Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 3263–3274
Bioorganic & Medicinal Chemistry

Synthesis and anticonvulsant activity of a class of 2-amino 3-hydroxypropanamide and 2-aminoacetamide derivatives

Eleonora Ghidini,[a,*] Maurizio Delcanale,[a] Renato De Fanti,[a] Andrea Rizzi,[a] Manuela Mazzuferi,[b] Donata Rodi,[b] Michele Simonato,[b] Milco Lipreri,[a] Franco Bassani,[a] Loredana Battipaglia,[a] Marco Bergamaschi[a] and Gino Villetti[a]

[a]*R&D Department, Chiesi Farmaceutici S.p.a., Via Palermo 26/A, 43100 Parma, Italy*
[b]*Department of Clinical and Experimental Medicine, Section of Pharmacology and Neuroscience Center, Via Fossato di Mortara 17-19, University of Ferrara, 44100 Ferrara, Italy*

Received 12 January 2005; accepted 16 December 2005
Available online 3 February 2006

Abstract—Several studies have demonstrated that N-substituted aminoacid derivatives exhibit weak anticonvulsant activities in vivo. In the present study, a series of amides of aminoacids structurally related to aminoacetamide have been synthesised and investigated for anticonvulsant activity. Among the molecules investigated, those containing a bicyclic (tetralinyl, indanyl) group linked to the aminoacetamide chain (**40**, **47** and **59**) were among the most active as anticonvulsants ($ED_{50} > 10$, <100 mg/kg after oral administration) against tonic seizures in the mouse maximal electroshock, bicuculline and picrotoxin tests at doses devoid of neurotoxic activity. Altogether, these results suggest the described compounds as a class of orally available anticonvulsants. The ability of these compounds to partially block veratridine-induced aspartate efflux from rat cortical synaptosomes suggests that their anticonvulsant activity may be only partly the consequence of an interaction with neuronal voltage-dependent sodium channels. Some of the most potent compounds appear worthy of a further investigation aimed at assessing their anticonvulsant activity in other models and at elucidating the underlying mechanism of action.

1. Introduction

The conditions grouped under the term epilepsy constitute an area of continuous medical need. Various literature reports estimate that approximately 25–30% of all epileptic patients do not respond to current therapeutic treatments,[1] and therefore new, less toxic and more effective drugs are required.[2] Even some of the most recently introduced drugs retain significant adverse effects.[3] Therefore, first-generation drugs, such as valproic acid and carbamazepine, are still widely used.

The strategy of investigating compounds structurally related to first generation of antiepileptic drugs seems appropriate to better understand the mechanism displayed by these old drugs and possibly improve them. The work reported in the present paper aimed to further explore the potential of the α-aminoacetamide derivatives as anticonvulsant agents.

Milacemide (*n*-pentylaminoacetamide, **1**, Fig. 1) was reported some years ago[4] as a promising antiepileptic agent, but its full development was not pursued due to marginal effects in clinical trials. However, further studies[5,6] led to the development of safinamide (NW 1015, **2**, Fig. 1) currently in Phase I trials.[3]

In this work, the anticonvulsant activity of a new series of compounds, most of which contain either the 2-amino-3-hydroxypropanamide (serinamide) or 2-acetamide (glycinamide) group, was evaluated.

2. Chemistry

The synthesis of most compounds (Tables 1–3) was accomplished by reductive amination of an aldehyde or ketone with the appropriate α-aminoacid ester using a literature protocol[7] (Scheme 1). The ester derivative was purified by simple trituration with a solvent and

Keywords: Milacemide; α-Aminoamides; Anticonvulsant activity; MES.
* Corresponding author. E-mail: E.Ghidini@chiesigroup.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.12.064

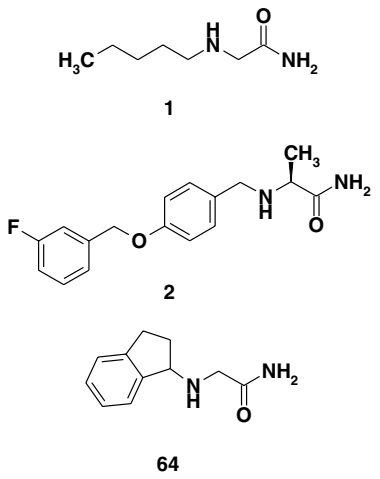


Figure 1.

dissolved in an ammonia–methanol solution to convert the ester into amide. The reductive amination of aldehydes occurred with moderate to good yields using Pd on carbon in methanol as a catalyst. With ketones (Scheme 2) it was necessary to use sodium cyanoborohydride in a EtOH/MeOH mixture as a reducing agent to obtain good yields. Previously unreported aldehydes or ketones were synthesised by literature methods.

3. Pharmacology

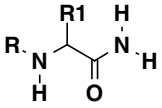
Compounds were tested and selected for an extensive pharmacological evaluation on the basis of a widely accepted screening programme for the identification of anticonvulsant activity.[8]

Initial screening was carried out by the maximal electroshock test (MES) in mice (see Tables 1–3 for results). The horizontal screen test was employed to quantify motor impairment induced by the most promising compounds (see Table 3).

A subset of three compounds that were found to have a significant potency and a good protective index were then evaluated in a series of secondary tests. These tests, whose methods have been described in the literature,[9] included the following: clonic convulsions induced by subcutaneous injection of pentylentetrazole (scPTZ) and tonic convulsions induced by subcutaneous injection of bicuculline (scBIC) and picrotoxin (scPIC).

The new compounds appeared to antagonise specifically the tonic seizures induced by both supramaximal electrical and chemical stimuli. For these reasons, the biochemical tests performed to elucidate their mechanism/s of action included in vitro *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole

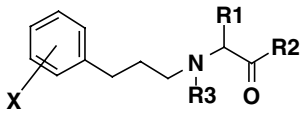
Table 1. Structural and physical data and MES activity (ED_{50}, mg/kg) for compounds **3**–**24** (Scheme 1)



Compound	R	R1	Configuration[a]	$[\alpha]_D$	ED_{50}[b]
Milacemide	Pentyl	H	—	—	698 (402–1212)
3	Heptyl	Phenyl	*R*	—	206 (109–389)
4	Heptyl	Benzyl	*rac*	—	421 (303–587)
5	Heptyl	Methyl	*rac*	—	>300
6	Heptyl	2-Thienyl	*rac*	—	∼500
7	Pentyl	CH_2OH	*R*	—	>1000
8	Heptyl	CH_2OH	*R*	+3.75[c]	>1000
9	*iso*-Propyl	CH_2OH	*R*	+17.0[d]	>500
10	Cyclohexylmethyl	CH_2OH	*R*	+12.6[d]	427 (337–542)
11	1-Methylhexyl	CH_2OH	*R*	−0.3[d]	101 (62–164)
12	2-Phenylethyl	CH_2OH	*R*	+11.5[d]	65 (46–93)
13	Octyl	CH_2OH	*rac*	—	138 (71–268)
14	Nonyl	CH_2OH	*rac*	—	161 (64–402)
15	1-Ethylheptyl	CH_2OH	*rac*	—	>500
16	1-Methylnonyl	CH_2OH	*rac*	—	295 (203–430)
17	1-Methyl-3-phenylpropyl	CH_2OH	*R* (*R*,*S*)	—	>500
18	1-Methyl-2-phenylethyl	CH_2OH	*R* (*S*)	−28.8[d]	∼300
19	3-Phenylbutyl	CH_2OH	*R*	—	172 (115–257)
20	1-Methylbenzyl	CH_2OH	*R*	—	144 (82–254)
21	1,1-Diphenylmethyl	CH_2OH	*R*	+12.1[d]	41 (33–50)
22	4-Phenylbutyl	CH_2OH	*R*	—	159 (69–364)
23	2-Phenoxyethyl	CH_2OH	*R*	—	217 (156–302)
24	3-Phenylpropyl	CH_2OH	*R*	—	47(29–76)

[a] The configuration between brackets is referred to the chiral centre in the R chain; when not indicated, it is a racemic mixture.
[b] Values in brackets are 95% confidence limits.
[c] 0, 4% in $CHCl_3/CH_3OH$.
[d] 1% in methanol.

Table 2. Structural and physical data and MES activity (ED_{50}, mg/kg) for compounds **25**–**38** (Scheme 1)



Compound	R1	R2	R3	X	Configuration	ED_{50}[a]
24	CH_2OH	NH_2	H	H	*R*	47 (29–76)
25	CH_2OH	NH_2	H	H	*S*	51 (33–80)
26	CH_2OH	NH_2	H	*p*-OCH_3	*RS*	>300
27	CH_2OH	NH_2	H	*o*-OCH_3	*RS*	~300
28	CH_2OH	NH_2	H	*m*-OCH_3	*RS*	>300
29	CH_2OH	NH_2	H	*p*-CH_3	*RS*	>300
30	CH_2OH	NH_2	H	*p*-F	*RS*	>300
31	CH_2OH	NH_2	H	*p*-Cl	*RS*	>300
32	CH_2OH	NH_2	H	*p*-CF_3	*R*	291 (159–532)
33	CH_2OH	NCH_3	H	H	*R*	79 (48–131)
34	CH_2OH	$N(CH_3)_2$	H	H	*RS*	>100
35	CH_2OH	OH	H	H	*R*	>300
36	CH_2OCOCH_3	NH_2	H	H	*S*	~300
37	CH_2OH	NH_2	CH_3	H	*RS*	71 (45–113)
38	C_6H_5	NH_2	H	H	*RS*	>100

[a] Values in brackets are 95% confidence limits.

propionate (AMPA), kainic acid (KA), γ-aminobutyric acid (GABA) and voltage-dependent sodium channel receptor binding. Furthermore, effects on veratridine-induced [^{3}H]D-aspartate release in rat synaptosomes were investigated, in order to analyse the compounds ability to functionally block neuronal voltage-dependent sodium channels.

On the basis of the collected data, several tested compounds have been investigated not only in epilepsy,[10] but also in hypoxic-ischaemic brain injury, inflammatory and neuropathic pain and degenerative disorders.[11,12]

4. Discussion

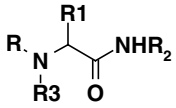
With the aim to identify compounds more potent and with a wider range of anticonvulsant spectra than milacemide, different substitution first introduced in position 2 on the basis that glycinamide analogues bearing substitutions in the carbon at that position have been shown[13] to be resistant to the monoamino oxidase activity.

