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1-Aminocylopropane-1-carboxylic acid derivatives as ligands at the glycine-binding site of the *N*-methyl-D-aspartate receptor

Cesarino Balsamini [a], Annalida Bedini [a], Gilberto Spadoni [a], Giorgio Tarzia [a,*], Andrea Tontini [a], Walter Balduini [b], Mauro Cimino [b]

[a] *Istituto di Chimica Farmaceutica, Università di Urbino, Piazza Rinascimento 6, 61029 Urbino, Italy*

[b] *Istituto di Farmacologia, Università di Urbino, Via Santa Chiara 27, 61029 Urbino, Italy*

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Abstract

Several 1-aminocyclopropane-1-carboxylic acid derivatives were prepared and tested for activity at the glycine-binding site of the *N*-methyl-D-aspartate (NMDA) receptor complex. Structural modifications involved the amino group, the carboxylic function or position 2 of the ring. When tested in a [^{3}H]-MK-801 binding assay in the presence of glutamic acid, some of the compounds were able to activate the receptor. Two of them (**3e** and **6**) are selective ligands for the glycine site of the NMDA receptor.

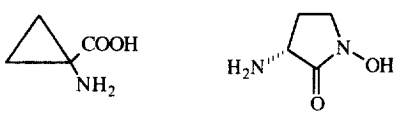
Keywords: 1-Aminocyclopropane-1-carboxylic acid (ACPC); Strychnine-insensitive glycine site; *N*-Methyl-D-aspartate (NMDA) receptor

1. Introduction

Molecules acting at the glycine site of the *N*-methyl-D-aspartate (NMDA) receptor have therapeutic potential for a number of central nervous system (CNS) disorders, including stroke, epilepsy and schizophrenia [1–3]. The clinical interest in partial agonists, e.g. R-(+)-HA-966 [4] and 1-aminocyclopropane-1-carboxylic acid (ACPC) [5] (Fig. 1) is due to their ability to act as modulators of the NMDA receptor, either by enhancing or by decreasing responses, according to their dosage and efficacy.

In particular, ACPC, which behaved as a partial agonist in vitro when tested in the presence of glutamate [5], shows antagonistic properties in vivo since it blocks NMDA-induced convulsions [6]. The clinical use of ACPC, however, is hampered by its relatively poor penetration of the blood–brain barrier [7,8].

Despite its interesting pharmacological profile, ACPC has only occasionally been considered as a template for the synthesis of new ligands potentially able to modulate NMDA receptor activity. A few *E* and *Z* isomers of 2-substituted ACPC (R = $-CH_3$, $-i$-Pr, $-C_6H_5$) were previously prepared and tested in our laboratory and the results of binding studies indicated that 2-methyl derivatives retain a moderate affinity for the glycine receptor, with a slight diastereoselectivity in favour of the *Z* isomer ($pK_i = 4.85$) [9].



ACPC **R-(+)-HA-966**

Fig. 1. Partial agonists ACPC and R-(+)-HA-966.

On the other hand, a large number of natural and synthetic amino acids structurally related to glycine have been tested [10–15]. Structural requirements for the activation of the glycine coagonist site, obtained from biological data, allowed the proposal of a pharmacophoric three-point interaction model, with an anionic and a cationic site, and a cooperative binding region, most likely hydrophobic in nature, which, however, tolerates hydroxylic function [14].

In this paper we report the synthesis and pharmacological activity of several new ACPC derivatives (Fig. 2) with structural modifications inferred from the structure–activity relationships (SARs) of the glycine site, as follows:

(a) the *N*-alkyl and *N*-phenyl substituents in compounds **3a–e** were selected in analogy to *N*-methylglycine and to *N*-(3,5-dichlorophenyl)glycine, which bind to the receptor with IC_{50} values of 0.85 [10] (a different value, $IC_{50} = 44$ μM, was reported by other authors [11]) and 49 μM [13], respectively;

* Corresponding author.

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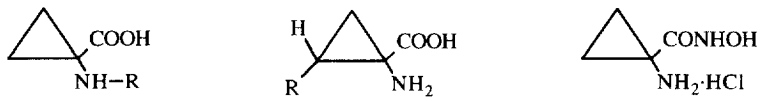
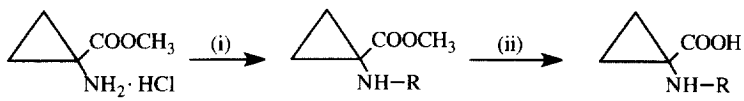


3a-e **9a-e** **6**

a: $CH_2C(CH_3)_3$ **a**: $CH_2S(CH_2)_2OH$
b: $CH_2CH{=}CHCH_3$ **b**: CH_2OBtz
c: $CH_2C_6H_5$ **c**: CH_2SBtz
d: C_6H_5 **d**: $CH_2NHCONHC_6H_5$
e: CH_3 **e**: $CH_2NHCOCH_2C_6H_5$

Fig. 2. New ACPC derivatives studied in the paper.



