular smooth muscle cells [3] (which is an important feature of atherosclerosis, restenosis, and hypertension), and bipolar and unipolar mood disorders [4]. Furthermore, enhanced formation and accumulation of advanced glycation endproducts (AGE's) have been proposed to play a major role in the pathogenesis of diabetic complications, ageing, atherosclerosis, and Alzheimer disease leading to progressive and irreversible intermolecular protein crosslinkings [5]. Finally, AGE's may elicit activation of the polyol pathway

[6], while about 29% of human liver cancers overexpress AR which might contribute to their resistance to chemotherapy [7]. Thus, compounds that combine inhibitory activity on both AR and protein glycation processes could be of pharmaceutical interest.

In view of the above, in the present study, [5-(4-pyrrol-1-yl-benzoyl)-1*H*-pyrrol-2-yl)]-acetic acid (**5**) was synthesised and its relevant biological activity was evaluated *in vitro*.

2. Investigations, results and discussion

The design of **5** was based on the reported [8] AR inhibitory activity of (5-benzoyl-1*H*-pyrrol-2-yl)-acetic acid [9]. The addition of the second pyrrole ring in **5** was based on the consideration that a biaryl structure (e.g. biphenyl [10]) is a privileged moiety for protein binding. Furthermore, pyrrole − containing compounds have shown good antioxidant activity [11], a property which is present in a number of protein glycation inhibitors [12].

The main steps in the preparation of **5** (Scheme) were the introduction of a *p*-nitrobenzoyl substituent on (1*H*-pyrrol-2-yl)-acetic acid ethyl ester (**1**), the formation of an anilinic group under hydrogen transfer reduction conditions [13], the formation of a pyrrole ring with a Clauson-Kaas

Scheme
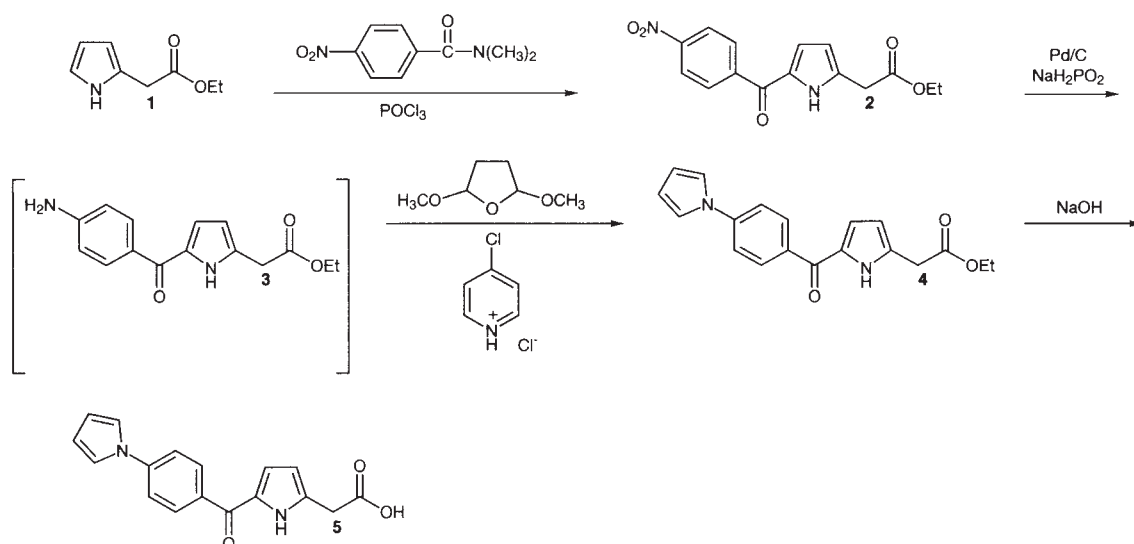
Table 1: Aldose reductase inhibitory activity

Compd.	Concentration % Inhibition (SEM)[a]		
	10^{-5} M	10^{-6} M	10^{-7} M
(5-Benzoyl-1*H*-pyrrol-2-yl)-acetic acid	60% (1.4)	4% (2.7)	0
5	65% (2.2)	49% (3.6)	0
Trolox	12% (0)		
Sorbinil			48% (0.5) at: 2.5×10^{-7} M

[a] $n = 3$

type reaction [14], and the alkaline hydrolysis of the ester functionality in a mixture of dioxane-water.

For developing optimum conditions for the introduction of the *p*-nitrobenzoyl substituent in **1**, both Friedel-Crafts [9] and Vilsmeier-Haack [9, 15] type aroylation reactions were studied. Best results were obtained with the Vilsmeier-Haack type reaction, which was implemented after an appropriate combination of the two [9, 15] previously reported methodologies.

