SUPPORTING INFORMATION

Solid Phase Synthesis and Insights into Structure-Activity Relationships of Safinamide Analogs as Potent and Selective Inhibitors of Type-B Monoamine Oxidase

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^{1}H-NMR spectral data of compounds **1-20**

N^2-[4-(benzyloxy)benzyl]glycinamide (1). ^{1}H-NMR (DMSO-d_6) δ 2.68 (br, 1H), 2.97 (s, 2H), 3.56 (s, 2H), 5.06 (s, 2H), 6.92 (d, 2H, *J* = 8.5), 7.03 (s, 1H), 7.21 (d, 2H, *J* = 8.5), 7.26 (s, 1H), 7.29-7.43 (m, 5H).

N^2-{4-[(3-fluorobenzyl)oxy]benzyl}glycinamide (2). ^{1}H-NMR (DMSO-d_6) δ 2.74 (br, 1H), 2.97 (s, 2H), 3.56 (s, 2H), 5.09 (s, 2H), 6.93 (d, 2H, *J* = 8.3), 7.03 (s, 1H), 7.10-7.16 (m, 1H), 7.21-7.27 (m, 5H), 7.38-7.45 (m, 1H).

N^2-{4-[(3-chlorobenzyl)oxy]benzyl}glycinamide (3). ^{1}H-NMR (DMSO-d_6) δ 3.32 (s, 2H), 3.76 (d, 2H, *J* = 6.1), 5.17 (s, 2H), 7.00 (s, 1H), 7.07 (d, 2H, *J* = 9.1), 7.33 (s, 1H), 7.34-7.42 (m, 3H), 7.51 (s, 1H), 7.83 (d, 2H, *J* = 9.1), 8.51 (t, 1H, *J* = 6.1).

N^2-{4-[(4-chlorobenzyl)oxy]benzyl}glycinamide (4). ^{1}H-NMR (DMSO-d_6) δ 2.79 (br, 1H), 2.97 (s, 2H), 3.56 (s, 2H), 5.06 (s, 2H), 6.92 (d, 2H, *J* = 8.5), 7.02 (br, 1H), 7.21 (d, 2H, *J* = 8.2), 7.25 (br, 1H), 7.44 (s, 4H).

N^2-{4-[(4-nitrobenzyl)oxy]benzyl}glycinamide (5). ^{1}H-NMR (Acetone-d_6) δ 2.78 (br, 1H), 3.14 (s, 2H), 3.70 (s, 2H), 5.31 (s, 2H), 6.35 (br, 1H), 6.95-7.05 (m, 4H), 7.08 (br, 1H), 7.31 (d, 2H, *J* = 8.8), 7.78 (d, 2H, *J* = 8.8), 8.28 (d, 2H, *J* = 8.8).

(*S*)-N^2-[4-(benzyloxy)benzyl]alaninamide (6). Already described in ref.[12]

(*S*)-N^2-{4-[(3-fluorobenzyl)oxy]benzyl}alaninamide (7). ^{1}H-NMR (Acetone-d_6) δ 1.59 (d, 3H, *J* = 6.6), 3.78-3.83 (m, 1H), 4.22 (d, 2H, *J* = 12.2), 5.19 (s, 2H), 7.04 (d, 2H, *J* = 8.5), 7.09 (s, 1H), 7.24-7.33 (m, 2H), 7.41-7.46 (m, 1H), 7.49 (d, 2H, *J* = 8.5), 7.96 (s, 1H), 7.67 (s, 2H).

(*S*)-N^2-{4-[(3-chlorobenzyl)oxy]benzyl}alaninamide (8). ^{1}H-NMR ($CDCl_3$) δ 1.36 (d, 3H, *J* = 7.2), 3.23 (q, 1H, *J* = 7.2), 3.67 (d, 1H, *J* = 12.9), 3.73 (d, 1H, *J* = 12.9), 5.03 (s, 2H), 5.40 (br, 1H), 6.90-6.94 (m, 2H), 7.06 (br, 1H), 7.18-7.32 (m, 5H), 7.43 (s, 1H).