In a first group of compounds, the effect of alkyl or aryl substitution at C2 of milacemide was assessed (Table 1). The four compounds **3**–**6** showed only a weak activity, so that further investigation of this kind of compounds was not pursued.

Instead the introduction of a CH_2OH substituent increased the activity when the alkyl group linked to the amino nitrogen reached a sufficient length (**13**, **14** and **16**). A further improvement was obtained, up to two-digit activity, when a phenyl group was tethered to the amino nitrogen by a short (C1–C2, C1–C3) alkyl chain (**12**, **24** vs **22** and **23**). Alkyl (**17**–**19**) but not phenyl (**21**) substitution on the tether chain was deleterious. The good activity of the *R*-3-phenylpropylderivative (**24**) prompted us to investigate the effect of the amino-acid configuration and of the substitution on the aromatic ring (Table 2). Whereas the *S*-enantiomer **25** showed substantially the same activity of **24**, no positive effect was obtained by substituting the aromatic ring of **24** with a series of either electron-donating or electron-withdrawing groups in the various positions (**26**–**32**). Methylation of the amide (**33**) and of the amino nitrogen (**37**), respectively, maintained the activity, although a second methyl group (**34**) on the former was detrimental.

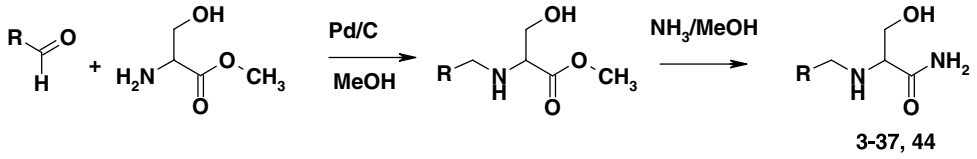
The best results were obtained by constraining the propyl chain of **24** into a ring. In the case of the 2-tetralinyl derivatives (Table 3, **39**–**42**), all the four stereoisomers were prepared and tested. The most active compounds are those with opposite configuration at the two centres (**40** and **42**) and this finding holds also for the *N*-methylamino derivative **47** versus **46**. The activity was maintained when the CH_2OH substituent was reduced to CH_3 (**43**, **44**), or in the case of compounds with a naphthylmethyl (**45**) or a 1-tetralinyl (**48**) ring. The best series appeared to be that of the 2-indanyl derivatives. Here again it was confirmed that the configuration of the aminoacid carbon has no relevance (compare **49** with **50**, **51** with **52** and **53** with **54**) for the anticonvulsant activity. It was also confirmed that reduction of the CH_2OH substituent to CH_3 (**55**–**58**) and even its deletion (**59**, **60**) was well tolerated, compounds **53** and **59** being the most active in the whole series. A similar conclusion holds for the N-methylation either at the amino (**51**, **52**, **57** and **58**) or at the amide (**53**, **54** and **60**) nitrogen. Again the introduction of a bulky substituent, such as a cycloalkyl (**61**, **62**) or phenyl (**63**), was detrimental. Shifting the chain onto the 1-position of indane to give the Teva compound, 2-[(1-indanyl)-amino]acetamide (**64**), allows to maintain a good activity.[14] The overall result is that

Table 3. Structural and physical data, MES activity (ED_{50}, mg/kg) and neurologic toxicity (TD_{50}, mg/kg) for carbocyclic derivatives **39–63** (Scheme 2)

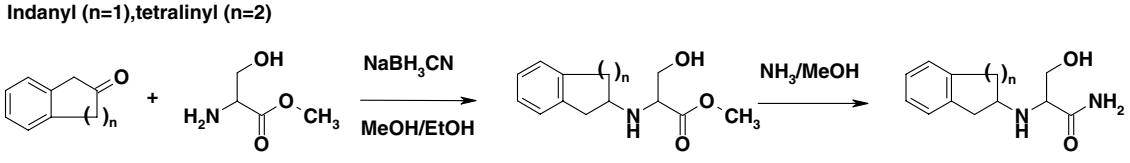


Compound	R	R1	R2	R3	Tetralinyl configuration	Aminoacid configuration	$[\alpha]_D$[a]	ED_{50}[b]	TD_{50}
39	2-Tetralinyl	CH_2OH	H	H	*R*	*R*	−105.1[c]	143 (81–251)	—
40	2-Tetralinyl	CH_2OH	H	H	*S*	*R*	+92.0[c]	43 (24–74)	1300
41	2-Tetralinyl	CH_2OH	H	H	*S*	*S*	−94.2[c]	∼150	—
42	2-Tetralinyl	CH_2OH	H	H	*R*	*S*	+110.0[c]	44 (32–62)	1099
43	2-Tetralinyl	CH_3	H	H	*rac*	*S*	—	76 (46–118)	—
44	2-Tetralinyl	CH_3	H	H	*rac*	*R*	—	100 (62–160)	—
45	2-Naphthylmethyl	CH_2OH	H	H	—	*rac*	—	113 (76–170)	—
46	2-Tetralinyl	CH_2OH	CH_3	H	*R*	*R*	+48.5[d]	10% at 100[e]	—
47	2-Tetralinyl	CH_2OH	CH_3	H	*S*	*R*	−93.0[d]	70% at 100[e]	—
48	1-Tetralinyl	CH_2OH	H	H	—	*rac*	—	85 (49–149)	—
49	2-Indanyl	CH_2OH	H	H	—	*S*	+15.8[c]	36 (22–61)	1172
50	2-Indanyl	CH_2OH	H	H	—	*R*	+26.2[c]	34 (21–55)	>1500
51	2-Indanyl	CH_2OH	CH_3	H	—	*R*	+2.0[c]	40% at 100[e]	—
52	2-Indanyl	CH_2OH	CH_3	H	—	*S*	−2.0[c]	40% at 100[e]	—
53	2-Indanyl	CH_2OH	H	CH_3	—	*R*	−15.6[d]	35 (25–48)	689
54	2-Indanyl	CH_2OH	H	CH_3	—	*S*	+19.8[d]	70% at 100[e]	—
55	2-Indanyl	CH_3	H	H	—	*S*	+22.6[d]	22 (11–42)	251
56	2-Indanyl	CH_3	H	H	—	*R*	−24.9[d]	20% at 100[e]	—
57	2-Indanyl	CH_3	CH_3	H	—	*R*	−10.6[d]	60% at 100[e]	—
58	2-Indanyl	CH_3	CH_3	H	—	*S*	+10.1[d]	80% at 100[e]	—
59	2-Indanyl	H	H	H	—	—	—	21 (14–31)	274
60	2-Indanyl	H	CH_3	H	—	—	—	70% at 100[e]	—
61	2-Indanyl	Cyclopropyl	H	H	—	*rac*	—	40% at 100[e]	—
62	2-Indanyl	Cyclopentyl	H	H	—	*rac*	—	30% at 100[e]	—
63	2-Indanyl	C_6H_5	H	H		*rac*	—	10% at 100[e]	—
64[11]	1-Indanyl	H	H	H	—	—	−1.4[d]	38 (27–55)	—

[a] See Experimental section.
[b] Values in brackets are 95% confidence limits.
[c] 1% in methanol.
[d] % in DMSO.
[e] At mg/kg.



Scheme 1.



Scheme 2.

a bulky, cyclic, lipophilic group linked to the nitrogen of the aminoacetamide chain is determinant for activity in this series, but without too stringent spatial requisites.

A lead optimisation exploration was performed to improve the noncompetitive antagonism of **59** (K_i = 8.8 μM) in the phencyclidine site of NMDA

receptor, as in parallel research **59** (CHF 3381) was found to be a NMDA functional antagonist.[12] However, data (not reported) for the displacement or [^{3}H]TCP from the phencyclidine site of NMDA receptor showed clearly that modifications in the structure of **59** did not improve the potency of the analogues. Some of the compounds which exhibited an excellent activity in the MES test (ED_{50} < 40 mg/kg) and a low neurotoxic potential in the horizontal screen test (TD_{50} > 250 mg/kg), namely **40**, **49** and **59**, were subjected to other in vitro and in vivo tests to better characterise the anticonvulsant profile of this new series.

In in vivo studies, compounds **40**, **49** and **59** antagonised BIC- and PIC-induced tonic convulsions in mice (Table 4), whereas they appeared inactive against pentylenetetrazole-induced clonic convulsions in mice (data not shown), thus appearing similar to the commonly used antiepileptic drugs carbamazepine, lamotrigine and phenytoin in their behaviour.

In in vitro studies with molecular targets which may be linked to the anticonvulsant effect, none of them (at 10^{-5} M concentration), but compound **59**, showed a significant interaction with molecular targets which may be linked to the anticonvulsant effect. Indeed, compound **59** was found to be a low-affinity noncompetitive *N*-methyl-D-aspartate receptor antagonist (K_i = 8.8 μM).

Even if the binding of the radioligand [^{3}H]batrachotoxin in rat brain membrane preparations was not displaced by our compounds, we sought to investigate their ability to functionally inhibit veratridine-dependent release of [^{3}H]D-aspartate from rat cortical synaptosomes, a model to investigate pharmacological blockade of neuronal voltage-sensitive sodium channels.[15] At a concentration of 300 μM, compounds **40** and **59** significantly reduced veratridine-induced [^{3}H]D-aspartate release to 87% and 77% of controls, respectively, indicating that part of their effects may depend upon sodium channel blockade (for results, see Fig. 2). A similar activity, ranging from 74% to 80% of control, was shown by compounds **25**, **41**, **50** and **55**. However, the well-established sodium channel blocker lamotrigine showed a slightly superior effect, being the reduction of [^{3}H]D-aspartate efflux equal to 51%.

Table 4. Anticonvulsant activity of selected compounds (ED_{50}, po) versus reference and antiepileptic drugs (mice, po)[a]

Compound	sc BIC[b] ED_{50}[c] (mg/kg)	sc PIC[b] ED_{50}[c] (mg/kg)
Na-Valproate	214 (189-242)	108 (68–170)
Phenytoin	18[d]	1.14 (0.55–2.36)
Lamotrigine	4[d]	1.4 (1–2.4)
Vigabatrin	N.A. up to 1500	N.A. up to 1000
Safinamide (**2**)	20 (11–38)	15 (10–22)
49	65 (48–87)	14 (6–31)
59	128[d]	13 (7–22)
40	68 (55–83)	53 (37–74)

[a] Values are as ED_{50} (mg/kg po) with 95% c.l. at the time of the peak effect in antagonising the tonic convulsion.
[b] BIC, bicuculline test; PIC, picrotoxin test; N.A., not active.
[c] Values in brackets are 95% confidence limits.
[d] Indicative value calculated from regression analysis.