The synthesised target compound **5** was tested *in vitro* for its 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 [16]. The assay performed was based on the spectrophotometric monitoring of NADPH oxidation, which has been proved to be quite a reliable method [17].

It was found (Table 1) that the target compound **5** was an inhibitor of AR, with potency approximately 10 times higher than the lead compound considered for its design (5-benzoyl-1*H*-pyrrol-2-yl)-acetic acid. However, the therapeutic index of these compounds has not been determined, and thus the advantage of **5** has not yet been proven.

Compound **5** was also tested for its ability to inhibit *in vitro* the irreversible modification of the model protein albumin (the most abundant in serum) in the presence of fructose (fructation) as the glycating monosacharide. Fructose was chosen in the assay rather than glucose (glucation), because is known to be a more potent glycation agent [12]. This derives from the fact that its acyclic (open chain) form, which is the reactive species, is approximately 10 times more potent than glucose. Fructose is also elevated in those tissues where the polyol pathway is active.

It was found (Table 2) that the compound examined is as effective (and for some measured parameters more effective) than the known [18] protein glycation inhibitor trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid). However, trolox is a weak AR inhibitor (Table 1).

The above results, taken together, are considered encouraging, as [5-(4-pyrrol-1-yl-benzoyl)-1*H*-pyrrol-2-yl]-acetic acid (**5**) favourably combines two biological activities directly connected to a number of pathological conditions.

3. Experimental

3.1. Apparatus

M.p.s. 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, ^{1}H NMR spectra with a Bruker AW-80 spectrometer with internal TMS standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, USA. Fluorescence was recorded on a Hitachi F-2000 spectrophotometer. All the results were in an acceptable range.

3.2. [5-(4-Nitrobenzoyl)-1H-pyrrol-2-yl]-acetic acid ethyl ester (2)

3.2.1. With Vilsmeier-Haack methodology

A mixture of *N,N*-dimethyl-4-nitrobenzamide [19] (1.67 g, 8.62 mmol) and phosphoryl chloride (2.88 g, 18.81 mmol) was stirred at room temperature for 4 h under a nitrogen atmosphere. A solution of (1*H*-pyrrol-2-yl)-acetic acid ethyl ester (**1**) [20] (0.6 g, 3.92 mmol) in 1,2-dichloroethane (20 ml) was added and the mixture was stirred at room temperature for 24 h under a nitrogen atmosphere. A solution of AcONa (9.8 g, 119.47 mmol) in H_2O (39 ml) and 1,2-dichloroethane (78 ml) was added and the mixture was refluxed for 2 h under a nitrogen atmosphere. The organic phase was separated and combined with a dichloromethane extract of the aqueous phase. The organic phase was washed with saturated aqueous NaCl solution, and dried over anh. Na_2SO_4 and the solvents were evaporated under reduced pressure. The residue was flash cromatographed with AcOEt/petroleum ether (3 : 1) followed by recrystallization from AcOEt/petroleum ether. Yield: 1.003 g (87%), m.p. 150–152 °C. IR (Film, cm^{-1}): 3260 (NH); 1752 (CO); 1623 (CO). ^{1}H NMR (CDCl$_3$), (δ ppm): 1.25 (t, J = 7.2 Hz, 3 H, CH$_3$); 3.74 (s, 2 H, CH$_2$); 4.20 (q, J = 7.2 Hz, 2 H, CH$_2$O); 6.15–6.29 (m, 1 H, pyrrole C-3-H, after addition of D$_2$O changes to d, J = 3.5 Hz); 6.74–6.91 (m, 1 H, pyrrole C-4-H, after addition of D$_2$O changes to d, J = 3.5 Hz); 7.94–8.13 (m, 2 H, phenyl C-2-H and C-6-H); 8.26–8.51 (m, 2 H, phenyl C-3-H and C-5-H); 10.57 (br s, 1 H, NH, exchangeable with D$_2$O). C$_{15}$H$_{14}$N$_2$O$_5$

3.2.2. With Friedel-Crafts methodology

Method A: 4-Nitrobenzoyl choride (2.07 g, 11.18 mmol) was slowly added, at room temperature, to a stirred suspension of anh. AlCl$_3$ (2.48 g, 18.63 mmol) in 1,2-dichloroethane (75 ml) under a nitrogen atmosphere. After 10 min, a solution of (1*H*-pyrrol-2-yl)-acetic acid ethyl ester (**1**) (1.43 g, 9.32 mmol) in 1,2-dichloroethane (19 ml) was added at 0 °C, and the mixture was stirred at room temperature for 12 h under a nitrogen atmosphere. The reaction was quenched with ice, CH$_2$Cl$_2$ (88 ml), Et$_3$N (18 ml) and EtOH (44 ml) and the product was extracted with CH$_2$Cl$_2$ (3 × 200 ml). The combined organic extracts were washed with saturated aqueous NaCl solution, dried over anh. Na$_2$SO$_4$ and evaporated under reduced pressure. The residue was flash chromatographed with AcOEt/petroleum ether (8 : 1 → 3 : 1) as the eluent to afford products in the following order.

