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Short communication

Synthesis and aldose reductase inhibitory activity of benzoyl-amino acid derivatives

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Abstract

A series of *N*-(4-methoxy, 4-fluoro, 4-trifluoromethyl and 4-nitrobenzoyl)-L-amino acids was synthesized and their inhibitory activity towards bovine lens aldose reductase (ALR2) was tested.

Keywords: Benzoyl-L-amino acid derivatives; Aldose reductase inhibitors

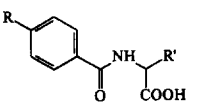
1. Introduction

The use of insulin and oral hypoglycemic agents has afforded relief for the control of glycemia in people suffering from diabetes, but the long-term side effects such as neuropathy, retinopathy, nephropathy and cataract that can occur are still a matter of concern. The cause would appear to be the increased glucose flux through the polyol pathway and/or the high intracellular accumulation of sorbitol [1]. Sorbitol is formed by reduction of glucose by aldose reductase (ALR2), the first enzyme of the polyol pathways that catalyses the transformation of aldoses into the corresponding polyalcohols [2].

Thus, several ALR2 inhibitors have been studied as therapeutic agents in order to reduce or to delay the development of long-term diabetic complications [3,4]. These compounds belong to different chemical classes and they can be divided into two general groups, those containing rigid spirohydantoins or a related ring system, such as Sorbinil, and those containing a carboxylic acid moiety, like Alrestatin, Tolrestat and Zopolrestat; in these molecules a planar aromatic structure with a carboxylic or another acid proton appears to be essential to the inhibitory effect [5].

Considering that some *N*-benzoylglycines were reported to be weak inhibitors of ALR2 [6], we decided to synthesize a series of *N*-(4-substituted)benzoyl-L-amino acids as ALR2 inhibitors (Table 1).

Table 1
The series of *N*-(4-substituted)benzoyl-L-amino acids synthesized as ALR2 inhibitors



Comp.	R	R′
1	OCH_3	H
2	OCH_3	CH_3
3	OCH_3	CH_2–C_6H_5
4	OCH_3	CH_2–C_6H_4OH
5	OCH_3	CH_2–$CH(CH_3)_2$
6	OCH_3	$CH(CH_3)$–CH_2–CH_3
7	OCH_3	$CH(CH_3)_2$
8	OCH_3	CH_2–CH_2SCH_3
9	F	H
10	F	CH_3
11	F	CH_2–C_6H_5
12	F	CH_2–C_6H_4OH
13	F	CH_2–$CH(CH_3)_2$
14	CF_3	H
15	CF_3	CH_3
16	CF_3	CH_2–C_6H_5
17	CF_3	CH_2–$CH(CH_3)_2$
18	NO_2	H
19	NO_2	CH_3
20	NO_2	CH_2–C_6H_5
21	NO_2	CH_2–C_6H_4OH
22	NO_2	CH_2–$CH(CH_3)_2$

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2. Experimental

2.1. Chemistry

2.1.1. Material and methods

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Structural assignments for compounds are based on UV, mass spectral, and ^{1}H NMR data (Table 2). The UV spectra were recorded on a Perkin-Elmer Lambda 15 spectrophotometer using 1 cm quartz cells in a 10^{-5} M ethanol solution. The ^{1}H NMR spectra were recorded in DMSO-d_6 solution with a Bruker AMX-400 WB spectrometer. Chemical shifts δ are reported in ppm from tetramethylsilane used as internal standard. Mass spectra were obtained with a Finningan MAT SSQ 710 instrument.

Microanalyses were within $\pm$.0.4% of the theoretical values.

The compounds were separated by flash-chromatography with silica gel 60 (particle size 0.040–0.063 mm, Merck) and the column was connected to an LKB Multirac 2111 fraction collector. The fractions were monitored using thin-layer chromatography plates.

2.1.2. General procedure for synthesis

L-Amino acid (8.5 mmol), 1N NaOH (18 mmol) and acetone (10 ml) for **1–8** or ethyl ether (25 ml) for **9–22** were added dropwise to a solution of 4-methoxybenzoyl chloride (8.5 mmol) in acetone (5 ml) for **1–8** or ethyl ether (25 ml) for **9–22**. The resulting mixture was stirred at room temperature for 60 minutes. The solution was acidified to pH 1 with conc. HCl and concentrated in vacuo to eliminate the organic solvent.