1 **2a-d** **3a-d**

2a,3a, R= $-CH_2-C(CH_3)_3$
2b,3b, R= $-CH_2CH{=}CHCH_3$
2c,3c, R= $-CH_2-C_6H_5$
2d,3d, R= $-C_6H_5$

Scheme 1. Reagents and conditions: (i) **2a–c**: (a) Et_3N, R–CHO, dry $CHCl_3$, molecular sieves, (b) $NaBH_4$, MeOH; **2d**: (a) dry CH_2Cl_2, Et_3N, (b) dry CH_2Cl_2, Cu, $BiPh_3(OAc)_2$, r.t.; (ii) 3N KOH, MeOH, r.t./Dowex 50-H^+, 2N NH_4OH.

(b) 1-aminocyclopropane-1-carbohydroxamic acid hydrochloride **6** [16] was included in our study because of its structural resemblance to both the partial agonist R-(+)-HA-966 (Fig. 1) and the corresponding hydroxamic acid of glycine [12];

(c) compound **9a**, the (*Z*)-2-[(2-hydroxyethylthio)-methyl]-1-aminocyclopropane-1-carboxylic acid, was modelled on γ-substituted vinylglycine derivatives [14], many of which showed affinities and potencies similar to those of glycine;

(d) the (*Z*)-2-substituted ACPC derivatives **9b–e**, initially prepared as putative glycine agonists, were also successively tested as antagonists according to a recent paper [17] describing a new class of diiodotyrosine-derived glycine antagonists.

The *Z* geometry of the 2-substituted compounds was preferred on the basis of our previous results on (*E*)- and (*Z*)-2-methyl derivatives [9].

2. Chemistry

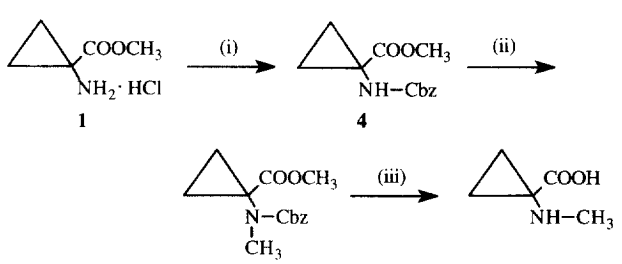
Compounds **3a–d** were prepared according to Scheme 1 using methyl 1-aminocyclopropane-1-carboxylate hydrochloride **1** [9] as starting material.

The synthesis of **2a–c** is based on a reductive amination: by using the appropriate aldehyde we obtained the imine intermediate (not isolated) which was directly reduced to the *N*-substituted-methyl-1-aminocyclopropane-1-carboxylate by treatment with $NaBH_4$ in MeOH. Metallic copper-catalyzed *N*-arylation with triarylbismuth diacetate [18] was applied to prepare **2d**. The esters thus obtained were subjected to alkaline hydrolysis to provide the corresponding amino acids **3a–d**.

N-Methyl-ACPC **3e** was obtained via methylation of *N*-benzyloxycarbonyl-ACPC **4** followed by hydrogenolysis and alkaline hydrolysis (Scheme 2). 1-Aminocyclopropane-1-carbohydroxamic acid hydrochloride **6** was prepared according to a literature method [16].

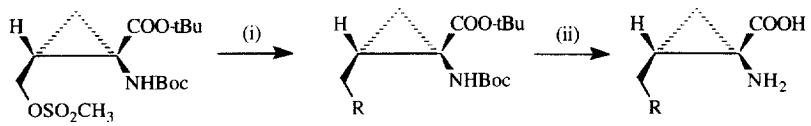
The synthesis of **9a–c** is depicted in Scheme 3: t-butyl (*Z*)-1-(*N-Boc*)-amino-2-(methanesulfonylmethyl)-cyclopropane-1-carboxylate **7** [19] was subjected to a nucleophilic substitution with 2-mercaptoethanol **9a** or the appropriate benzothiazole **9b,c** to afford derivatives **8a–c**, which were then deprotected using CF_3COOH.

Derivatives **9d,e** were synthesized from the known t-butyl (*Z*)-1-(*N-Boc*)-amino-2-(aminomethyl)-cyclopropane-1-carboxylate **10** [20] by treatment with phenyl isocyanate or phenylacetyl chloride, respectively, to give intermediates **11d,e** which were subjected to a one-step deprotection with CF_3COOH (Scheme 4).



1 **4** **5** **3e**

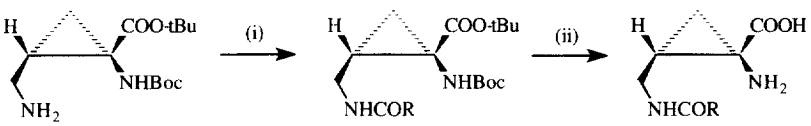
Scheme 2. Reagents and conditions: (i) benzyl chloroformate, aqueous $NaHCO_3$, r.t.; (ii) Ag_2O (molar excess), CH_3I (molar excess), dry DMF, 50°C, 3–4 days; (iii) (a) H_2, 1.5 atm, 5% Pd/C, absolute EtOH, (b) 3N KOH, MeOH, r.t./Dowex 50-H^+, 2N NH_4OH.