(*S*)-N^2-{4-[(4-chlorobenzyl)oxy]benzyl}alaninamide (9). ^{1}H-NMR (Acetone-d_6) δ 1.22 (d, 3H, *J* = 6.9), 3.11 (q, 1H, *J* = 6.9), 3.60 (d, 1H, *J* = 13.1), 3.70 (d, 1H, *J* = 13.1), 5.12 (s, 2H), 6.27 (br, 1H), 6.90-7.00 (m, 2H), 7.11 (br, 1H), 7.28 (d, 2H, *J* = 8.8), 7.40-7.46 (m, 2H), 7.50 (d, 2H, *J* = 8.3).

(*S*)-N^2-{4-[(4-nitrobenzyl)oxy]benzyl}alaninamide (10). ^{1}H-NMR (Acetone-d_6) δ 1.22 (d, 3H, *J* = 6.9), 3.11 (q, 1H, *J* = 6.9), 3.62 (d, 1H, *J* = 12.9), 3.72 (d, 1H, *J* = 12.9), 5.31 (s, 2H), 6.25 (br,

1H), 6.97-7.00 (m, 2H), 7.09 (br, 1H), 7.31 (d, 2H, *J* = 8.8), 7.78 (d, 2H, *J* = 8.8), 8.20-8.30 (m, 2H).

(*S*)-N^2-[4-(benzyloxy)benzyl]serinamide (11). Already described in ref.[12]

(*S*)-N^2-{4-[(3-fluorobenzyl)oxy]benzyl}serinamide (12). ^{1}H-NMR (Acetone-d_6) δ 2.32 (br, 1H), 3.14 (t, 1H, *J* = 5.8), 3.55-3.85 (m, 4H), 3.98 (br, 1H), 6.46 (br, 1H), 6.94-7.02 (m, 2H), 7.09 (dt, 1H, *J* = 8.8, 2.75), 7.20-7.36 (m, 5H), 7.38-7.50 (m, 1H).

(*S*)-N^2-{4-[(3-chlorobenzyl)oxy]benzyl}serinamide (13). ^{1}H-NMR (Acetone-d_6) δ 3.15 (dd, 1H, *J* = 6.6, 5.0), 3.59-3.80 (m, 5H), 5.14 (s, 2H), 6.47 (br, 1H), 6.95-7.00 (m, 2H), 7.25 (br, 1H), 7.29-7.44 (m, 5H), 7.52 (s, 1H).

(*S*)-N^2-{4-[(4-chlorobenzyl)oxy]benzyl}serinamide (14). ^{1}H-NMR (DMSO-d_6) δ 2.28 (br, 1H), 2.93-2.97 (m, 1H), 3.41-3.66 (m, 4H), 4.72 (t, 1H, *J* = 5.5), 5.07 (s, 2H), 6.90-6.94 (m, 2H), 7.07 (s, 1H), 7.21-7.25 (m, 2H), 7.29 (s, 1H), 7.41-7.47 (m, 4H).

(*S*)-**N^2-{4-[(4-nitrobenzyl)oxy]benzyl}serinamide (15)**. ^{1}H-NMR (DMSO-d_6) δ 2.34 (br, 1H), 2.93-2.97 (m, 1H), 3.40-3.67 (m, 4H), 4.72 (t, 1H, *J* = 5.5), 5.25 (s, 2H), 6.95 (d, 2H, *J* = 8.8), 7.07 (s, 1H), 7.25 (d, 2H, *J* = 8.8), 7.30 (s, 1H), 7.70 (d, 2H, *J* = 8.8), 8.24 (d, 2H, *J* = 8.8).

(*S*)-2-{[4-(benzyloxy)benzyl]amino}-2-phenylacetamide (16). ^{1}H-NMR (Acetone-d_6) δ 2.55 (br, 1H), 3.67 (s, 2H), 4.19 (s, 1H), 5.11 (s, 2H), 6.45 (br, 1H), 6.95-6.98 (m, 2H), 7.15 (br, 1H), 7.26-7.50 (m, 12H).