For compounds **1–8** the precipitate was purified by flash-chromatography (ethyl acetate/cyclohexane/acetic acid

Table 2
Physical data of compounds **1–22**

Comp.	M.p. (°C)	Spectral data
1	160–162 (172 [11])	UV: 252.8 (log ϵ=4.19) MS, *m/z*: 209 (M^+)$^{(19)}$, 165$^{(36)}$, 164$^{(38)}$, 135$^{(100)}$, 107$^{(19)}$ ^{1}H NMR: 3.91 (3H, OCH_3, s), 4.00 (2H, CH_2, d), 7.10 (2H, Ar, m), 7.94 (2H, Ar, m), 8.75 (1H, NH, d), 12.64 (1H, COOH, s)
2	141–143	UV: 252.0 (log ϵ=4.21) MS, *m/z*: 223 (M^+)$^{(3)}$, 179$^{(27)}$, 178$^{(17)}$, 135$^{(100)}$, 107$^{(7)}$ ^{1}H NMR: 1.48 (3H, CH_3, m), 3.91 (3H, OCH_3, s), 4.49 (1H, CH, d), 7.09 (2H, Ar, m), 7.96 (2H, Ar, m), 8.56 (1H, NH, d), 12.58 (1H, COOH, s)
3	90	UV: 252.0 (log ϵ=4.20) MS, *m/z*: 299 (M^+)$^{(4)}$, 151$^{(41)}$, 135$^{(100)}$, 107$^{(6)}$ ^{1}H NMR: 3.19 (2H, CH_2, m), 3.83 (3H, OCH_3, s), 4.61 (1H, CH, m), 7.02 (2H, Ar, m), 7.25 (5H, Ar, m), 7.82 (2H, Ar, m), 8.44 (1H, NH, d), 12.75 (1H, COOH, s)
4	110–112	UV: 252.8 (log ϵ=4.20) MS, *m/z*: 315 (M^+)$^{(4)}$, 164$^{(17)}$, 152$^{(80)}$, 135$^{(100)}$, 107$^{(57)}$ ^{1}H NMR: 3.02 (2H, CH_2, m), 3.82 (3H, OCH_3, s), 4.53 (1H, CH, d), 6.64 (2H, Ar, m), 6.96 (2H, Ar, m), 7.10 (2H, Ar, m), 7.82 (2H, Ar, m), 8.43 (1H, NH, d), 12.61 (1H, COOH, s)
5	133–136	UV: 252.0 (log ϵ=4.21) MS, *m/z*: 265 (M^+)$^{(<1)}$, 209$^{(25)}$, 151$^{(5)}$, 135$^{(100)}$, 107$^{(6)}$ ^{1}H NMR: 0.92 (6H, $2CH_3$, dd),1.7 (3H, CH_2–CH, m), 3.83 (3H, OCH_3, s), 4.43 (1H, CH_α, d), 7.03 (2H, Ar, m), 7.87 (2H, Ar, m), 8.41 (1H, NH, d), 12.55 (1H, COOH, s)
6	105–107	UV: 252.0 (log ϵ=4.22) MS, *m/z*: 265 (M^+)$^{(4)}$, 221$^{(15)}$, 220$^{(7)}$, 209$^{(20)}$, 191$^{(18)}$, 151$^{(65)}$, 135$^{(100)}$, 107$^{(14)}$ ^{1}H NMR: 0.92 (6H, $2CH_3$, dd), 1.37 (2H, CH_2, m), 2.01 (1H, CH_β, m), 3.83 (3H, OCH_3, s), 4.37 (1H, CH_α, m), 7.03 (2H, Ar, m), 7.90 (2H, Ar, m), 8.20 (1H, NH, d), 12.55 (1H, COOH, s)
7	147–151	UV: 251.0 (log ϵ=4.08) MS, *m/z*: n.d. ^{1}H NMR: 0.97 (6H, $2CH_3$, dd), 2.22 (1H, CH_β, m), 3.83 (3H, OCH_3, s), 4.29 (1H, CH_α, m), 7.01 (2H, Ar, m), 7.90 (2H, Ar, m), 8.20 (1H, NH, d), 12.52 (1H, COOH, s)
8	117–120	UV: 252.8 (log ϵ=4.24) MS, *m/z*: 283 (M^+)$^{(4)}$, 209$^{(28)}$, 192$^{(3)}$, 191$^{(25)}$, 135$^{(100)}$, 107$^{(7)}$ ^{1}H NMR: 2.05 (3H, SCH_3, s), 2.06 (2H, CH_2, m), 2.61 (2H, CH_2, m), 3.83 (3H, OCH_3, s), 4.51 (1H, CH_α, m), 7.03 (2H, Ar, m), 7.87 (2H, Ar, m), 8.45 (1H, NH, d), 12.59 (1H, COOH, s)
9	163–165	UV: 234.0 (log ϵ=3.69) MS, *m/z*: 197 (M^+)$^{(2)}$, 153$^{(59)}$, 152$^{(40)}$, 123$^{(100)}$ ^{1}H NMR: 3.93 (2H, CH_2, d), 7.31 (2H, Ar, m), 7.92 (2H, Ar, m), 8.85 (1H, NH, t), 12.50 (1H, COOH, s)
10	103–104	UV: 230.4 (log ϵ=4.06) MS, *m/z*: 211 (M^+)$^{(2)}$, 167$^{(13)}$, 166$^{(27)}$, 123$^{(100)}$ ^{1}H NMR: 1.41 (3H, CH_3, d), 4.43 (1H, CH, m), 7.32 (2H, Ar, m), 7.98 (2H, Ar, m), 8.67 (1H, NH, d), 12.50 (1H, COOH, s)