7 8a-c 9a-c

8a,9a, R= -S-CH_2CH_2OH
8b,9b, R= -OBtz
8c,9c, R= -SBtz

Scheme 3. Reagents and conditions: (i) **8a**: DBU, C_6H_6, 2-mercaptoethanol, r.t.; **8b**: DMSO, 2-hydroxybenzothiazole, KOH, r.t. to 60–70°C; **8c**: DBU, C_6H_6, 2-mercaptobenzothiazole, r.t.; (ii) CF_3COOH, 0°C to r.t., CH_2Cl_2/Dowex 50-H^+, 2N NH_4OH.



10 11d,e 9d,e

11d,9d, R= -NH-C_6H_5
11e,9e, R= -CH_2-C_6H_5

Scheme 4. Reagents and conditions: (i) **11d**: PhNCO, CH_2Cl_2, r.t.; **11e**: phenylacetyl chloride, $CHCl_3$, r.t.; (ii) CF_3COOH, 0°C to r.t., CH_2Cl_2/Dowex-50-H^+, 2N NH_4OH.

Table 1
Effect of different 1-aminocyclopropane-1-carboxylic acid (ACPC) analogues in stimulating [^{3}H]-MK-801 binding to the glycine modulatory site of the NMDA receptor complex in the rat forebrain membranes [a]

Compound	% of control	Intrinsic activity	EC_{50} [c,d] (nM)
Glu (10 μM)	100 ± 7		
Gly [b]	171 ± 2*	1.0	22.8
ACPC [b]	169 ± 3*	0.97	73.5
3a [b]	95 ± 2	0.00	n.d.
3b [b]	123 ± 5*	0.32	20.7
3c [b]	125 ± 9*	0.35	8.2
3d [b]	106 ± 4	0.08	n.d.
3e [b]	127 ± 8*	0.38	50.3
6 [b]	165 ± 7*	0.91	365.0
9a [b]	115 ± 3	0.21	n.d.
9b [b]	110 ± 9	0.14	n.d.
9c [b]	102 ± 4	0.02	n.d.
9d [b]	97 ± 6	0.00	n.d.
9e [b]	96 ± 5	0.00	n.d.

[a] Experiments were performed as described in the experimental protocols (Section 5). Results represent the mean (±SEM) of the three different experiments and are expressed as % of control (specific binding obtained in the presence of glutamate).
[b] All compounds were tested at a concentration of 10 μM in the presence of 10 μM glutamate. Basal values, without the addition of glutamate or glycine, were 85.3 ± 13 fmol/mg protein. In the presence of 10 μM glutamate the specific binding was 478 ± 67 fmol/mg protein.
[c] EC_{50} values were calculated by non-linear fitting analysis of concentration response curves (1 nM–10 μM). Results are the mean of two different experiments performed in triplicate.
[d] n.d. = not determined.
* Significantly different from 10 μM glutamate.

3. Pharmacology

We report herein a preliminary pharmacological evaluation, performed with the aid of the [^{3}H]-MK-801 paradigm [21], of compounds **3a–e**, **6** and **9a–e**. The results obtained are reported in Table 1.

Compounds **9b–e** were also tested as antagonists of the glycine modulatory site of the NMDA receptor complex (Table 2).

Compounds showing a positive modulation of NMDA receptor activity (**3b,c,e**, **6** and **9a**) were further tested for affinity at the glycine-binding site (^{3}H-MDL105119) and

Table 2
Antagonism of [^{3}H]-MK-801 binding to the glycine modulatory site of the NMDA receptor complex in rat forebrain membranes [a]

Compound	% of control
Glu (10 μM)	100 ± 5
Glu (10 μM) + Gly (1 μM)	155 ± 3
DCKA	99 ± 5
9b	149 ± 6
9c	160 ± 8
9d	151 ± 4
9e	148 ± 6

[a] Experiments were performed as described in the experimental protocols (Section 5). Results represent the mean (±SEM) of the three or four different experiments and are expressed as % of control (specific binding obtained in the presence of glutamate). Compounds **9b–e** were tested at a concentration of 100 μM, DCKA (20 μM), 10 μM glutamate and 1 μM glycine.

Table 3
Receptor profile of compounds **3b,c,e, 6** and **9a** on excitatory amino acid binding sites [a]

Compound	^{3}H-CGP39653	^{3}H-AMPA	^{3}H-KA	^{3}H-MDL105519
3b	> 100	> 100	> 100	> 100
3c	> 100	> 100	> 100	> 100
3e	> 100	> 100	> 100	8.6
9a	> 100	> 100	> 100	> 100
6	> 100	> 100	> 100	36.3

[a] Affinity is expressed as IC_{50} (μM).

selectivity for the glutamate-binding site (^{3}H-CGP39653) as well as for the other excitatory amino acid receptor subtypes, i.e. DL-[^{3}H]α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate. The results obtained are reported in Table 3.