(*S*)-2-({4-[(3-fluorobenzyl)oxy]benzyl}amino)-2-phenylacetamide (17). ^{1}H-NMR (Acetone-d_6) δ 2.52-2.59 (m, 1H), 3.68 (d, 2H, *J* = 5.2), 4.19 (d, 1H, *J* = 5.8), 5.15 (s, 2H), 6.49 (br, 1H), 6.95-7.00 (m, 2H), 7.09 (ddd, 1H, *J* = 8.5, 8.5, 2.5), 7.15 (br, 1H), 7.35-7.44 (m, 7H), 7.40-7.47 (m, 3H).

(*S*)-**2-({4-[(3-chlorobenzyl)oxy]benzyl}amino)-2-phenylacetamide (18)**. ^{1}H-NMR (Acetone-d_6) δ 2.52-2.59 (m, 1H), 3.68 (d, 2H, *J* = 5.2), 4.19 (d, 1H, *J* = 5.8), 5.14 (s, 2H), 6.46 (br, 1H), 6.95-7.00 (m, 2H), 7.26-7.46 (m, 11H), 7.52 (s, 1H).

(*S*)-2-({4-[(4-chlorobenzyl)oxy]benzyl}amino)-2-phenylacetamide (19). ^{1}H-NMR (DMSO-d_6) δ 3.43-3.58 (m, 2H), 4.17-4.19 (m, 1H), 4.42 (s, 1H), 5.06 (s, 2H), 6.91-6.93 (m, 2H), 7.15-7.34 (m, 9H), 7.41-7.47 (m, 4H).

(*S*)-2-({4-[(4-nitrobenzyl)oxy]benzyl}amino)-2-phenylacetamide (20). ^{1}H-NMR (Acetone-d_6) δ 3.69 (s, 2H), 4.19 (s, 1H), 5.31 (s, 2H), 6.43 (s, 1H), 6.98-7.02 (m, 2H), 7.13 (s, 1H), 7.24-7.35 (m, 5H), 7.43-7.46 (m, 2H), 7.76-7.79 (m, 2H), 8.27-8.30 (m, 2H), NH not detected.

Elemental analysis of (*R*)**and** (*S*) **enantiomer of 21** ($C_{19}H_{21}FN_2O_2$) **.**

	Calculated, %			Found, %		
Comp.	C	H	N	C	H	N
(*R*)**-21**	69.49	6.45	8.53	69.80	6.68	8.81
(*S*)**-21**	69.49	6.45	8.53	69.77	6.79	8.87

Table 1 Chromatographic data for chiral separation and enantiopurity control

Compound [a]	**Column**	**Φ**[b]	**k'**[c]	**α**[d]	**Rs**[e]
(*R*)+(*S*) **8**	Chiralpak IA	30	2.22	1.11	1.5
(*R*)+(*S*) **7**	Chiralpak IA	20	2.42	1.12	1.1
(*R*)+(*S*) **13**	Chiralpak IA	20	2.85	1.13	1.3
(*R*)+(*S*) **21**	Chirobiotic TAG	65[f]	7.29	1.10	1.1

[a] Reconstituted racemic form. [b] Percentage (v/v) of ethanol in *n*-hexane. [c] Capacity factor of the first eluted (R)-enantiomer; flow rate 1mL/min, except for **7** (0.5 mL/min). [d] Separation factor. [e] Resolution factor.[f] Percentage (v/v) of methanol in water.

The experimental conditions delineated in Table 1 were used to measure the enantiomeric excess of all the alaninamide and serinamide chiral compounds. Chiral phenylglycinamides derivatives were examined on Chiralpak IA column by using a 80/20, EtOH/hexane eluent mixture.

Biological assays

Membrane preparations (crude mitochondrial fraction)

Male Wistar rats (Harlan, Italy weighting 175-200 g) were sacrificed under light anaesthesia and brains were rapidly removed and homogenized in 8 volumes of ice-cold 0.32 M sucrose buffer containing 0.1 M EDTA, pH 7.40. The crude homogenate was centrifuged at 2220 rpm for 10 minutes and the supernatant recovered. The pellet was homogenized and centrifuged again. The two supernatants were pooled and centrifuged at 9250 rpm for 10 minutes at +4 °C. The pellet was re-suspended in fresh buffer and centrifuged at 11250 rpm for 10 minutes at +4 °C. The resulting pellet was stored at –80 °C.