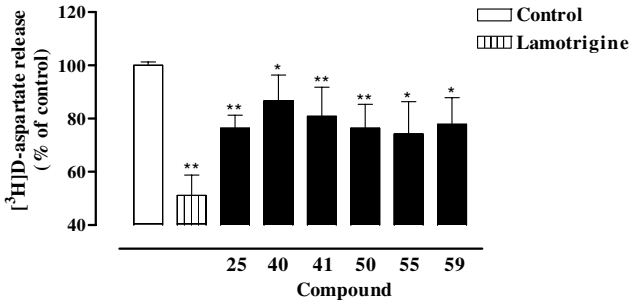


Figure 2. Effect of compounds **25**, **40**, **41**, **50**, **55** and **59** on veratridine-induced [^{3}H]D-aspartate release in rat cortical synaptosomes. Results (n = 3–5) are expressed as the mean percentage (±SEM) of the release observed in the control and statistical analysis (*p < 0.05; **p < 0.01) was determined by Student's t test for unpaired data.

Compound **59** has demonstrated also to be a very promising drug candidate for the treatment of chronic pain. Clinical trials are ongoing to investigate the therapeutic potential of the compound.

5. Conclusions

In conclusion, we have identified a new series of bicycloalkyl-substituted aminoacetamides that are active against tonic convulsions induced by electrical and chemical stimuli in mice. Preliminary data suggest that this class of compounds only partially exerts its anticonvulsant activity by functionally blocking neuronal voltage-sensitive sodium channels. Some of the most potent compounds appear worthy of a further investigation aimed at assessing their anticonvulsant activity in other models and at elucidating the underlying mechanism of action.

6. Experimental

6.1. Chemistry

Melting points were determined in open glass capillaries with a Büchi melting point apparatus and are uncorrected. The IR spectra were determined on a Perkin-Elmer 1310 spectrophotometer. ^{1}H NMR spectra were recorded at 200.13 MHz on a Bruker ACF 200 spectrometer; chemical shifts are in δ (ppm), with tetramethylsilane as internal standard. Several spectra do not show N–H and O–H proton signals because they are in rapid exchange with the protons of the water present in the solvent (DMSO). Mass spectra were measured with a Fisons-VG Trio 2000 single quadrupole spectrometer equipped with a dual EI/CI source. Optical rotations were measured on a Perkin-Elmer 241 and 241-MC polarimeter. Sodium sulfate was employed as a drying agent for diethyl ether extracts. The petroleum ether used throughout this work had a boiling point of 40–70 °C. TLC on silica gel plates (Merck, 60F 254) was used to check product purity and the spots were detected

with ninhydrin or visualized by iodine vapour. Preparative chromatography was carried out on silica gel ICN (32–63 μm). The structures of all compounds were consistent with their analytical and spectroscopic data.

6.1.1. General reductive amination of α-aminoacid derivatives (Scheme 1). To the appropriate α-aminoacid ester (0.9 mol) in dry methanol (350 ml) at room temperature was added the corresponding aldehyde (0.9 mol) and the mixture was hydrogenated under 45 psi in the presence of 10% Pd/C, until the hydrogen absorption ceased. The composition of the crude product was determined by t.l.c. and infrared spectroscopy. When the conversion was complete, the catalyst was filtered off and the filtrate was evaporated to dryness under vacuum. The resulting oil was taken up with methylene chloride (500 ml) and extracted in acidic aqueous phase by means of a 0.1 N HCl solution. The separated aqueous phase was basified with sodium bicarbonate and the product was extracted again with methylene chloride (2× 250 ml), the combined organic solutions were washed with water and dried. The solvent was removed in vacuo at 35 °C. The recovered pale yellow oil was used without further purification, only in a few cases it was purified by crystallization or by flash chromatography. The residue was dissolved in dry methanol (150 ml). Ammonia was bubbled through the solution, cooled at −5 °C, to a ∼15 M concentration. The hermetically sealed system was reacted for 5 days at room temperature and then evaporated to dryness under vacuum. The product was recovered generally as hydrochloride by dissolution in ethanol (40 ml), acidification with diethyl ether saturated with HCl and precipitation with diethyl ether (400 ml). The following compounds were prepared according to this procedure; the reported yields calculated from the corresponding starting aminoacid ester:

6.1.1.1. (2*R*)-2-Phenyl-2-(heptylamino)acetamide (3). Mp 113–116 °C; (45% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.8–0.9 (t, J = 5.3 Hz, 3H, CH_3–(CH$_2$)$_6$–NH), 1.1–1.6 (br, 10H, CH$_3$–(CH_2)$_5$–CH$_2$–NH), 2.2–2.4 (br, m, 3H, N–H, CH$_3$–(CH$_2$)$_5$–CH_2–NH), 4.1 (s, 1H, NH–CH–CO), 6.9–7.5 (br, m, 7H, Ar-H e CO–NH_2); MS/EI$^+$ 249 [MH]$^+$ 205 [M−(CH$_3$–(CH$_2$)$_2$)]$^+$.

6.1.1.2. (2*R*,*S*)-3-Phenyl-2-(heptylamino)propanamide (4). Mp 89–93 °C; (40% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.7–0.8 (t, J = 5.8 Hz, 3H, CH_3–(CH$_2$)$_6$–NH), 1.1–1.4 (br, 10H, CH$_3$–(CH_2)$_5$–CH$_2$–NH), 2.2–2.4 (m, 2H, CH$_3$–(CH$_2$)$_5$–CH_2–NH), 2.6–2.9 (m, m, 3H, NH–CH–CH_2-Ar), 6.8–7.4 (br, m, 7H, Ar-H e CO–NH_2).

6.1.1.3. (2*R*,*S*)-2-(Heptylamino)propanamide (5). Mp 68–72 °C; (39% yield); ^{1}H NMR (200 MHz, CHCl$_3$-d_1) δ 0.7–1.0 (t, J = 5.8 Hz, 3H, CH_3–(CH$_2$)$_6$–NH), 1.1–1.6 (br, 13H, CH$_3$–(CH_2)$_5$-CH$_2$–NH and CH_3–CH–NH), 2.4–2.7 (m, 3H, CH$_3$–(CH$_2$)$_5$–CH_2–NH and CH$_3$–CH–NH), 5.9 and 7.1 (2br, 2H, CO–NH_2).

6.1.1.4. (2*R*,*S*)-[2-Thienyl]-2-[heptylamino]acetamide (6). Mp 74–76 °C; (34% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.7–0.9 (t, J = 5.9 Hz, 3H, CH_3–(CH$_2$)$_6$–NH), 1.1–1.6 (br, 10H, CH$_3$–(CH_2)$_5$–CH$_2$–NH), 2.3–2.5 (m, 2H, CH$_3$–(CH$_2$)$_5$–CH_2–NH, overlay with solvent), 4.3 (s, 1H, NH–CH–CO), 6.3–7.0 (m, 2H, C=H), 7.3–7.4 (dd, 1H, C = H), 7.1 and 7.5 (2br, 2H, CO–NH_2).

6.1.1.5. (2*R*)-3-Hydroxy-2-[pentylamino]propanamide hydrochloride (7). Mp 58–63 °C; (48% yield); ^{1}H NMR (300 MHz, DMSO-d_6/CHCl$_3$-d_3 1:1, TMS) δ 0.95 (t, J = 6.0 Hz, 3H, CH_3–(CH$_2$)$_4$–NH), 1.2–1.5 (m, 4H, CH$_3$–(CH_2)$_2$–(CH$_2$)$_2$–NH), 2.8–3.0 (m, 2H, CH$_3$–(CH$_2$)$_2$–CH_2–CH$_2$–NH), 3.7–4.0 (m, 3H, HN–CH_1–CH_2–OH), 5.3–5.6 (br, 1H, OH), 7.5–7.9 (br, 2H, CO–CH_2), 8.1–8.6 (br, 2H, N$^+$–H_2); MS/EI$^+$ 175 [MH]$^+$, 130 [M−(CONH$_2$)]$^+$.

6.1.1.6. (2*R*)-3-Hydroxy-2-[heptylamino]propanamide hydrochloride (8). Mp 62–65 °C; (53% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.6–1.0 (t, J = 6.5 Hz, 3H, CH_3–(CH$_2$)$_6$–NH), 1.1–1.7 (br, 10H, CH$_3$–(CH_2)$_5$–CH$_2$–NH), 2.3–2.7 (br, 2H, CH$_3$–(CH$_2$)$_5$–CH_2–NH), 2.8–3.1 (t, J = 6 Hz, 1H, HN–CH_1–CH$_2$–OH), 3.4–3.7 (t, J = 8 Hz, 2H, HN–CH–CH_2–OH), 4.5–5.1 (br, 1H, OH), 7.0–7.4 (br, 2H, CO–NH_2).

6.1.1.7. (2*R*)-3-Hydroxy-2-[isopropylamino]propanamide (9). Mp 65–69 °C; (38% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.9–1.0 (d, J = 6.5 Hz, 6H, (CH_3)$_2$–CH–NH), 2.6–2.8 (m, 1H, CH–(CH$_3$)$_2$), 3.0–3.12 (t, J = 6.0 Hz, 1H, NH–CH–CH$_2$OH), 3.3–3.53 (m, 2H, NH–CH–CH_2–OH), 6.93–7.4 (br, 2H, CO–NH_2); MS/EI$^+$ 147 [MH]$^+$, 115 [M−(CH$_2$–OH)]$^+$.

6.1.1.8. (2*R*)-3-Hydroxy-2-[cyclohexylmethylamino]propanamide (10). Mp 106–109 °C; (37% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.7–2.0 (m, 11H, cyclohexyl), 2.2–2.4 (d, J = 6.0 Hz, 2H, cyclohexyl–CH_2–NH), 2.8–3.0 (t, J = 6.0 Hz, 1H, NH–CH–CH$_2$–OH), 3.3–3.7 (m, 2H, NH–CH–CH_2–OH), 4.5–4.8 (br, 1H, OH), 6.9–7.3 (br, 2H, CO–NH_2); MS/EI$^+$ 201 [MH]$^+$, 169 [M−(CH$_2$–OH)]$^+$.