4. Results and discussion

In agreement with previous data [5], the increase in [^{3}H]-MK-801 binding induced by ACPC in the presence of glutamate was similar to that induced by glycine (Table 1). The *N*-substituted derivatives **3**, with exclusion of the 2,2-dimethylpropyl and phenyl terms, behave as positive modulators of the NMDA receptor, retaining 32–38% of glycine activity; *N*-methyl-ACPC **3e** is the most active compound of the series. Interestingly, in an electrophysiological test on oocytes [12], *N*-methylglycine was able to evoke only 6% of the current evoked by glycine.

The effect of the increase in size of the *N*-substituents is unclear: the *trans*-2-buten-1-yl group in **3b** and the benzyl group in **3c** maintain the activity otherwise suppressed by 2,2-dimethylpropyl and phenyl substituents, i.e. **3a,d**. As for the (*Z*)-2-[(2-hydroxyethylthio)methyl]-1-aminocyclopropane-1-carboxylic acid **9a**, it should be noted that the introduction of a chain similar to that of γ-substituted vinylglycine derivatives [14] gives a compound retaining activity (20% of that afforded by glycine).

1-Aminocyclopropane-1-carbohydroxamic acid hydrochloride **6** is the compound showing the highest intrinsic activity in our series. It is noteworthy that, in the case of glycine, the same isosteric substitution generated a compound with an activity of only 7% that of glycine in the above-mentioned electrophysiological test [12].

Compounds **9b–e** were tested as agonists and antagonists; however, under our conditions they were found to be inactive (see Tables 1 and 2).

The most interesting positive modulators, **3b,c,e, 6** and **9a**, were submitted to binding studies in order to verify and evaluate the affinity for the glycine-binding site of the NMDA receptor, as well as the selectivity for other excitatory amino acid binding sites. The results reported in Table 3 confirm that compounds **3e** and **6** bind to the glycine site with IC_{50} of 8.6 and 36.3 μM, respectively, and not to the other excitatory amino acid sites included in our experiments. No affinity was observed for compounds **9a** and **3b,c** even at high concentration (100 μM); however, the increase in [^{3}H]-MK-801 binding induced by **3b,c,e** was completely blocked when the glycine-binding site antagonist 5,7-dichlorokynurenic acid (DCKA) was added to the assay (data not shown), indicating that, notwithstanding the loss of affinity in the [^{3}H]-MDL105519 assay, their effect is due to modulation of the glycine-binding site.

In conclusion, we have shown that compounds endowed with a varying degree of agonist activity at the strychnine-insensitive glycine-binding site on the NMDA receptor can be obtained by a structural modification of 1-amino-1-cyclopropanecarboxylic acid. The small set of compounds used and the preliminary nature of the pharmacological tests performed in this study do not allow us to draw conclusive SARs; however, steric factors appear critical for agonism of compounds derived from ACPC, as for glycine and pyrrolidinone derivatives. Interestingly, the SARs of the glycine agonists obtained from the examination of glycine derivatives are not predictive in an absolute manner: in fact, the *N*-methyl substitution is tolerated in ACPC as well as in glycine, but the carbohydroxamic substitution, which causes an almost complete inactivation in glycine, leads to the active compounds **6** when introduced in ACPC. It is noteworthy that the glycine partial agonist R-(+)-HA-966, which retains from 40 to 70% of the efficacy of glycine, depending on the experimental protocol used, and shows an affinity (IC_{50} = 27.2 μM) [2] similar to that of our partial agonists **3e** and **6**, is an acknowledged lead for the development of therapeutics.

A more complete pharmacological profile of compounds **3e** and **6** would be necessary in order to assess their potential.

5. Experimental

5.1. Chemistry

Melting points were taken on a Büchi SMP-510 capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Bruker FT-48 spectrometer and absorbances ν are reported in cm^{-1}. Elemental analyses were performed on a Carlo Erba analyser and were within ±0.4% of the theoretical values. Electron ionization mass spectra (70 eV) were taken on a Fisons Trio 1000 instrument. ^{1}H NMR spectra were recorded on a Bruker 200 spectrometer; chemical shifts δ are reported in ppm. Column chromatography purifications were performed under 'flash' conditions using Merck 230–400 mesh silica gel. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates.

5.1.1. General procedure for the synthesis of methyl N-substituted-aminocyclopropane-1-carboxylates 2a–c

Activated molecular sieves and 1.2 mmol of the appropriate aldehyde were added to a solution of 151 mg (1 mmol)

of methyl 1-aminocyclopropane-1-carboxylate hydrochloride **1** and 0.139 ml (1 mmol) of Et_3N in 5 ml of $CHCl_3$. The mixture was stirred at room temperature for 4 h (**2a,c**) or 16 h (**2b**), after which 6 ml of MeOH and 115 mg (3 mmol) of $NaBH_4$ were added and the mixture was refluxed for 0.5 h. The mixture was neutralized using 2N HCl, filtered on Celite® and then evaporated to give a residue which was purified by flash chromatography using EtOAc with cyclohexane mixtures as eluant.

