

## IL FARMACO

Il Farmaco 53 (1998) 439-442

### Short communication

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

Stefania Benvenuti\*, Fabio Severi, Luca Costantino, Gabriella Vampa, Michele Melegari

Dipartimento di Scienze Farmaceutiche, Università di Modena, Via Campi 183, 41100 Modena, Italy

Received 5 December 1997; accepted 26 March 1998

#### 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. © 1998 Elsevier Science S.A. All rights reserved.

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 <sub>3</sub>	Н
2	OCH <sub>3</sub>	CH <sub>3</sub>
3	OCH <sub>3</sub>	CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>
4	OCH <sub>3</sub>	CH <sub>2</sub> –C <sub>6</sub> H₄OH
5	OCH <sub>3</sub>	$CH_2$ - $CH(CH_3)_2$
6	OCH <sub>3</sub>	CH(CH <sub>3</sub> )-CH <sub>2</sub> -CH <sub>3</sub>
7	OCH <sub>3</sub>	$CH(CH_3)_2$
8	OCH <sub>3</sub>	CH <sub>2</sub> -CH <sub>2</sub> SCH <sub>3</sub>
9	F	н
10	F	CH <sub>3</sub>
11	F	CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>
12	F	CH <sub>2</sub> -C <sub>6</sub> H <sub>4</sub> OH
13	F	$CH_2-CH(CH_1)_2$
14	CF <sub>3</sub>	H
15	CF <sub>3</sub>	CH <sub>3</sub>
16	CF <sub>3</sub>	CH <sub>2</sub> -C <sub>4</sub> H <sub>5</sub>
17	CF <sub>3</sub>	$CH_2 - CH(CH_3)_2$
18	NO <sub>2</sub>	н
19	NO <sub>2</sub>	CH <sub>3</sub>
20	NO <sub>2</sub>	CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>
21	NO <sub>2</sub>	CH <sub>2</sub> -C <sub>6</sub> H <sub>4</sub> OH
22	NO <sub>2</sub>	$CH_2 - CH(CH_3)_2$

<sup>\*</sup> Corresponding author. Tel.: + 39 59 378 575; fax: + 39 59 378 560; e-mail: melegari@unimo.it

#### 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 <sup>1</sup>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 <sup>1</sup>H NMR spectra were recorded in DMSO-d<sub>6</sub> solution with a Bruker AMX-400 WB spectrometer. Chemical shifts  $\delta$  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.

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

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 thinlayer 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 flashchromatography (ethyl acetate/cyclohexane/acetic acid

Comp.	M.p. (°C)	Spectral data
1	160–162	UV: 252.8 (log $\epsilon$ = 4.19)
	(172 [11])	MS, $m/z$ : 209 $(M^+)^{(19)}$ , 165 <sup>(36)</sup> , 164 <sup>(38)</sup> , 135 <sup>(100)</sup> , 107 <sup>(19)</sup>
	, <u> </u>	<sup>1</sup> H NMR: 3.91 (3H, OCH <sub>3</sub> , s), 4.00 (2H, CH <sub>2</sub> , 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 $\epsilon = 4.21$ )
		MS, $m/z$ : 223 $(M^+)^{(3)}$ , 179 <sup>(27)</sup> , 178 <sup>(17)</sup> , 135 <sup>(100)</sup> , 107 <sup>(7)</sup>
		<sup>1</sup> H NMR: 1.48 (3H, CH <sub>3</sub> , m), 3.91 (3H, OCH <sub>3</sub> , 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 $\epsilon = 4.20$ )
		$MS, m/z: 299 (M^+)^{(4)}, 151^{(41)}, 135^{(100)}, 107^{(6)}$
		<sup>1</sup> H NMR: 3.19 (2H, CH <sub>2</sub> , m), 3.83 (3H, OCH <sub>3</sub> , 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 $\epsilon = 4.20$ )
		$MS, m/z: 315 (M^+)^{(4)}, 164^{(17)}, 152^{(80)}, 135^{(100)}, 107^{(57)}$
		<sup>1</sup> H NMR: 3.02 (2H, CH <sub>2</sub> , m), 3.82 (3H, OCH <sub>3</sub> , 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 $\epsilon$ = 4.21)
		$MS, m/z; 265 (M^+)^{(<1)}, 209^{(25)}, 151^{(5)}, 135^{(100)}, 107^{(6)}$
		<sup>1</sup> H NMR: 0.92 (6H, 2CH <sub>3</sub> , dd), 1.7 (3H, CH <sub>2</sub> -CH, m), 3.83 (3H, OCH <sub>3</sub> , s), 4.43 (1H, CH <sub>a</sub> , 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 $\epsilon$ = 4.22)
		$MS, m/z; 265 (M^+)^{(4)}, 221^{(15)}, 220^{(7)}, 209^{(20)}, 191^{(18)}, 151^{(65)}, 135^{(100)}, 107^{(14)}$
		<sup>1</sup> H NMR: 0.92 (6H, 2CH <sub>3</sub> , dd), 1.37 (2H, CH <sub>2</sub> , m), 2.01 (1H, CH <sub>β</sub> , m), 3.83 (3H, OCH <sub>3</sub> , s), 4.37 (1H, CH <sub>α</sub> ,
		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 $\epsilon$ = 4.08)
		MS, m/z: n.d.
		<sup>1</sup> H NMR: 0.97 (6H, 2CH <sub>3</sub> , dd), 2.22 (1H, CH <sub>p</sub> , m), 3.83 (3H, OCH <sub>3</sub> , s), 4.29 (1H, CH <sub>a</sub> , 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 $\epsilon$ = 4.24)
-		MS, $m/z$ : 283 $(M^+)^{(4)}$ , 209 <sup>(28)</sup> , 192 <sup>(3)</sup> , 191 <sup>(25)</sup> , 135 <sup>(100)</sup> , 107 <sup>(7)</sup>
		<sup>1</sup> H NMR: 2.05 (3H, SCH <sub>3</sub> , s), 2.06 (2H, CH <sub>2</sub> , m), 2.61 (2H, CH <sub>2</sub> , m), 3.83 (3H, OCH <sub>3</sub> , s), 4.51 (1H, CH <sub>a</sub> ,
		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 $\epsilon$ =3.69)
-		MS, $m/z$ : 197 $(M^+)^{(2)}$ , 153 <sup>(59)</sup> , 152 <sup>(40)</sup> , 123 <sup>(100)</sup>
		<sup>1</sup> H NMR: 3.93 (2H, CH <sub>2</sub> , 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 $\epsilon = 4.06$ )
		MS, $m/z$ : 211 $(M^+)^{(2)}$ , 167 <sup>(13)</sup> , 166 <sup>(27)</sup> , 123 <sup>(100)</sup>
		<sup>1</sup> H NMR: 1.41 (3H, CH <sub>3</sub> , 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	(continue	d)
---------	-----------	----