6.1.1.9. (2*R*)-3-Hydroxy-2-[1-methylhexylamino]propanamide hydrochloride (11). Mp 88–92 °C; (34% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.9–1.0 (t, J = 6.2 Hz, 3H, CH_3–(CH$_2$)$_5$–CH–NH), 1.1–1.9 (m, 13H, CH$_3$–(CH_2)$_5$–CH–CH_3), 3.3–3.25 (br, 1H, CH$_3$–CH–NH), 3.7–3.8 (br, 2H, CH_2–OH), 3.81–4.1 (br, 1H, CO–CH–NH), 4.9–5.1 (br, 1H, OH), 7.5–8.2 (2br, 2H, CO–NH_2), 8.3–9.4 (2br, 2H, N$^+$–H_2); MS/EI+ 217 [MH]$^+$, 172 [M−(CH$_2$OH)]$^+$.

6.1.1.10. (2*R*)-3-Hydroxy-2-[2-phenylethylamino]propanamide hydrochloride (12). Mp 94–99 °C; (51% yield); ^{1}H NMR (200 MHz, DMSO-d_6 and CF3COOH-d$_1$) δ 3.4–3.7 (m, 4H, Ar-CH_2–CH_2–NH), 4.1–4.6 (m, 3H, NH–CH–CH_2–OH), 7.7–8.5 (m, br, 7H, Ar-H, CO–NH_2); MS/EI+ 209 [MH$^+$], 164 [M−(CO–NH$_2$)]$^+$.

6.1.1.11. (2*R*,*S*)-3-Hydroxy-2-[octylamino]propanamide (13). Mp 71–72 °C; (59% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.7–1.0 (t, J = 6.0 Hz, 3H, CH_3–(CH$_2$)$_7$–NH), 1.1–1.6 (m, 12H, CH$_3$–(CH_2)$_6$–

CH_2–NH), 2.3–2.6 (m, 2H, CH_3–$(CH_2)_6$–CH_2–NH), 2.9–3.0 (t, J = 6.3 Hz, 1H, NH–C*H*–CH_2–OH), 3.3–3.6 (m, 2H, NH–CH–CH_2–OH), 4.2–4.8 (br, 1H, O*H*), 7.0 and 7.3 (2br, 2H, CO–NH_2).

6.1.1.12. (2*R*,*S*)-3-Hydroxy-2-[nonylamino]propanamide (14). Mp 63–65 °C; (51% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.7–0.9 (t, J = 6.0 Hz 3H, CH_3–$(CH_2)_8$–NH), 1.1–1.5 (br, 14H, CH_3–$(CH_2)_7$–CH_2–NH), 2.3–2.6 (m, 2H, CH_3–$(CH_2)_7$–CH_2–NH), 2.8–3.0 (t, J = 6.4 Hz, 1H, NH–C*H*–CH_2–OH), 3.3–3.6 (m, 2H, NH–CH–CH_2–OH), 4.2–5.0 (br, 1H, O*H*), 7.0 and 7.3 (2br, 2H, CO–NH_2).

6.1.1.13. (2*R*,*S*)-3-Hydroxy-2-[1-ethylheptylamino]propanamide (15). Mp 60–62 °C; (32% yield); ^{1}H NMR (200 MHz, $CHCl_3$-d_3) δ 0.7–1.0 (m, 6H, CH_3–CH_2–CH–$(CH_2)_5$–CH_3), 1.1–1.6 (m, 12H, CH_3–CH_2–CH–$(CH_2)_5$–CH_3), 2.3–2.6 (t, J = 6.2 Hz, 1H, CH_3–CH_2–C*H*–NH), 3.1–3.3 (t, J = 6.5 Hz, 1H, NH–C*H*CH_2–OH), 3.6–3.9 (m, 2H, NH–CH–CH_2–OH), 6.6 and 7.4 (2br, 2H, CO–NH_2).

6.1.1.14. (2*R*,*S*)-3-Hydroxy-2–[1-methylnonylamino]propanamide (16). Mp 71–73 °C; (34% yield); ^{1}H NMR (200 MHz, $CHCl_3$-d_3) δ 0.8–0.9 (t, J = 6.2 Hz, 3H, CH_3–$(CH_2)_7$–CH–NH), 1.0–1.1 (dd, J = 7 Hz, 3H, CH_3–CH–NH), 1.2–1.5 (br, 14H, CH_3–$(CH_2)_7$–CH–NH), 2.5–2.7 (m, 1H, CH_3–C*H*–NH), 3.1–3.3 (m, 1H, NH–C*H*–CH_2–OH), 3.6–3.9 (m, 2H, NH–CH–CH_2–OH), 6.0 e 7.4 (2br, 2H, CO–NH_2).

6.1.1.15. (2*R*)-3-Hydroxy-2–[1-methyl-3-phenylpropylamino]propanamide (17). (34% yield); ^{1}H NMR (200 MHz, $CHCl_3$-d_1) δ 0.9–1.1 (dd, J = 6.1 Hz, 3H, CH_3–CH–NH), 1.5–1.9 (m, 2H, Ar-CH_2–CH_2–CH–NH), 2.5–2.7 (m, 3H, Ar-CH_2–CH_2–CH–NH), 2.8–3.1 (br, 1H, O*H*), 3.2–3.3 (m, 1H, NH–C*H*–CH_2–OH), 3.6–3.9 (m, 2H, NH–CH–CH_2–OH), 6.4 and 7.5 (br, 2H, CO–NH_2), 7.0–7.3 (m, 5H, Ar-*H*).

6.1.1.16. (2*R*)-3-Hydroxy-2–[1-methyl-2-phenylethylamino]propanamide hydrochloride (18). Mp 105–107 °C; (37% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.0–1.2 (d, J = 6.2 Hz, 3H, CH_3–CH–NH), 2.6–2.8 (m, 1H, CH_3–C*H*–NH), 3.2–3.4 (m, 2H, Ar-CH_2–CH–NH), 3.8–3.9 (br, 2H, NH–CH–CH_2–OH), 4.1–4.2 (br, 1H, NH–C*H*–CH_2–OH), 5.5–5.8 (br, 1H, O*H*), 7.1–7.4 (m, 5H, Ar-*H*), 7.7 and 8.2 (2br, 2H, CO–NH_2), 8.7–9.5 (2br, 2H, N^+–H_2). Anal Calcd. for $C_{13}H_{20}N_2O_2$·HCl: C, 57.24; H, 7.76; Cl, 13.00; N, 10.27; O, 11.73. Found: C, 55.82; H, 7.74; Cl, 13.82; N, 13.91.

6.1.1.17. (2*R*)-3-Hydroxy-2-[-3-phenybutylamino]propanamide hydrochloride (19). Mp 135–139 °C; (33% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.1–1.2 (d, J = 6.0 Hz, 3H, Ar-CH–CH_3), 1.8–2.1 (m, 2H, NH–CH_2–CH_2), 2.6–3.0 (m, 3H, Ar-C*H*–CH_2–CH_2–NH), 3.7–3.9 (br, 3H, NH–C*H*–CH_2–OH), 5.3–5.6 (br, 1H, O*H*), 7.1–7.4 (m, 5H, Ar-*H*), 7.1 and 8.1 (2br, 2H, CO–NH_2), 8.6–9.5 (2br, 2H, N^+–H_2); MS/EI$^+$ 237 $[MH]^+$ 192 $[M-(CO–NH_2)]^+$.

6.1.1.18. (2*R*)-3-Hydroxy-2–[1-methylbenzylamino]propanamide hydrochloride (20). Mp 180–183 °C; (45% yield); ^{1}H NMR (200 MHz, CH_3OH-d_4) δ 1.6–1.7 (d, J = 6.3 Hz, 3H, NH–CH–CH_3), 3.4–4.1 (m, 3H, NH–C*H*–CH_2–OH), 4.4–4.6 (m, 1H, Ar-C*H*–NH), 7.4–7.6 (m, 5H, Ar-*H*); MS/EI$^+$ 209 $[MH]^+$ 164 $[M-(CO–NH_2)]^+$.

6.1.1.19. (2*R*)-3-Hydroxy-2–[1,1-diphenylmethylamino]propanamide (21). Mp 134–137 °C; (36% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.6–2.7 (br, 1H, NH–C*H*–CH_2–OH), 2.8–3.0 (br, 1H, NH–C*H*–$(Ar)_2$), 3.4–3.6 (t, J = 4.5 Hz, 2H, NH–CH–CH_2–OH), 4.6–4.7 (t, J = 5.5 Hz, 1H, O*H*), 4.8–4.9 (br, 1H, N*H*), 6.9–7.6 (br, m, 12H, Ar-*H*, CO–NH_2); MS/EI$^+$ 226 $[M-(CO–NH_2)]^+$.

6.1.1.20. (2*R*)-3-Hydroxy-2-[4-phenylbutylamino]propanamide (22). Mp 159–161 °C; (42% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.3–1.7 (m, 4H, NH–CH_2–CH_2–CH_2), 2.4–2.7 (m, 4H, Ar-CH_2–$(CH_2)_2$–CH_2–NH overlay with solvent), 2.9–3.1 (t, J = 5.8 Hz, 1H, NH–C*H*–CH_2–OH), 3.3–3.6 (m, 2H, NH–CH–CH_2–OH), 4.4–5.0 (br, 1H, NH–CH–CH_2–O*H*), 6.9– 7.4 (br, m, 7H, Ar-*H*, CO–NH_2); MS/EI$^+$ 237 $[MH]^+$ 192 $[M-(CONH_2)]^+$.

6.1.1.21. (2*R*)-3-Hydroxy-2-[2-Phenoxyethylamino]propanamide hydrochloride (23). Mp 59–61 °C; (42% yield); ^{1}H NMR (200 MHz, DMSO-d_6 and CF3COOH-d_1) δ 3.1–3.6 (t, J = 5.2 Hz, 2H, NH–CH_2–CH_2–O), 3.7–4.1 (m, 3H, NH–C*H*–CH_2–OH), 4.2–4.4 (t, J = 5.2 Hz, 2H, NH–CH_2), 5.0–6.0 (br, 1H, O*H*), 6.9–7.1 (m, 3H, Ar-*H*), 7.2–7.4 (m, 2H, Ar-*H*), 7.6 and 8.0 (2br, 2H, CO–NH_2), 8.7–9.5 (2br, 2H, N^+–H_2); MS/EI+ 225 $[MH]^+$ 193 $[M-(CH_2OH)]^+$.