5.1.2. Methyl 1-pivaloylaminocyclopropane-1-carboxylate ***2a***

Yield 59%, oil. IR (film): 3357, 1728. ^1H NMR ($CDCl_3$) δ: 0.83 (s, 9H); 0.92 (m, 2H); 1.21 (m, 2H); 2.43 (s, 2H); 3.65 (s, 3H). MS: 185 (M^+), 128 (100%).

5.1.3. Methyl 1-(2-buten-1-ylamino)cyclopropane-1-carboxylate ***2b***

Yield 72%, oil. IR (film): 3338, 1728. ^1H NMR ($CDCl_3$) δ: 0.99 (m, 2H); 1.27 (m, 2H); 1.65 (m, 3H); 3.23 (m, 2H), 3.68 (s, 3H); 5.54 (m, 2H). MS: 169 (M^+), 110 (100%).

5.1.4. Methyl 1-benzylaminocyclopropane-1-carboxylate ***2c***

Yield 64%, oil. IR (film): 3338, 1724. ^1H NMR ($CDCl_3$) δ: 1.06 (m, 2H); 1.30 (m, 2H); 3.72 (s, 3H), 3.88 (s, 2H); 7.27–7.34 (m, 5H). MS: 205 (M^+), 91 (100%).

5.1.5. Methyl 1-phenylaminocyclopropane-1-carboxylate ***2d***

151 mg (1 mmol) of **1** were suspended in 3 ml of dry CH_2Cl_2. Et_3N (1 mmol) was added carefully, then $BiPh_3(OAc)_2$ [22] (630 mg, 1.13 mmol) and metallic copper powder (4 mg). The resulting white suspension, which shortly after turned into a more fluid blue–green suspension, was filtered and purified by flash chromatography (eluant: cyclohexane/EtOAc 98:2, then 80:20) to yield **2d** as a pale-yellow oil. Yield 94%. IR (film): 3388, 1724. ^1H NMR ($CDCl_3$) δ: 1.21 (m, 2H); 1.61 (m, 2H); 3.66 (s, 3H); 6.68–7.27 (m, 5H). MS: 205 (M^+), 130 (100%). *Anal.* $C_{11}H_{13}NO_2$ (C, H, N).

5.1.6. General procedure for the synthesis of N-substituted-aminocyclopropane-1-carboxylic acids ***3a–d***

1 mmol of methyl esters **2a–d** was solubilized in 5 ml of MeOH, then 1 ml of a 3N KOH solution was added and stirred at room temperature. The solvent was evaporated to give a residue which was solubilized in water and washed twice with EtOAc. The product was purified using a cation exchange resin column (Dowex-50-H^+); after washing with water, the product was eluted with 2N aqueous ammonia; the eluate was collected and concentrated in vacuo to afford the amino acid, which was further purified by crystallization (**3a–c**) or flash chromatography (**3d**) (EtOAc/cyclohexane 9:1) followed by washing the product twice with 0.6 ml of a mixture of Et_2O and hexane (1:9).

5.1.7. 1-Pivaloylaminocyclopropane-1-carboxylic acid ***3a***

Yield 72%, m.p. 180°C subl. (MeOH/Et_2O). ^1H NMR (DMSO-d_6) δ: 0.828 (s, 9H); 0.845 (m, 2H); 1.06 (m, 2H); 2.42 (s, 2H). MS: 171 (M^+), 68 (100%). *Anal.* $C_9H_{17}NO_2$ (C, H, N).

5.1.8. 1-(2-Buten-1-ylamino)cyclopropane-1-carboxylic acid ***3b***

Yield 44%, m.p. 210°C subl. (MeOH). ^1H NMR (DMSO-d_6/D_2O) δ: 0.92 (m, 2H); 1.04 (m, 2H); 1.64 (d, J=6.24 Hz, 3H); 3.23 (m, 2H), 3.48 (d, J=6 Hz, 2H); 5.45 (m, 1H); 5.75 (m, 1H). MS: 156 ($M+H^+$), 55 (100%). *Anal.* $C_8H_{13}NO_2 \cdot 0.25\ H_2O$ (C, H, N).

5.1.9. 1-Benzylaminocyclopropane-1-carboxylic acid ***3c***

Yield 78%, m.p. 219°C (decomp.) (H_2O). ^1H NMR (DMSO-d_6) δ: 0.89 (m, 2H); 1.1 (m, 2H); 3.83 (s, 2H); 7.27–7.3 (m, 5H). MS: 191 (M^+), 91 (100%). *Anal.* $C_{11}H_{13}NO_2$ (C, H, N).

5.1.10. 1-Phenylaminocyclopropane-1-carboxylic acid ***3d***

Yield 72%, m.p. 73–79°C. ^1H NMR ($CDCl_3$) δ: 1.24 (m, 2H); 1.68 (m, 2H); 7.27–6.69 (m, 5H). MS: 177 (M^+), 130 (100%). *Anal.* $C_{10}H_{11}NO_2$ (C, H, N).