(*continued*)

Table 2 (*continued*)

Comp.	M.p. (°C)	Spectral data
11	134–135	UV: 269.6 (log ϵ=4.12) MS, *m/z*: 287 (M^+)$^{(3)}$, 148$^{(76)}$, 147$^{(37)}$, 123$^{(100)}$ ^{1}H NMR: 3.12 (2H, CH_2, m), 4.64 (1H, CH, m), 7.13–7.35 (7H, Ar, m), 7.88 (2H, Ar, m), 8.72 (1H, NH, d), 12.52 (1H, COOH, s)
12	149–151	UV: 270.4 (log ϵ=3.86) MS, *m/z*: 303 (M^+)$^{(2)}$, 164$^{(83)}$, 140$^{(25)}$, 123$^{(70)}$, 107$^{(100)}$ ^{1}H NMR: 3.02 (2H, CH_2, m), 4.53 (1H, CH, d), 6.64 (2H, Ar, m), 7.11 (2H, Ar, m), 7.30 (2H, Ar, m), 7.88 (2H, Ar, m), 8.62 (1H, NH, d), 9.13 (1H, OH, s), 12.64 (1H, COOH, s)
13	159–162	UV: 222.4 (log ϵ=3.84) MS, *m/z*: 253 (M^+)$^{(<1)}$, 208$^{(7)}$, 197$^{(24)}$, 179$^{(12)}$, 139$^{(2)}$, 123$^{(100)}$ ^{1}H NMR: 0.93 (6H, $2CH_3$, dd), 1.73 (3H, CH_2–CH, m), 4.48 (1H, CH, m), 7.87 (2H, Ar, m), 8.11 (2H, Ar, m), 8.83 (1H, NH, d), 12.55 (1H, COOH, s)
14	153–156	UV: 222.4 (log ϵ=3.95) MS, *m/z*: 247 (M^+)$^{(1)}$, 203$^{(26)}$, 202$^{(23)}$, 173$^{(100)}$, 145$^{(55)}$ ^{1}H NMR: 3.98 (2H, CH2, d), 7.88 (2H, Ar, m), 8.08 (2H, Ar, m), 9.06 (1H, NH, t), 12.60 (1H, COOH, s)
15	146–147	UV: 223.2 (log ϵ=4.08) MS, *m/z*: 261 (M^+)$^{(3)}$, 2170$^{(8)}$, 216$^{(50)}$, 173$^{(100)}$, 145$^{(70)}$ ^{1}H NMR: 1.43 (3H, CH_3, d), 4.47 (1H, CH, m), 7.87 (2H, Ar, m), 8.10 (2H, Ar, m), 8.90 (1H, NH, d), 12.58 (1H, COOH, s)
16	133–135	UV: 212.0 (log ϵ=4.03) MS, *m/z*: 337 (M^+)$^{(2)}$, 173$^{(97)}$, 148$^{(100)}$, 147$^{(41)}$, 145$^{(65)}$ ^{1}H NMR: 3.20 (2H, CH_2, m), 4.68 (1H, CH, m), 7.16–7.36 (5H, Ar, m), 7.85 (2H, Ar, m), 7.99 (2H, Ar, m), 8.93 (1H, NH, d), 12.78 (1H, COOH, s)
17	82	UV: 237.0 (log ϵ=3.40) MS, *m/z*: 303 (M^+)$^{(<1)}$, 258$^{(14)}$, 247$^{(27)}$, 229$^{(18)}$, 173$^{(100)}$, 145$^{(38)}$ ^{1}H NMR: 0.88 (6H, $2CH_3$, dd), 1.72 (3H, CH_2–CH, m), 4.54 (1H, CH, m), 7.34 (2H, Ar, m), 7.99 (2H, Ar, m), 8.59 (1H, NH, d), 12.60 (1H, COOH, s)
18	133–135 (135 [12])	UV: 257.0 (log ϵ=3.67) MS, *m/z*: 224 (M^+)$^{(1)}$, 180$^{(39)}$, 179$^{(46)}$, 150$^{(100)}$, 120$^{(11)}$, 104$^{(42)}$ ^{1}H NMR: 3.97 (2H, CH_2, d), 8.10 (2H, Ar, m), 8.34 (2H, Ar, m), 9.15 (1H, NH, t), 12.65 (1H, COOH, s)
19	166–167	UV: 258.0 (log ϵ=3.64) MS, *m/z*: 238 (M^+)$^{(1)}$, 194$^{(4)}$, 193$^{(71)}$, 150$^{(100)}$, 120$^{(9)}$, 104$^{(32)}$ ^{1}H NMR: 1.43 (3H, CH_3, d), 4.47 (1H, CH, m), 8.13 (2H, Ar, m), 8.35 (2H, Ar, m), 9.00 (1H, NH, d), 12.60 (1H, COOH, s)
20	137–138	UV: 258.0 (log ϵ=3.67) MS, *m/z*: 314 (M^+)$^{(6)}$, 150$^{(73)}$, 148$^{(100)}$, 147$^{(50)}$, 120$^{(19)}$, 104$^{(82)}$ ^{1}H NMR: 3.19 (1H, CH, m), 4.69 (2H, CH_2, m), 7.16–7.36 (5H, Ar, m), 8.03 (2H, Ar, m), 8.33 (2H, Ar, m), 9.05 (1H, NH, d), 12.82 (1H, COOH, s)
21	148–150 dec (163–164 [13])	UV: 252.0 (log ϵ=3.22) MS, *m/z*: 330 (M^+)$^{(1)}$, 164$^{(83)}$, 150$^{(14)}$, 120$^{(6)}$, 107$^{(100)}$, 104$^{(17)}$ ^{1}H NMR: 3.06 (2H, CH_2, m), 4.59 (1H, CH, m), 6.66 (2H, Ar, m), 7.10 (2H, Ar, m), 8.02 (2H, Ar, m), 8.32 (2H, Ar, m), 8.99 (1H, NH, d), 9.13 (1H, OH, s), 12.70 (1H, COOH, s)
22	155	UV: 256.0 (log ϵ=3.30) MS, *m/z*: 280 (M^+)$^{(<1)}$, 235$^{(29)}$, 224$^{(33)}$, 206$^{(23)}$, 150$^{(100)}$, 120$^{(9)}$, 104$^{(39)}$ ^{1}H NMR: 0.94 (6H, $2CH_3$, dd), 1.73 (3H, CH_2–CH, m), 4.41 (1H, CH, m), 8.13 (2H, Ar, m), 8.34 (2H, Ar, m), 8.94 (1H, NH, d), 12.61 (1H, COOH, s)