6.1.1.22. (2*R*)-3-Hydroxy-2-[3-phenylpropylamino]propanamide hydrochloride (24). Mp 70–72 °C; (53% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.8–2.1 (m, 2H, Ar-CH_2–CH_2–CH_2–NH), 2.6–2.7 (t, J = 6.4 Hz 2H, Ar-CH_2–CH_2–CH_2_NH), 2.8–3.0 (m, 2H, CH_2–NH), 3.8–4.0 (br, 3H, NH–C*H*–CH_2–OH), 5.4–5.7 (br, 1H, O*H*), 7.1–7.3 (m, 5H, Ar-*H*), 7.6 and 8.1 (2br, 2H, CO–NH_2), 8.6–9.4 (2br, 2H, N^+–H_2); MS/EI+ 223 $[MH^+]$, 178 $[M-(CO–NH_2)]^+$.

6.1.1.23. (2*S*)-3-Hydroxy-2-[3-phenylpropylamino]propanamide (25). Mp 70–72 °C; (53% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.8–2.1 (m, 2H, Ar-CH_2–CH_2–CH_2–NH), 2.6–2.7 (t, J = 6.4 Hz 2H, Ar-CH_2–CH_2–CH_2_NH), 2.8–3.0 (m, 2H, CH_2–NH), 3.8–4.0 (br, 3H, NH–C*H*–CH_2–OH), 5.4–5.7 (br, 1H, O*H*), 7.1–7.3 (m, 5H, Ar-*H*), 7.6 and 8.1 (2br, 2H, CO–NH_2), 8.6–9.4 (2br, 2H, N^+–H_2); MS/EI+ 223 $[MH^+]$, 178 $[M-(CO–NH_2)]^+$.

6.1.1.24. (2*R*)-3-Hydroxy-2-[3-(4-methoxyphenyl)propylamino]propanamide hydrochloride (26). Mp 168–170 °C; (33% yield); ^{1}H NMR (200 MHz, CH_3OH-d_4) δ 1.9–2.1 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–2.8 (t, J = 6.2 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.9–3.2 (m, 2H, NH–CH_2–CH_2–CH_2), 3.7 (s, 3H, O–CH_3), 3.8–4.1 (m,

3H, NH–C*H*–CH_2–OH), 6.8–6.9 (d, *J* = 8.2 Hz, 2H, Ar-*H*), 7.1–7.2 (d, *J* = 8.2 Hz, 2H, Ar-*H*); MS/EI$^+$ 253 [MH]$^+$ 208 [M−(CO–NH_2)]$^+$.

6.1.1.25. (2*R*,*S*)-3-Hydroxy-2-[3-(2-methoxyphenyl)propylamino]propanamide hydrochloride (27). Mp 154–156 °C; (30% yield); ^{1}H NMR (200 MHz, CH_3OH-d_4) δ 1.9–2.2 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–2.8 (t, *J* = 6.4 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.9–3.2 (m, 2H, NH–CH_2–CH_2–CH_2), 3.8 (S, 3H, O–CH_3), 3.8–4.1 (m, 3H, NH–C*H*–CH_2–OH), 6.8–7.0 (m, 2H, Ar-*H*), 7.1–7.3 (m, 2H, Ar-*H*); MS/EI$^+$ 253 [MH]$^+$ 208 [M−(CO–NH_2)]$^+$.

6.1.1.26. (2*R*,*S*)-3-Hydroxy-2-[3-(3-methoxyphenyl-)propylamino]propanamide hydrochloride (28). Mp 142–145 °C; (33% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.8–2.1 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–2.7 (t, *J* = 6.1 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.7–3.0 (br, 2H, NH–CH_2–CH_2–CH_2), 3.7 (s, 3H, O–CH_3), 5.0–5.7 (br, 1H, O–*H*), 6.7–6.9 (m, 3H, Ar-*H*), 7.1–7.3 (m, 1H, Ar-*H*), 7.6 and 8.0 (2br, 2H, CO–NH_2), 8.1–9.3 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 253 [MH]$^+$ 208 [M−(CO–NH_2)]$^+$.

6.1.1.27. (2*R*,*S*)-3-Hydroxy-2-[3-(4-methylphenyl)propylamino]propanamide hydrochloride (29). Mp 183–186 °C; (35% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.8–2.1 (br, 2H, NH–CH_2–CH_2–CH_2), 2.2 (s, 3H, Ar-CH_3), 2.6–2.7 (t, *J* = 6.2 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.8–3.0 (br, 2H, NH–CH_2–CH_2–CH_2), 3.8–4.0 (br, 3H, NH–C*H*–CH_2–OH), 5.3–5.7 (br, 1H, O–*H*), 7.1 (s, 4H, Ar-*H*), 7.6 and 8.1 (2br, 2H, CO–NH_2), 8.6–9.4 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 237 [MH]$^+$ 192 [M−(CO–NH_2)]$^+$.

6.1.1.28. (2*R*,*S*)-3-Hydroxy-2-[3-(4-fluorophenyl)-propylamino]propanamide hydrochloride (30). Mp 188–191 °C; (37% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.8–2.1 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–2.7 (t, *J* = 6.2 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.8–3.0 (br, 2H, NH–CH_2–CH_2–CH_2), 3.7–3.9 (br, 3H, NH–C*H*–CH_2–OH), 5.3–5.7 (br, 1H, O–*H*), 6.9–7.2 (2m, 4H, Ar-*H*), 7.6 and 8.1 (2br, 2H, CO–NH_2), 8.6–9.5 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 241 [MH]$^+$ 196 [M−(CO–NH_2)]$^+$.

6.1.1.29. (2*R*,*S*)-3-Hydroxy-2-[3-(4-chlorophenyl)-propylamino]propanamide hydrochloride (31). Mp 198–200 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.7–2.1 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–2.7 (t, *J* = 6.1 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.8–3.0 (br, 2H, NH–CH_2–CH_2–CH_2), 3.7–3.9 (br, 3H, NH–C*H*–CH_2–OH), 5.3–5.7 (br, 1H, O–*H*), 7.1–7.4 (2m, 4H, Ar-*H*), 7.6 and 8.0 (2br, 2H, CO–NH_2), 8.6–9.4 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 256 M$^+$ 212 [M−(CO–NH_2)]$^+$.

6.1.1.30. (2*R*)-3-Hydroxy-2-[3-(4-trifluoromethylphenyl)-propylamino]propanamide hydrochloride (32). Mp 222–224 °C; (42% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.8–2.1 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–3.7 (t, *J* = 6.2 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.8–3.1 (br, 2H, NH–CH_2–CH_2–CH_2), 3.7–3.9 (br, 3H, NH–C*H*–CH_2–OH), 5.4–5.6 (br, 1H, O–*H*), 7.3–7.5 (d, *J* = 8.1 Hz, 2H, Ar-*H*), 7.5–7.7 (d, br, *J* = 8.1 Hz, Ar-*H*, CO–NH_2), 8.0 (br, 1H, CO–NH_2), 8.5–9.4 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 291 [MH]$^+$ 259 [M−(CH_2–OH)]$^+$.

6.1.1.31. (2*R*)-3-Hydroxy-2-[3-phenyl-propylamino]*N*-methyl propanamide (33). Mp 76–78 °C; (41% yield); 41%; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.6–1.8 (m, 2H, NH–CH_2–CH_2–CH_2), 1.8–2.0 (br,1H, N–*H*–$(CH_2)_3$), 2.3–2.5 (t, *J* = 6.3 Hz, 2H, NH–CH_2–CH_2–CH_2 overlay with solvent), 2.6–2.7 (t, *J* = 6.3 Hz, d, *J* = 6.0 Hz, 5H, NH–CH_2–CH_2–CH_2, N–CH_3), 2.9–3.1 (t, *J* = 5.8 Hz, 1H, NH–C*H*–CH_2–OH), 3.4–3.6 (m, 2H, NH–CH–CH_2–OH), 4.6–4.8 (t, *J* = 6.0 Hz, 1H, O–*H*), 7.0–7.4 (m, 5H, Ar-*H*), 7.6–7.8 (br, 1H, CO–N*H*–CH_3); MS/EI$^+$ 237 [MH]$^+$ 205 [M−(CH_2–OH)]$^+$.

6.1.1.32. (2*R*,*S*)-3-Hydroxy-2-[3-phenylpropylamino]*N*,*N*-dimethyl propanamide hydrochloride (34). Mp 125–129 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.8–2.1 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–2.7 (t, *J* = 6.1 Hz, 8H, NH–CH_2–CH_2–CH_2 and N(CH_3)$_2$), 2.8–3.3 (m, 4H, NH–CH_2–CH_2–CH_2), 3.7–3.8 (d, *J* = 5.2 Hz, 2H, NH–CH–CH_2–OH), 4.3–4.4 (t, *J* = 5.2 Hz, 1H, NH–C*H*–CH_2–OH), 4.8–5.8 (br, 1H, -O*H*), 7.1–7.4 (m, 5H, Ar-*H*), 8.4–9.8 (br, 2H, N$^+$–H_2); MS/EI$^+$ 251 [MH]$^+$ 219 [M−(CH_2–OH)]$^+$.

6.1.1.33. (2*R*)-3-Hydroxy-2-[3-phenylpropylamino]propionic acid hydrochloride (35). Mp 165–168 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.8–2.1 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–2.7 (t, *J* = 6.2 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.9–3.1 (m, 2H, NH–CH_2–CH_2–CH_2), 3.8–4.0 (m, 3H, NH–C*H*–CH_2–OH), 7.1–7.4 (m, 5H, Ar-*H*), 8.0–10.0 (br, N-*H*, COO*H*, chemical exchange); MS/EI$^+$ 223 M$^+$ 179 [M−(CO–NH_2)]$^+$.

6.1.1.34. (2*S*)-3-(Acetoxy)-2-[3-phenylpropylamino]propanamide hydrochloride (36). Mp 165–167 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.9–2.1 (m, 5H, CO–CH_3, NH–CH_2–CH_2–CH_2), 2.6–2.7 (t, *J* = 6.4 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.8–3.0 (br, 2H, NH–CH_2–CH_2–CH_2), 4.0–4.2 (br, 1H, NH–C*H*–CH_2), 4.3–4.6 (m, 2H, NH–CH–CH_2), 7.0–7.3 (m, 5H, Ar-*H*), 7.6 and 8.3 (2br, 2H, CO–NH_2), 9.0 and 9.8 (br, 2H, N$^+$–H_2); MS/DEI$^+$ 265 [MH]$^+$ 220 [M−(CO–NH_2)]$^+$.