5.1.11. 1-Methylaminocyclopropane-1-carboxylic acid ***3e***

1 mmol of **5** was dissolved in 15 ml of absolute EtOH. 50 mg of 5% Pd/C were added and the mixture was hydrogenated at 1.5 atm for 90 min before being acidified with 2N HCl, filtered on Celite®, concentrated, washed with EtOAc, and evaporated to give an oil which was dissolved in MeOH (6 ml), to which 1.5 ml of 3M aqueous KOH were carefully added. The mixture was stirred for 20 h at room temperature, then evaporated; water was then added and the mixture was washed with EtOAc; the pH of the water phase was adjusted to 6 with 2N HCl. Evaporation of water afforded crude **3e**, which was purified using a cation exchange resin column (Dowex-50-H^+). After washing with water, the product was eluted with 2N aqueous ammonia and the eluate was collected and concentrated in vacuo to afford the amino acid, which was further purified by crystallization (MeOH). Yield 58%, decomposition was observed before melting. IR (nujol): 1603. ^1H NMR (DMSO-d_6/D_2O) δ: 1.02 (m, 2H); 1.12 (m, 2H); 2.55 (s, 2H). MS: 115 (M^+, 100%). *Anal.* $C_5H_9NO_2$ (C, H, N).

5.1.12. Methyl 1-(benzyloxycarbonylamino)cyclopropane-1-carboxylic acid ***4***

1 mmol of **1** was carefully added to a solution of $NaHCO_3$ (2.9 mmol) in water (5 ml); after the addition of benzyl chloroformate (1.2 mmol) the mixture was stirred overnight, then extracted with EtOAc. The organic phase was washed with a solution of citric acid in water (5%), dried and evaporated to give **4** as a crude oil, which was purified by flash chromatography (EtOAc/cyclohexane 6:4). Yield 94%, m.p. 98–100°C (Et_2O/hexane). IR (nujol): 3286, 1716,

1682. ^{1}H NMR ($CDCl_3$) δ: 1.15 (m, 2H); 1.45 (m, 2H); 3.6 (s, 3H); 5.15 (s, 2H); 5.5 (br s, 1H); 7.25 (s, 5H). MS: 249 (M^+), 91 (100%). *Anal.* $C_{13}H_{15}NO_4$ (C, H, N).

5.1.13. Methyl 1-(N-benzyloxycarbonyl-N-methyl)-aminocyclopropane-1-carboxylic acid **5**

1 mmol of **4** was dissolved in dry DMF (1.5 ml). Ag_2O (6.5 mmol) and CH_3I (32 mmol) were added and the mixture heated at 50–60°C for 3–4 days. The mixture was then cooled and filtered, and the solid washed with acetone; the filtrate was evaporated to give an oil which, after flash chromatography (cyclohexane/EtOAc 6:4), gave 54 mg of pure (TLC) **5** together with 300 mg of unreacted **4**; this sample was again subjected to the treatment just described to give a further 86 mg of **5** and a practically quantitative recovery of unreacted **4**. Overall yield 38%, oil. ^{1}H NMR ($CDCl_3$) δ: 1.25 (s, 2H); 1.62 (s, 2H); 2.97 (s, 3H); 3.66 (m, 3H); 5.17 (s, 2H); 7.34 (d, 5H). MS: 263 (M^+), 91 (100%).

5.1.14. t-Butyl (Z)-1-(N-Boc)-amino-2-[(2-hydroxyethylthio)methyl]-cyclopropane-1-carboxylate ***8a***

t-Butyl (*Z*)-1-(*N-Boc*)-amino-2-(methanesulfonylmethyl)cyclopropane-1-carboxylate **7** (220 mg, 0.55 mmol) was dissolved in benzene (2.5 ml), before the addition of 2-mercaptoethanol (0.55 mmol) and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (0.55 mmol), after which the mixture was stirred at room temperature for 2 h, then poured into EtOAc/water. After extracting the aqueous phase with EtOAc, the collected organic phases were washed with water, dried and evaporated to give a crude material which was purified by flash chromatography (cyclohexane/EtOAc 7:3) to give **8a** as a yellow oil which, on standing, yielded a waxy solid. Yield 68%. IR (nujol): 3520, 3342, 1685. ^{1}H NMR ($CDCl_3$) δ: 0.94 (m, 1H); 1.45 (s, 18H); 1.72 (m, 1H); 1.90 (m, 1H); 2.68 (br d, 2H); 2.74 (t, 2H); 3.77 (t, 2H); 5.23 (br s, 1H). MS: 291 (M–C_4H_8), 57 (100%).