Comp.	M.p. (°C)	Spectral data
11	134-135	UV: 269.6 (log $\epsilon = 4.12$ )
		MS, $m/z$ : 287 $(M^+)^{(3)}$ , 148 <sup>(76)</sup> , 147 <sup>(37)</sup> , 123 <sup>(100)</sup>
		<sup>1</sup> H NMR: 3.12 (2H, CH <sub>2</sub> , 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 $\epsilon = 3.86$ )
		MS, $m/z$ : 303 $(M^+)^{(2)}$ , 164 <sup>(83)</sup> , 140 <sup>(25)</sup> , 123 <sup>(70)</sup> , 107 <sup>(100)</sup>
		<sup>1</sup> H NMR: 3.02 (2H, CH <sub>2</sub> , 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 $\epsilon = 3.84$ )
		$MS, m/z; 253 (M^+)^{(<1)}, 208^{(7)}, 197^{(24)}, 179^{(12)}, 139^{(2)}, 123^{(100)}$
		<sup>1</sup> H NMR: 0.93 (6H, 2CH <sub>3</sub> , dd), 1.73 (3H, CH <sub>2</sub> -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 $(\log \epsilon = 3.95)$
		$MS, m/z: 247 (M^+)^{(1)}, 203^{(26)}, 202^{(23)}, 173^{(100)}, 145^{(55)}$
		<sup>1</sup> 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 $(\log \epsilon = 4.08)$
		$MS, m/z; 261 (M^+)^{(3)}, 2170^{(8)}, 216^{(50)}, 173^{(100)}, 145^{(70)}$
		<sup>1</sup> H NMR: 1.43 (3H, CH <sub>3</sub> , 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 $\epsilon = 4.03$ )
		$MS, m/z: 337 (M^+)^{(2)}, 173^{(97)}, 148^{(100)}, 147^{(41)}, 145^{(65)}$
		<sup>1</sup> H NMR: 3.20 (2H, CH <sub>2</sub> , 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 $\epsilon = 3.40$ )
		$MS, m/z; 303 (M^+)^{(<1)}, 258^{(14)}, 247^{(27)}, 229^{(18)}, 173^{(100)}, 145^{(38)}$
		<sup>1</sup> H NMR: 0.88 (6H, 2CH <sub>3</sub> , dd), 1.72 (3H, CH <sub>2</sub> -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	UV: 257.0 ( $\log \epsilon = 3.67$ )
	(135 [12])	$MS, m/z; 224 (M^+)^{(1)}, 180^{(39)}, 179^{(46)}, 150^{(100)}, 120^{(11)}, 104^{(42)}$
		<sup>1</sup> H NMR: 3.97 (2H, CH <sub>2</sub> , 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 ( $\log \epsilon = 3.64$ )
		$\mathbf{MS}, m/z; 238 \ (M^+)^{(1)}, 194^{(4)}, 193^{(71)}, 150^{(100)}, 120^{(9)}, 104^{(32)}$
		<sup>1</sup> H NMR: 1.43 (3H, CH <sub>3</sub> , 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 $\epsilon = 3.67$ )
		$MS, m/z; 314 (M^+)^{(6)}, 150^{(73)}, 148^{(100)}, 147^{(50)}, 120^{(19)}, 104^{(82)}$
		<sup>1</sup> H NMR: 3.19 (1H, CH, m), 4.69 (2H, CH <sub>2</sub> , 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	UV: 252.0 (log $\epsilon = 3.22$ )
	(163–164 [13])	$\mathbf{MS}, m/z; 330 \ (M^+)^{(1)}, 164^{(83)}, 150^{(14)}, 120^{(6)}, 107^{(100)}, 104^{(17)}$
		<sup>1</sup> H NMR: 3.06 (2H, CH <sub>2</sub> , 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 $\epsilon = 3.30$ )
		MS, $m/z$ : 280 $(M^+)^{(<1)}$ , 235 <sup>(29)</sup> , 224 <sup>(33)</sup> , 206 <sup>(23)</sup> , 150 <sup>(100)</sup> , 120 <sup>(9)</sup> , 104 <sup>(39)</sup>
		<sup>1</sup> H NMR: 0.94 (6H, 2CH <sub>3</sub> , dd), 1.73 (3H, CH <sub>2</sub> 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  $\mu$ M in the assay. Sorbinil, Tolrestat, and quercetin show the following IC<sub>50</sub> values: Sorbinil 2.58  $\mu$ M, Tolrestat 0.096  $\mu$ M, and quercetin 39.9  $\mu$ M.