6.1.1.35. (2*R*,*S*)-3-Hydroxy-2-[3-phenyl-propyl-(*N*-methyl)-amino]propanamide hydrochloride (37). Mp 111–113 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.7–2.1 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–2.7 (t, *J* = 6.3 Hz, 2H, NH–CH_2–CH_2–CH_2), 2.9 (s, 3H, N–CH_3), 3.8–4.1 (br, 3H, NH–C*H*–CH_2–OH), 5.5–5.8 (br, 1H, O–*H*), 7.1–7.4 (m, 5H, Ar-*H*), 7.7 and 8.3 (br, d, 2H, CO–NH_2), 9.7 and 10.3 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 236 M$^+$ 192 [M−(CO–NH_2)]$^+$.

6.1.1.36. (2*R*,*S*)-2-Phenyl-2-[3-phenylpropyl-amino]acetamide hydrochloride (38). Mp 230–231 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.8–2.1 (m, 2H, NH–CH_2–CH_2–CH_2), 2.6–2.9 (t, *J* = 6.3 Hz, m, 4H, NH–CH_2–CH_2–CH_2), 5.0 (br, 1H, Ar-C*H*–CO), 7.1–7.7 (3m, 12H, Ar-*H*, CO–NH_2), 8.1 (br, 2H,

CO–NH_2), 9.3–9.7 (br, 2H, N^+–H_2); MS/EI$^+$ 269 $[MH]^+$ 224 $[M-(CO-NH_2)]^+$.

6.1.2. General reductive amination of α-aminoacid derivatives (Scheme 2). The appropriate aminoacid ester (13 mmol) was dissolved in a dry mixture of 10:1 ethanol/methanol (110 ml), added with corresponding ketone (13 mmol) and, after half an hour, with sodium cyanoborohydride in 3 portions at room temperature. The suspension was left overnight. The solvent was evaporated in vacuo and the residue was treated in the same way as described in Section 6.1.1. The final products were tested as either the free base or its hydrochloride salt. Following the procedure above these compounds were synthesised:

6.1.2.1. 3-Hydroxy-(2*R*)-2-[(1,2,3,4-tetrahydro-2-(*R*)-naphthalenyl)amino]propanamide hydrochloride (39). mp 226–231 °C; (41% yield); ^{1}H NMR (200 MHz, CH_3OH-d_4) δ 1.7–2.0 and 2.3–2.5 (2m, 2H, Ar-CH_2–CH_2–CH), 2.8–3.1 and 3.2–3.3 (2m, 4H, Ar-CH_2–CH and Ar-CH_2–CH–NH), 3.4–3.7 (m, 1H, Ar-CH_2–C*H*–NH), 3.7–4.1 (m, 2H NH–CH–CH_2–OH), 4.2–4.3 (m, 1H, NH–C*H*–CH_2–OH), 7.1–7.2 (m, 4H, Ar-*H*); MS/EI$^+$ 235 $[MH]^+$ 190 $[M-CO-NH_2]^+$.

6.1.2.2. 3-Hydroxy-(2*R*)-2-[(1,2,3,4-tetrahydro-2-(*S*)-naphthalenyl)amino]propanamide hydrochloride (40). Mp 143–146 °C; (40% yield); ^{1}H NMR (200 MHz, CH_3OH-d_4) δ 1.5–1.7 and 1.9–2.1 (2m, 2H, Ar-CH_2–CH_2–CH), 2.5–3.1 (2m, 5H, Ar-CH_2–CH_2–C*H*–NH and Ar-CH_2–CH–NH), 3.4–3.5 (t, J = 5.8 Hz, 1H, NH–C*H*–CH_2–OH), 3.6–3.8 (m, 2H, NH–CH–CH_2–OH), 6.9–7.1 (m, 4H, Ar-*H*); MS/EI$^+$ 235 $[MH]^+$ 190 $[M-CO-NH_2]^+$.

6.1.2.3. 3-Hydroxy-(2*S*)-2-[(1,2,3,4-tetrahydro-2-(*S*)-naphthalenyl)amino]propanamide hydrochloride (41). Mp 232–236 °C; (41% yield); ^{1}H NMR (200 MHz, CH_3OH-d_4) δ 1.7–2.0 and 2.3–2.5 (2m, 2H, Ar-CH_2–CH_2–CH), 2.8–3.1 and 3.2–3.3 (2m, 4H, Ar- CH_2–CH and Ar-CH_2–CH–NH), 3.4–3.7 (m, 1H, Ar-CH_2–C*H*–NH), 3.8–4.1 (m, 2H, NH–CH–CH_2–OH), 4.2–4.3 (m,1H, NH–C*H*–CH_2–OH), 7.0–7.2 (m, 4H, Ar-*H*).

6.1.2.4. 3-Hydroxy-(2*S*)-2-[(1,2,3,4-tetrahydro-2-(*R*)-naphthalenyl)amino]propanamide (42). Mp 143–148 °C; (41% yield); ^{1}H NMR (200 MHz, CH_3OH-d_4) δ 1.5–1.7 and 1.9–2.1 (2m, 2H, Ar-CH_2–CH_2–CH), 2.5–3.1 (2m, 5H, Ar-CH_2–CH_2–C*H*–NH and Ar-CH_2–CH–NH), 3.4–3.5 (t, J = 5.8 Hz, 1H, NH–C*H*–CH_2–OH), 3.6–3.8 (m, 2H, NH–CH–CH_2–OH), 6.9–7.1 (m, 4H, Ar-*H*).

6.1.2.5. (2*S*)-2-[(1,2,3,4-Tetrahydro-2-(*R*,*S*)-naphthalenyl)amino]propanamide hydrochloride (43). Mp 247–249 °C; (35% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.4–1.6 (d, J = 8.2 Hz, 3H, CH_3), 1.7–2.0 and 2.1–2.4 (2m, 2H, Ar-CH_2–CH_2–CH–NH), 2.7–3.2 (m, 4H, Ar-(CH_2)$_2$), 3.3–3.5 (br, 1H, Ar-CH_2–C*H*–NH), 4.0–4.2 (br, 1H, C*H*–CH_3), 7.0–7.2 (m, 4H, Ar-*H*), 7.6 and 8.1 (2br, 2H, CO–NH_2), 8.9–9.6 (2br, 2H, N^+–H_2); MS/EI$^+$ 219 $[MH]^+$, 174 $[M-CO-NH_2]^+$.

6.1.2.6. (2*R*)-2-[(1,2,3,4-Tetrahydro-2-(*R*,*S*)-naphthalenyl)amino]propanamide hydrochloride (44). Mp 244–246 °C; (37% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.4–1.6 (d, J = 8.2 Hz, 3H, CH_3), 1.7–2.0 and 2.1–2.4 (2m, 2H, Ar-CH_2–CH_2–CH–NH), 2.7–3.2 (m, 4H, Ar-(CH_2)$_2$), 3.3–3.5 (br, 1H, Ar-CH_2–C*H*–NH), 4.0–4.2 (br, 1H, C*H*–CH_3), 7.0–7.2 (m, 4H, Ar-*H*), 7.6 and 8.1 (2br, 2H, CO–NH_2), 8.9–9.6 (2br, 2H, N^+–H_2); MS/EI$^+$ 219 $[MH]^+$, 174 $[M-CO-NH_2]^+$.

6.1.2.7. 3-Hydroxy-(2*R*,*S*)-2-[(2-naphthyl)-methyl-amino]propanamide hydrochloride (45). Mp 240–241 °C; (39% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.7–4.0 (2m, 3H, NH–C*H*–CH_2–OH), 4.3–4.4 (br, 2H, Ar-CH_2–NH), 7.5–7.7 and 7.8–8.2 (2br, 9H, Ar-*H* and CO–NH_2), 9.0–9.8 (2br, 2H, N^+–H_2); MS/EI$^+$ 244 $[M]^+$, 200 $[M-CO-NH_2]^+$.

6.1.2.8. 3-Hydroxy-(2*R*)-2-[(1,2,3,4-tetrahydro-2-(*R*)-naphthalenyl)-amino]*N*-methyl propanamide hydrochloride (46). Mp 179–182 °C; (43% yield); ^{1}H NMR (200 MHz, CH_3OH-d_4) δ 1.8–2.1 and 2.3–2.4 (2m, 2H, Ar-CH_2–CH_2–CH), 2.8 (s, 3H, CO–NH–CH_3), 2.9–3.1 and 3.2–3.4 (2m, 4H, Ar-[CH_2]$_2$), 3.5–3.7 (m, 1H, Ar-CH_2–C*H*–NH), 3.7–4.1 (m, 2H, CH–CH_2–OH), 4.2–4.3 (m, 1H, NH–C*H*–CH_2–OH), 7.1 (s, 4H, Ar-*H*); MS/EI$^+$ 217 $[M-CH_2OH]^+$.

6.1.2.9. 3-Hydroxy-(2*R*)-2-[(1,2,3,4-tetrahydro-2-(*S*)-naphthalenyl)amino]*N*-methyl propanamide hydrochloride (47). Mp 163–167 °C; (45% yield);^{1}H NMR (200 MHz, CH_3OH-d_4) δ 1.8–2.1 and 2.3–2.4 (2m, 2H, Ar-CH_2–CH_2–CH), 2.8 (s, 3H, CO–NH–CH_3), 2.9–3.1 and 3.2–3.4 (2m, 4H, Ar-[CH_2]$_2$), 3.5–3.7 (m, 1H, Ar-CH_2–C*H*–NH), 3.7–4.1 (m, 2H, CH–CH_2–OH), 4.2–4.3 (m, 1H, NH–C*H*–CH_2–OH), 7.1 (s, 4H, Ar-*H*); MS/EI$^+$ 217 $[M-CH_2OH]^+$.

6.1.2.10. 3-Hydroxy-(2*R*,*S*)-2-[(1,2,3,4-tetrahydro-1-(*R*,*S*)-naphthalenyl)amino]propanamide (48). Mp 115–122 °C; (45% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.5–2.0 (m, 5H, Ar-CH_2–CH_2–CH_2–CH–N*H*), 2.9–2.9 (br, 4H. Ar-CH_2–CH_2 and NH–CH–CH_2–OH), 3.6–3.7 (q, J = 3.4 Hz, 1H, Ar-C*H*–NH), 3.8–4.0 (m, 1H, NH–C*H*–CO), 5.2–5.5 (br, 1H, O*H*), 7.0–7.4 (m, 6H, Ar-*H*, CO–NH_2).