5.1.15. t-Butyl (Z)-1-(N-Boc)-amino-2-[(benzothiazol-2-yl)oxymethyl]-cyclopropane-1-carboxylate ***8b***

A solution of 2-hydroxybenzothiazole (0.58 mmol) and KOH (1 mmol) in DMSO (0.5 ml) was stirred at room temperature for 30 min; t-butyl (*Z*)-1-(*N-Boc*)-amino-2-(methanesulfonylmethyl)cyclopropane-1-carboxylate **7** (0.68 mmol) was added, and the mixture stirred at room temperature for 4 h and at 60–70°C for 3 h, then cooled before the addition of water and extraction with EtOAc. Subsequently, the organic phase was dried and evaporated to give a residue which was purified by flash chromatography (cyclohexane/EtOAc 7:3) to give pure **8b** as a white solid. Yield 33%, m.p. 205°C (decomp.). IR (nujol): 3336, 1716, 1690. ^{1}H NMR ($CDCl_3$) δ: 1.18 (m, 1H); 1.43 (s, 9H); 1.50 (s, 9H); 1.64 (m, 1H); 2.60 (m, 1H); 4.09 (m, 2H); 5.95 (br s, 1H); 7.20–7.48 (m, 4H). MS: 364 (M^+), 57 (100%). *Anal.* $C_{21}H_{28}N_2O_5S \cdot 0.5H_2O$ (C, H, N).

5.1.16. t-Butyl (Z)-1-(N-Boc)-amino-2-[(benzothiazol-2-yl)thiomethyl]-cyclopropane-1-carboxylate ***8c***

t-Butyl (*Z*)-1-(*N-Boc*)-amino-2-(methanesulfonylmethyl)cyclopropane-1-carboxylate **7** (360 mg, 0.9 mmol) was dissolved in toluene (2.5 ml). 2-Mercaptobenzothiazole (0.9 mmol) and DBU (0.9 mmol) were added. The mixture was stirred at room temperature for 2 h, then diluted with EtOAc and washed with water; the organic phase was dried and evaporated to give a residue which was purified by flash chromatography (cyclohexane/EtOAc 85:15) to give pure **8c** as a colourless oil. Yield 37%. ^{1}H NMR ($CDCl_3$) δ: 1.38 (s, 9H); 1.54 (s, 9H); 0.82–2.01 (m, 3H); 3.35 (m, 1H); 3.59 (m, 1H); 6.98 (br s, 1H); 7.32–8.02 (m, 4H). MS: 436 (M^+), 193 (100%).

5.1.17. t-Butyl (Z)-1-(N-Boc)-amino-2-(N-carbamoyl-aminomethyl)-cyclopropane-1-carboxylate ***11d***

0.06 ml (0.525 mmol) of phenyl isocyanate was added to a solution of 100 mg (0.35 mmol) of t-butyl (*Z*)-1-(*N-Boc*)-amino-2-(aminomethyl)cyclopropane-1-carboxylate in 3 ml of dry CH_2Cl_2. The mixture was stirred overnight at room temperature; the solvent was then evaporated and the residue was purified by flash chromatography (EtOAc/cyclohexane 1:1). Yield 60%, m.p. 135°C (Et_2O/cyclohexane). ^{1}H NMR ($CDCl_3$) δ: 0.79 (m, 1H), 1.20 (m, 1H), 1.49 (s, 9H), 1.46 (s, 9H), 2.02 (m, 1H), 2.61 (m, 1H), 4.07 (m, 1H), 5.32 (br s, 1H), 5.91 (br d, 1H), 6.37 (br s, 1H), 7.25–7.38 (m, 5H). MS: 349 (M–C_4H_8), 113 (100%). *Anal.* $C_{21}H_{31}N_3O_5 \cdot 0.33\ C_6H_{12}$ (C, H, N).

5.1.18. t-Butyl (Z)-1-(N-Boc)-amino-2-(N-phenylacetyl-aminomethyl)-cyclopropane-1-carboxylate ***11e***

0.156 ml of Et_3N and 0.124 ml (0.94 mmol) of phenylacetylchloride were added to a solution of 180 mg (0.63 mmol) of t-butyl (*Z*)-1-(*N-Boc*)-amino-2-(aminomethyl)cyclopropane-1-carboxylate in 5 ml of dry $CHCl_3$. The mixture was stirred for 2 h at room temperature, then washed once with water and dried on Na_2SO_4. The solvent was evaporated and the residue was purified by flash chromatography (EtOAc/cyclohexane 1:1). Yield 61%, m.p. 110°C (Et_2O/light petroleum). ^{1}H NMR ($CDCl_3$) δ: 0.74 (m, 1H), 1.20 (m, 1H), 1.43 (s, 9H), 1.44 (s, 9H), 1.84 (m, 1H), 2.56 (m, 1H), 3.54 (s, 2H), 4.05 (m, 1H), 5.34 (br s, 1H), 6.74 (br s, 1H), 7.25-7.38 (m, 5H). MS: 348 (M–C_4H_8), 91 (100%). *Anal.* $C_{22}H_{32}N_2O_5$ (C, H, N).