7:1:0.05) to obtain the corresponding *N*-(4-methoxybenzoyl)amino acid.

For compounds **9–22** the residue obtained by evaporation of the organic phase was crystallized from ethyl acetate/n-heptane.

2.2. Enzyme inhibition assay

Quercetin was purchased from Fluka; Tolrestat was synthesized following the published procedure [7]; Sorbinil was a gift from Pfizer.

Aldose reductase (EC 1.1.1.21 ALR2) was partially purified from bovine lenses as reported in the literature [8,9].

The partially purified enzyme obtained had a specific activity of 6.5 mU/mg; no appreciable aldehyde reductase contamination was detected by sodium valproate assay [10].

A reference blank containing all the above reagents except the substrate was used to correct for the oxidation of NADPH not associated with the catalytic activity [8].

IC_{50} values were determined from least-squares analysis of the linear portion of the log dose–inhibition curves. Each curve was generated using at least three concentrations of

inhibitor causing an inhibition between 20% and 80% with two replicates at each concentration.

3. Results

All the compounds showed no activity when tested at a final concentration of 100 μM in the assay. Sorbinil, Tolrestat, and quercetin show the following IC_{50} values: Sorbinil 2.58 μM, Tolrestat 0.096 μM, and quercetin 39.9 μM.

Compounds **1**, **2** and **18** were reported to be weak inhibitors of rat lens ALR2 [6] (1/100 of the activity of Sorbinil), but they were found to be inactive with respect to bovine lens ALR2 and the introduction of different substituents showed no appreciable potentiating effects.

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