6.1.2.11. 3-Hydroxy-(2*S*)-2-[(2-indanyl)amino]propanamide hydrochloride (49). Mp 190–192 °C; (44% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.1–3.5 (m, 4H, Ar-(CH_2)$_2$), 3.8–4.1 (m, 4H, C*H*–NH–C*H*–CH_2–OH), 5.4–5.7 (br, 1H, O*H*), 7.1–7.3 (m, 4H, Ar-*H*), 7.7 and 8.1 (2br, 2H, CO–NH_2), 9.1–9.9 (2br, 2H, N^+–H_2). Anal. Calcd. for $C_{12}H_{16}N_2O_2$·HCl: C, 56.14; H, 6.67; Cl, 13.81; N, 10.91; O, 12.46. Found: C, 56.27; H, 6.52; Cl, 13.92; N, 10.78.

6.1.2.12. 3-Hydroxy-(2*R*)-2-[(2-indanyl)amino]propanamide hydrochloride (50). Mp 94–96 °C; (40% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.1–2.4 (br, 1H, N*H*), 2.6–2.8 (m, 2H, Ar-(CH_2)$_2$ H_{ax}), 2.9–3.1 (m, 3H, Ar-(CH_2)$_2$ H_{eq} and Ar-(CH_2)$_2$–C*H*–NH), 3.4–3.7 (m, 3H, NH–C*H*–CH_2–OH), 4.6–4.8 (br, 1H, O*H*), 6.9–7.5

(m,br, 6H, Ar-*H* and CO–NH_2); MS/EI$^+$ 221 [MH]$^+$, 189 [M−CH_2OH]$^+$. Anal Calcd. for $C_{12}H_{16}N_2O_2$·HCl: C, 56.14; H, 6.67; Cl, 13.81; N, 10.91; O, 12.46. Found: C, 56.08; H, 6.76; Cl, 13.62; N, 10.82.

6.1.2.13. 3-Hydroxy-(2*R*)-2-[(2-indanyl)-amino]*N*-methyl-propanamide hydrochloride (51). Mp 189–190 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.6–2.8 (d, J = 6.0 Hz, 3H, CH_3–NH–CO), 3.0–3.4 (m, 4H, Ar-[CH_2]$_2$–CH), 3.7–4.1 (m, 4H, C*H*–NH–C*H*–CH_2–OH), 5.4–5.7 (br, 1H, O*H*), 7.1–7.3 (m, 4H, Ar-*H*), 8.6–8.8 (q, 1H, CO–N*H*), 9.1–9.8 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 235 [MH]$^+$, 203 [M−CH_2–OH]$^+$.

6.1.2.14. 3-Hydroxy-(2*S*)-2-[(2-indanyl)-amino]*N*-methyl-propanamide hydrochloride (52). Mp 190–192 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.6–2.8 (d, J = 6.0 Hz, 3H, CH_3–NH–CO), 3.0–3.4 (m, 4H, Ar-[CH_2]$_2$–CH), 3.7–4.1 (m, 4H, C*H*–NH–C*H*–CH_2–OH), 5.4–5.7 (br, 1H, O*H*), 7.1–7.3 (m, 4H, Ar-*H*), 8.6–8.8 (q, 1H, CO–N*H*), 9.1–9.8 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 235 [MH]$^+$, 203 [M−CH_2–OH]$^+$.

6.1.2.15. 3-Hydroxy-(2*R*)-2-[(2-indanyl),-(*N*-methyl)amino]propanamide hydrochloride (53). Mp 208–210 °C; (41% yield);^{1}H NMR (200 MHz, DMSO-d_6+CF3COOD) δ 2.7–2.9 (s, 3H, N–CH_3), 2.9–3.5 (m, 4H, Ar-[CH_2]$_2$–CH), 3.9–4.1 (2br, 3H, N–C*H*–CH_2–OH), 4.2–4.4 (br, 1H, Ar-[CH_2]$_2$–C*H*), 7.0–7.3 (4H, Ar-*H*); MS/ES$^+$ 235 [MH]$^+$.

6.1.2.16. 3-Hydroxy-(2*S*)-2-[(2-indanyl)-(*N*-methyl)-amino]propanamide hydrochloride (54). Mp 209–211 °C; (41% yield);^{1}H NMR (200 MHz, CH_3OH-d_4) δ 2.7–3.0 (br, 3H, N–CH_3), 3.2–3.5 (m, 4H, Ar-[CH_2]$_2$), 3.9–4.2 (m, 3H, CO–C*H*–CH_2–OH), 4.3–4.5 (m, 1H, N–CH_3–C*H*–[CH_2]$_2$), 4.7–5.6 (br, 1H, O*H*), 7.1–7.3 (m, 4H, Ar-*H*), 7.6–7.8 and 8.1–8.4 (2br, 2H, CO–NH_2), 10.1–10.4 and 11.1–11.4 (2br, 1H, N$^+$–*H*).

6.1.2.17. (2*S*)-2-[(2-Indanyl)amino]propanamide hydrochloride (55). Mp 217–219 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.2–1.4 (d, 3H, J = 6.1 Hz, CH_3–CH), 2.9–3.3 (m, 4H, Ar-[CH_2]$_2$–CH), 3.7–3.9 (m, 2H, C*H*–N–C*H*–CH_3), 6.9–7.2 (m, 4H, Ar-*H*), 7.5 and 8.1 (2br, 2H, CO–NH_2), 8.9–10.0 (2br, 2H, N$^+$-H_2); MS/DEI$^+$ 205 [MH]$^+$, 160 [M−CO–NH_2]. Anal Calcd. for $C_{12}H_{16}N_2O$·HCl: C, 59.87; H, 7.12; Cl, 14.73; N, 11.64; O, 6.65. Found: C, 59.95; H, 7.24; Cl, 14.86; N, 11.57.

6.1.2.18. (2*R*)-2-[(2-Indanyl)amino]propanamide hydrochloride (56). Mp 217–219 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.2–1.4 (d, 3H, J = 6.1 Hz, CH_3–CH), 2.9–3.3 (m, 4H, Ar-[CH_2]$_2$–CH), 3.7–3.9 (m, 2H, C*H*–N–C*H*–CH_3), 6.9–7.2 (m, 4H, Ar-*H*), 7.5 and 8.1 (2br, 2H, CO–NH_2), 8.9–10.0 (2br, 2H, N$^+$–H_2). Anal Calcd. for $C_{12}H_{16}N_2O$·HCl: C, 59.87; H, 7.12; Cl, 14.73; N, 11.64; O, 6.65. Found: C, 59.74; H, 7.06; Cl, 14.66; N, 11.76.

6.1.2.19. (2*R*)-2-[(2-Indanyl)amino]*N*-methyl propanamide hydrochloride (57). Mp 194–196 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.3–1.6 (d, J = 5.8 Hz, 3H, CH_3–CH–CO), 2.6–2.7 (d, J = 4.9 Hz, 3H, CH_3–NH), 3.1–3.3 (m, 4H, Ar-[CH_2]$_2$), 3.7–4.1 (m, 2H, C*H*–N–C*H*–CH_3), 7.1–7.3 (m, 4H, Ar-*H*), 8.7–8.9 (q, 1H, CO–N*H*), 9.2–10.2 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 160 [M−CH_3–NH–CO]$^+$.

6.1.2.20. (2*S*)-2-[(2-Indanyl)amino]*N*-methyl propanamide hydrochloride (58). Mp 194–196 °C; (41% yield);^{1}H NMR (200 MHz, DMSO-d_6) δ 1.3–1.6 (d, J = 5.8 Hz, 3H, CH_3–CH–CO), 2.6–2.7 (d, J = 4.9 Hz, 3H, CH_3–NH), 3.1–3.3 (m, 4H, Ar-[CH_2]$_2$), 3.7–4.1 (m, 2H, C*H*–N–C*H*–CH_3), 7.1–7.3 (m, 4H, Ar-*H*), 8.7–8.9 (q, 1H, CO–N*H*), 9.2–10.2 (2br, 2H, N$^+$–H_2); MS/EI$^+$ 219 [MH]$^+$, 160 [M−CH_3–NH–CO]$^+$.

6.1.2.21. 2-[(2-Indanyl)amino]l-acetamide hydrochloride (59). Mp 207–209 °C; (48% yield);^{1}H NMR (200 MHz, DMSO-d_6) 3.0–3.2 (m, 4H, Ar-(CH_2)$_2$–CH) 3.7 (s, 2H, CO–CH_2–N$^+$) 3.9–4.1 (m, 1H, Ar-(CH_2)–C*H*) 7.1–7.3 (m, 4H, Ar-*H*) 7.5–7.6 and 7.9–8.1 (2br, 2H, CO–NH_2) 9.5–9.7 (br, 2H, N$^+$–H_2); MS/DEI$^+$ 191 [MH]$^+$ 146 [M−CO–NH_2]$^+$. Anal Calcd. for $C_{11}H_{14}N_2O$·HCl: C, 58.28; H, 6.67; Cl, 15.64; N, 12.36; O, 7.06. Found: C, 58.36; H, 6.73; Cl, 15.75; N, 12.49.

6.1.2.22. 2-[(2-Indanyl)amino]*N*-methylacetamide hydrochloride (60). Mp 219–220 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) 2.6–2.7 (d, J = 4.7 Hz, 3H, N–CH_3) 3.03–3.3 (m, 4H, Ar-(CH_2)$_2$–CH) 3.7 (s, 2H, CO–CH_2–N$^+$) 3.9–4.1 (m, 1H, AR-(CH_2)$_2$–C*H*) 7.0–7.3 (m, 4H, Ar-*H*) 8.5–8.7 (m, 1H, CO–N*H*) 9.4–9.8 (br, 2H, N$^+$–H_2) ; MS/ES$^+$ 205 [MH]$^+$.

6.1.2.23. 2-[(2-Indanyl)amino]-2-cyclopropylacetamide hydrochloride (61). Mp 195–198 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) 1.4–1.7 (2m, 4H, CO–C–(CH_2)$_2$) 3.1–3.4 (m, 4H, Ar-(CH_2)$_2$–CH) 4.1–4.3 (m, 1H, Ar-(CH_2)$_2$–C*H*) 7.1–7.3 (m, 4H, Ar-*H*) 7.4–7.7 (br, 2H, CO–NH_2) 9.8–10.2 (br, 2H, N$^+$–H_2); MS/DCI$^+$ 217 [MH]$^+$.