a) 0.968 g (34%) [5-(4-Nitrobenzoyl)-1*H*-pyrrol-2-yl]-acetic acid ethyl ester (**2**), identical with the compound isolated with the Vilsmeier-Haack methodology.

b) 0.034 g (1%) [4-(4-Nitrobenzoyl)-1*H*-pyrrol-2-yl]-acetic acid ethyl ester (isomer of **2**). M.p. 143–145 °C (recrystallization from AcOEt/petroleum ether). IR (Film, cm^{-1}): 3346 (NH); 1722 (CO); 1632 (CO). ^{1}H NMR

Table 2: Glycation-induced fluorescence changes of BSA and formation of DNPH-reactive carbonyl groups in BSA exposed to fructose. Effect of inhibitors

Compd.	C (mM)	Relative fluorescence		Carbonyl groups	
		(R.U.)	% Inhibition	(nmol/mg BSA)	% Inhibition
None	—	15.56 ± 1.8 (4)	—	4.46 ± 0.04 (3)	—
5	5	0 (6)	100%	Not detectable (6)	100%
5	2.5	2.4 ± 0.17 (5)[b]	85%	Not detectable (6)	100%
5	1	5.34 ± 0.4 (5)[b]	66%	Not detectable (5)	100%
Trolox	1	7.16 ± 1.4 (5)[b]	54%	3.05 ± 0.53 (5)[a]	32%

Results are means ±SD with number of samples in parentheses; [a] $P < 0.01$, [b] $P < 0.001$ vs. control according to Student's t test

(CDCl$_3$), (δ ppm): 1.28 (t, J = 7.3 Hz, 3 H, CH$_3$); 3.68 (s, 2 H, CH$_2$); 4.21 (q, J = 7.23 Hz, 2 H, CH$_2$); 6.51–6.60 (m, 1 H, pyrrole C-3-H, after addition of D$_2$O changes to d, J = 1 Hz); 7.23–7.34 (m, 1 H, pyrrole C-5-H, after addition of D$_2$O changes to d, J = 1 Hz); 7.84–8.05 (m, 2 H, phenyl C-2-H and C-6-H); 8.23–8.43 (m, 2 H, phenyl C-3-H and C-5-H); 9.62 (br s, 1 H, NH, exchangeable with D$_2$O).
C$_{15}$H$_{14}$N$_2$O$_5$

Method B: AlCl$_3$ (0.4 g, 3 mmol) was slowly added at 0 °C to a stirred solution of (1H-pyrrol-2-yl)-acetic acid ethyl ester (1) (0.23 g, 1.5 mmol) in 1,2-dichloroethane (12 ml) under a nitrogen atmosphere. After 10 min a solution of 4-nitrobenzoyl chloride (0.344 g, 1.8 mmol) in 1,2-dichloroethane (3 ml) was added and the mixture was stirred at room temperature for 12 h under a nitrogen atmosphere. The work up and isolation process was analogous to method A, and yielded products in the following order.
a) 0.118 g (26%) [5-(4-Nitrobenzoyl)-1H-pyrrol-2-yl]-acetic acid ethyl ester (2), identical with the compound isolated with the Vilsmeier-Haack methodology.
b) 0.022 g (5%) [4-(4-Nitrobenzoyl)-1H-pyrrol-2-yl]-acetic acid ethyl ester (isomer of 2), identical with the compound isolated with method A.

3.3. [5-(4-Aminobenzoyl)-1H-pyrrol-2-yl]-acetic acid ethyl ester (3)

To a stirred suspension of [5-(4-nitrobenzoyl)-1H-pyrrol-2-yl)-acetic acid ethyl ester (2) (0.46 g, 1.53 mmol) and Pd/C (10%, 0.092 g) in tetrahydrofuran (9 ml), was gradually added an aqueous solution of sodium phosphinate (30%). Addition of the phosphinate solution was continued until no starting material remained. The mixture was filtered and the filtrate poured into H$_2$O (13 ml) and extracted with CH$_2$Cl$_2$. The combined extracts were dried (Na$_2$SO$_4$) and the solvents were evaporated under reduced pressure to give crude [5-(4-aminobenzoyl)-1H-pyrrol-2-yl]-acetic acid ethyl ester (3). This product (0.433 g) was used directly in the next step without further purification, due to its low stability. IR (Film, cm^{-1}): 3474, 3367, 3260 (NH); 1730 (CO); 1640 (CO). ^{1}H NMR (CDCl$_3$), (δ ppm): 1.37 (t, J = 6.8 Hz, 3 H, CH$_3$); 3.71 (s, 2 H, CH$_2$); 4.15 (q, J = 6.8 Hz, 2 H, CH$_2$O); 6.14–6.28 (m, 1 H, pyrrole C-3-H, after addition of D$_2$O changes to d, J = 4.1 Hz); 6.57–7.02 (m, 5 H, pyrrole C-4-H, phenyl C-3-H and C-5-H, NH$_2$ after addition of D$_2$O changes to m, 3H); 7.68–8.00 (m, 2 H, phenyl C-2-H and C-6-H), 10.51 (br s, 1 H, NH, exchangeable with D$_2$O).