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.

#### References

- C.R. Rasmussen, B.E. Maryanoff, G.F. Tutwiler, Section IV, Metabolic diseases and endocrin function. Ch. 17, Diabetes mellitus, Ann. Rep. Med. Chem. 16 (1981) 173-187.
- [2] M. Brownlee, A. Cerami, The biochemistry of the complications of diabetes mellitus, Ann. Rev. Biochem. 50 (1981) 385-432.
- [3] P.F. Kador, The role of aldose reductase in the development of diabetic complications, Med. Res. Rev. 8 (1988) 325-352.

- [4] R. Sarges, P.J. Oates, Aldose reductase inhibitors: recent developments, Prog. Drug Res. 40 (1993) 99-161.
- [5] P.F. Kador, J.H. Kinoshita, N.E. Sharpless, Aldose reductase inhibitors: a potential new class of agents for the pharmacological control of certain diabetic complications, J. Med. Chem. 28 (1985) 841–849.
- [6] J. De Ruiter, B.E. Swearingen, V. Wandrekar, C.A. Mayfield, Synthesis and in vitro aldose reductase inhibitory activity of compounds containing an N-acylglycine moiety, J. Med. Chem. 32 (1989) 1033– 1038.
- [7] K. Sestanj, F. Bellini, S. Fung, N. Abraham, A. Treasurywala, L. Humber, N. Simard-Duquesns, D. Dvornik, N-[[5-(Trifluoromethyl)-6-methoxy-1-naphthalenyl]-N-methylglycine (Tolrestat), a potent orally active aldose reductase inhibitor, J. Med. Chem. 26 (1984) 255-256.
- [8] L. Costantino, G. Rastelli, K. Vescovini, G. Cignarella, P. Vianello, A. Del Corso, M. Cappiello, U. Mura, D. Barlocco, Synthesis, activity, and molecular modelling of a new series of tricyclic pyridazinones as selective aldose reductase inhibitors, J. Med. Chem. 39 (1996) 4396– 4405.
- [9] A. Del Corso, M. Camici, U. Mura, In vitro modification of bovine lens aldose reductase activity, Biochem. Biophys. Res. Commun. 148 (1987) 369–375.
- [10] W.H.J. Ward, C.M. Sennitt, H. Ross, A. Dingle, D. Timms, D.J. Mirrlees, D.P. Tuffin, Ponalrestat: a potent and specific inhibitor of aldose reductase, Biochem. Pharmacol. 39 (1990) 337-346.
- [11] H.G. Bray, M. Valda, S. Craddock, M.W. Thorpe, Biochem. J. 60 (1955) 225 [Beilstein 10, IV, 435].
- [12] N. Friedler, J.N. Smith, Biochem. J. 57 (1954) 396 [Beilstein, 9, IV, 1199].
- [13] M. Van der Schear, S. Landsteiner, J. Immunol. 29 (1935) 371 [Beilstein, 14, III, 1523].