6.1.2.24. 2-[(2-Indanyl)amino]-2-cyclopentylacetamide hydrochloride (62). Mp 235–239 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) 1.6–1.8 (m, 4H, 2CH_2) 2.0–2.3 (2m, 4H, 2CH_2) 3.1–3.4 (m, 4H, Ar-(CH_2)$_2$–CH) 3.8–4.0 (m, 1H, Ar-(CH_2)$_2$–C*H*) 7.1–7.3 (m, 4H, Ar-*H*) 7.7–7.9 (br, 2H, CO–NH_2) 9.5–9.8 (br, 2H, N$^+$–H_2); MS/DEI$^+$ 245 [MH]$^+$ 200 [M−CO–NH_2].

6.1.2.25. 2-[(2-Indanyl)amino]phenylacetamide hydrochloride (63). Mp > 250 °C; (41% yield); ^{1}H NMR (200 MHz, DMSO-d_6) 3.1–3.3 (m, 4H, Ar-(CH_2)$_2$–CH) 3.6–3.8 (m, 1H, Ar-(CH_2)$_2$–C*H*) 5.0–5.2 (br, 1H, N$^+$–C*H*–CO) 7.1–7.2 (m, 4H, Ar-*H*) 7.4–7.6 (m, 3H, Ar-*H*) 7.7–7.8 (m, 3H, Ar-*H*, CO–NH_2) 8.1–8.3 (br, 1H, CO–NH_2) 9.7–10.3 (br, 2H, N$^+$–H_2).

6.2. Pharmacology

6.2.1. Maximal electroshock seizure test in mice. Male CD-1 mice (20–30 g, Charles River Laboratories, Italy,

Calco) were used in this study. An electrical stimulus (50 mA, 60 Hz; 0.2 s ECT unit model 7801, Ugo Basile, Varese, Italy) was delivered through corneal electrodes after the application of a local anaesthetic (lidocaine in 0.9% sodium chloride solution). This stimulus was sufficient to produce a hind limb tonic extension in 100% of control animals. The anticonvulsant activity of orally administered test compounds was initially evaluated at different time points (15, 30, 60, 120, 180 and 240 min) from treatment to establish the time of peak effect. At least three doses of test compounds were then administered to groups of 10–20 mice at time of peak effect in a dose volume of 10 ml/kg to calculate the respective doses at which 50% of the animals were protected from seizures (ED_{50}). All controls received the identical volume of corresponding vehicle. The complete suppression of the hind limb tonic component of the seizures was taken as the end-point for this test. The method of Litchfield–Wilcoxon (1949) was used to calculate the ED_{50} values with 95% confidence interval.[16]

6.2.2. Horizontal screen test in mice. In this study, neural impairment was determined by failure of mice (male CD-1 mice of 20–30 g, Charles River Laboratories, Italy, Calco) to perform successfully the horizontal screen test.[17] The apparatus consisted of a 13×14 cm square wire screens which was mounted horizontally on a steel rod. The rod was supported at both ends and could be inverted through an arc of 180°. Untrained mice were placed individually on the top of the screen and the rod was then rotated (mice unable to climb to an upright position within 1 min were rated as failures). The neurotoxic activity of orally administered test compounds was initially evaluated at different time points from treatment to establish the time of peak effect. At least three doses of test compounds were then administered to groups of 10–20 mice at time of peak effect in a dose volume of 10 ml/kg to calculate the respective doses at which 50% All controls received the identical volume of corresponding vehicle. After drug treatment, two values were recorded: (1) the number of mice that fall from the screen and (2) the number of mice that fail to climb the top of the screen (i.e., the sum of those that remain clinging to the bottom of the screens and those that fall from the screen). The method of Litchfield–Wilcoxon (1949) was used to calculate the respective doses at which 50% of the animals failed the test (TD_{50}).[16]

6.2.3. Chemically induced tonic seizures in mice. Testing for efficacy against chemically induced tonic convulsions occurred at the time of the maximal anticonvulsant effect of the compounds obtained in previous studies or from published data showing anticonvulsant activity in mice (male CD-1 mice of 20–30 g, Charles River Laboratories, Italy, Calco). All the anticonvulsants were administered orally. Control animals received the vehicle in the same way. Chemical convulsants were administered sc at doses that caused convulsions in 97% of the animals (CD_{97} values), 2.2 mg/kg for bicuculline (BIC) and 13.4 mg/kg for picrotoxin (PIC). For po and sc administration a volume of 10 ml/kg was used. Following injections of chemical convulsants in a fold of skin in the midline of the neck, animals were placed in individual plexiglas cages and observed continuously for the presence or absence of hind limb tonic extension. Observation period was of 30 min for BIC and of 45 min for PIC.[18] Dose–response curves consisted of at least three dose levels for each compound, with 12–24 animals used for dose.

6.2.4. Modulation of veratridine-induced [^{3}H]D-aspartate release in cortical synaptosomes. Synaptosomes were prepared following previously described protocols.[19,20] Adult male Sprague–Dawley rats (200–250 g) were sacrificed by decapitation. Cortices (200–300 mg) was quickly dissected and homogenized in ice cold 0.32 M Tris-buffered sucrose (pH 7.4). The homogenate was centrifuged at 900 g for 5 min at 4 °C. The supernatant was stratified on a Percoll discontinuous gradient (20%, 10% and 6%) and then centrifuged at 36,000*g* for 5 min. The synaptosomal fraction (interface between Percoll 20% and 10%) was extracted, added with 5 ml cold, oxygenated (95% O_2, 5% CO_2) Krebs solution (mM NaCl 125; KCl 3; $CaCl_2$ 1.2; $MgSO_4$ 1.2; NaH_2PO_4 1; $NaHCO_3$ 22; glucose 10) and centrifuged again at 39,000*g* for 15 min. Finally, the resulting pellet was resuspended in 1 ml of oxygenated Krebs solution at room temperature (as above).

Synaptosomes were incubated in oxygenated Krebs solution containing 150 nM [^{3}H]D-aspartate (16. Ci/mmol) for 15 min at 37 °C. At the end of the incubation time they were transferred into superfusion chambers according to Simonato et al..[21] The preparation was stimulated by pulses (60 s duration) of 10 μM veratridine. Treatments were applied 6 min before stimulation. The perfusate was collected at 3 min intervals, and [^{3}H]D-aspartate content was determined using a scintillation β-spectrometer (LS1800 Beckman). Protein content of the preparation was determined by the method of Lowry.[22]

Veratridine-evoked overflow of [^{3}H]D-aspartate was calculated by subtracting the presumed basal outflow from the total tritium found in the sample at stimulus application and in the following sample. The presumed basal outflow was obtained by interpolation between the precedent and the following sample. Data have been expressed as % of the overflow obtained in the control (vehicle-treated) filters.

References and notes

1. Mattson, R. H. *Epilepsia* **1996**, *37*, S1.
2. Patsalos, P. N. *Emerging Drugs* **1999**, *4*, 87.
3. Bialer, M. *Therap. Drug Monitoring* **2002**, *24*, 85; Bialer, M. *Neurology* **2004**, *63*, 746–748.
4. Van Dorsser, W.; Barris, D.; Cordi, A.; Roba, J. *Arch. Int. Pharmacodyn.* **1983**, *266*, 239–249.
5. Pevarello, P.; Bonsignori, A.; Dostert, P.; Heidempergher, F.; Pinciroli, V.; Colombo, M.; McArthur, R. A.; Salvati, P.; Post, C.; Fariello, R. G.; Varasi, M. *J. Med. Chem.* **1998**, *41*, 579.
6. Pevarello, P.; Bonsignori, A.; Caccia, C.; Amici, R.; McArthur, R. A.; Fariello, R. G.; Salvati, P.; Varasi, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2521.
7. Baxter, E. W.; Reitz, A. B. *Org. React.* **2002**, *59*, 1.

8. White, H. S.; Woodhead, J. H.; Franklin, M. R.; Swinyard, E. A.; Wolf, H. H. In *Antiepileptic Drugs*; Levy, R. H., Mattson, R. H., Meldrum, B. S., Eds., 4th ed.; Raven Press: New York, 1994; pp 99–110.
9. Janssens de Varebeke, P.; Cavalier, R.; David-Remacle, M.; Youdim, M. B. H. *J. Neurochem.* **1988**, *50*, 1011.
10. Villetti, G.; Bregola, G.; Bassani, F.; Bergamaschi, M.; Rondelli, I.; Pietra, C.; Simonato, M. *Neuropharmacology* **2001**, *40*, 866.
11. Zucchini, S.; Buzzi, A.; Bergamaschi, M.; Pietra, C.; Villetti, C.; Simonato, M. *NeuroReport* **2002**, *13*, 2071.
12. Barbieri, M.; Bregola, G.; Buzzi, A.; Marino, S.; Zucchini, S.; Stables, J. P.; Bergamaschi, M.; Pietra, C.; Villetti, G.; Simonato, M. *Br. J. Pharmacol.* **2003**, *139*, 1333.
13. O'Brien, E. M.; Tipton, K. F.; Strolin Benedetti, M.; Bonsignori, A.; Marrani, P.; Dostert, P. *Biochem. Pharmacol.* **1991**, *41*, 1731–1737.
14. PCT Appl. No. WO 95/18617 and WO 96/21640.
15. Lingamaneni, R.; Hemmings, H. C., Jr. *Neurosci. Lett.* **1999**, *276*, 1217.
16. Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.
17. Coughenour, L. L.; McLean, J. R.; Parker, R. B. *Pharmacol. Biochem. Behav.* **1977**, *6*, 351.
18. Piredda, S. G.; Woodhead, J. H.; Swinyard, E. A. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 741.
19. Raiteri, M.; Bonanno, G.; Marchi, M.; Maura, G. *J. Pharmacol. Exp. Ther.* **1984**, *231*, 671.
20. Dunkley, P. R.; Jarvie, P. E.; Heath, J. W.; Kidd, G. J.; Rostas, J. A. *Brain Res.* **1984**, *372*, 115.
21. Simonato, M.; Bregola, G.; Muzzolini, A.; Bianchi, C.; Beani, L. *Neurochem. Int.* **1993**, *23*, 555.
22. Lowry, O. H.; Rosenburg, N. J.; Farr, A.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.