3.4. [5-(4-Pyrrol-1-yl-benzoyl)-1H-pyrrol-2-yl]-acetic acid ethyl ester (4)

A mixture of crude 3 (0.433 g), 4-chloropyridine hydrochloride (0.366 g, 2.44 mmol) and 2,5-dimethoxy-tetrahydrofuran (0.29 g, 2.2 mmol) in dioxane (20 ml) was refluxed for 3 h under a nitrogen atmosphere. Then, it was evaporated under reduced pressure and the residue was treated with several portions of ether. The ethereal layer was collected, dried over anh. Na$_2$SO$_4$ and evaporated under reduced pressure. The residue was flash cromatographed with AcOEt/petroleum ether (5 : 1) followed by recrystallization from CH$_2$Cl$_2$/petroleum ether. Yield: 0.58 g (59% from 2), m.p. 107–109 °C. IR (Film, cm^{-1}): 3282 (NH); 1735 (CO); 1615 (CO).
^{1}H NMR (CDCl$_3$), (δ ppm): 1.24 (t, J = 7.2 Hz, 3 H, CH$_3$); 3.77 (s, 2 H, CH$_2$); 4.17 (q, J = 7.2 Hz, 2 H, CH$_2$O); 6.16–6.48 (m, 3 H, pyrrole C-3-H and phenylpyrrole C-3-H and C-4-H); 6.80–6.92 (m, 1 H, pyrrole C-4-H, after addition of D$_2$O changes to d, J = 4 Hz); 7.12–7.24 (m, 2 H, phenylpyrrole C-2-H and C-5-H), 7.40–7.64 (m, 3 H, ArH–N and NH, after addition of D$_2$O changes to m, 2 H, ArH-N); 7.88–8.16 (m, 2 H, ArH–C=O).
C$_{19}$H$_{18}$N$_2$O$_3$

3.5. [5-(4-Pyrrol-1-yl-benzoyl)-1H-pyrrol-2-yl]-acetic acid (5)

A mixture of 4 (0.35 g, 1.09 mmol), dioxane (20 ml) and 5% aqueous NaOH solution (20 ml) was stirred at room temperature for 1 h. After this period, it was concentrated to half its volume, H$_2$O (20 ml) was added, and it was cooled (ice bath) and acidified with concentrated HCl. The precipitate formed was collected by filtration, and the filtrate was extracted with CH$_2$Cl$_2$ (2x40 ml). The organic phase was washed with saturated aqueous NaCl solution and evaporated under reduced pressure. The residue was combined with the precipitate and recrystallized from CHCl$_3$/petroleum ether. Yield: 0.305 g (95%), m.p. 201–203 °C. IR (Film, cm^{-1}): 3346 (NH); 2833–2042 (OH); 1705 (CO); 1615 (CO). ^{1}H NMR (CDCl$_3$/DMSO-d$_6$), (δ ppm): 3.72 (s, 2 H, CH$_2$); 6.04–6.35 (m, 3 H, pyrrole C-3-H and phenylpyrrole C-3-H and C-4-H); 6.66–6.86 (m, 1 H, pyrrole C-4-H, after addition of D$_2$O changes to d, J = 4 Hz); 7.12–7.24 (m, 2 H, phenylpyrrole C-2-H and C-5-H), 7.40–7.68 (m, 3 H, ArH–N and NH,

after addition of D$_2$O changes to m, 2 H, ArH–N); 7.84–8.12 (m, 2 H, ArH–C=O); 11.34 (br s, 1 H, COOH, exchangeable with D$_2$O).
C$_{17}$H$_{14}$N$_2$O$_3$ · 0.07 CHCl$_3$

3.6. In vitro aldose reductase enzyme assay

The test compounds 5, (5-benzoyl-1H-pyrrol-2-yl)-acetic acid, trolox, 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 [21] 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. All experiments were performed in triplicate. Results are shown in Table 1.

3.7. In vitro protein glycation assay

The assay was performed as previously described [18]. It involved incubation of bovine serum albumin (BSA, fraction V, essentially fatty acid free) with fructose for 28 days. The test compounds 5 and trolox (reference) were dissolved in water in the form of their potassium salts. Results are shown in Table 2.

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