5.1.19. General procedure for the deprotection of t-butyl (Z)-(N-Boc)-carboxylate derivatives ***9a-e***

1 mmol of the appropriate derivative was dissolved in 3.5 ml of CH_2Cl_2 at 0°C and 1 ml of CF_3COOH was added. The mixture was stirred at room temperature until the starting material had disappeared (TLC analysis); the solvent was then evaporated under reduced pressure and the crude residue was purified using a cation exchange resin column (Dowex-50-H^+). After washing with water, the product was eluted with 2N aqueous ammonia and the eluate was collected and

concentrated in vacuo to afford the amino acid, which was further purified by crystallization.

5.1.20. (Z)-1-Amino-2-[(2-hydroxyethylthio)methyl]-cyclopropane-1-carboxylate ***9a***

Yield 40%, m.p. 180–190°C (decomp.) (EtOH). ^{1}H NMR (DMSO-d_6) δ: 0.64 (q, 1H); 1.24 (q, 1H); 1.54 (m, 1H), 2.63 (m, 4H); 3.52 (t, 2H). MS: 114 (M–C_2H_5SO, 100%). *Anal.* $C_7H_{13}NO_3S$ (C, H, N).

5.1.21. (Z)-1-Amino-2-(benzoxazol-2-yl)-cyclopropane-1-carboxylate ***9b***

Yield 19%, m.p. 170°C (MeOH). ^{1}H NMR (DMSO-d_6) δ: 0.84 (q, 1H); 1.20 (q, 1H); 1.70 (m, 1H), 4.12 (q, 2H); 7.16–7.65 (m, 4H). MS: 264 (M^+), 113 (100%). *Anal.* $C_{12}H_{12}N_2O_3S \cdot 0.5H_2O$ (C, H, N).

5.1.22. (Z)-1-Amino-2-(benzothiazol-2-yl)-cyclopropane-1-carboxylate ***9c***

Yield 30%, m.p. 232–6°C (decomp.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 0.82 (q, 1H); 1.33 (q, 1H); 1.84 (m, 1H), 3.53 (d, 2H); 6.37 (br s, 2H); 7.31–8.02 (m, 4H). MS: 167 (100%). *Anal.* $C_{12}H_{12}N_2O_2S_2 \cdot H_2O$ (C, H, N).

5.1.23. (Z)-1-Amino-2-(N-carbamoylaminomethyl)-cyclopropane-1-carboxylate ***9d***

Yield 30%, m.p. 160–3°C (decomp.) (MeOH/EtOAc). ^{1}H NMR (DMSO-d_6) δ: 0.83 (m, 1H), 1.30 (m, 1H), 1.54 (m, 1H), 2.81 (m, 1H), 3.72 (m, 1H), 6.80–7.39 (m, 5H), 9.21 (br s, 1H). MS: 115, 93 (100%). *Anal.* $C_{12}H_{15}N_3O_3 \cdot 0.3H_2O \cdot 1EtOAc$ (C, H, N).

5.1.24. (Z)-1-Amino-2-(N-phenylacetylaminomethyl)-cyclopropane-1-carboxylate ***9e***

Yield 36%, m.p. 190–1°C (decomp.) (EtOH). ^{1}H NMR (DMSO-d_6/D_2O) δ: 0.81 (m, 1H), 1.18 (m, 1H), 1.55 (m, 1H), 2.93 (m, 1H), 3.32 (m, 1H), 3.43 (s, 2H), 7.19–7.25 (m, 5H), 8.56 (br s, 1H). MS: 249 (M+H), 113 (100%). *Anal.* $C_{13}H_{16}N_2O_3 \cdot 0.1\ H_2O$ (C, H, N).

5.2. Pharmacology

5.2.1. Radioligand binding assays

5.2.1.1. Membrane preparation and in vitro binding assays

Rats were sacrificed under light anaesthesia, the brain quickly removed and the cerebral cortex was dissected out. The inhibition of binding of the ^{3}H-radioligand to each receptor (final concentration), brain areas, non-specific agents (final concentration) and methods used in the present investigation are reported in Table 4. All assays were validated using appropriate reference standards.

5.2.1.2. Statistical analysis

IC_{50} values were calculated using the computer program LIGAND. The displacement curves were obtained by using at least eight concentrations of displacers.

5.2.2. [^{3}H]-MK-801 assays

Forebrains from Sprague-Dawley rats (150–200 g) were homogenized in 10 ml/g of 0.32 M sucrose, 1 mM Hepes-K^+, and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4 (buffer A). The homogenates were diluted 1:5 in the same buffer and centrifuged for 10 min at 1000 g, after which the supernatant was centrifuged for 20 min at 23 000 g. The resulting pellet was resuspended in 50 ml/g of 5 mM Hepes-K^+ and 1 mM EDTA buffer, pH 7.4 (buffer B) and centrifuged for 20 min at 23 000 g. The pellet was then washed four times by resuspension and centrifugation (20 min at 23 000 g): the first two times with buffer B, and the following two with a buffer containing only 5 mM Hepes-K^+, pH 7.4 (buffer C). The membranes were then stored at −20°C in 5 ml/g (original wet weight) of buffer C. On the day of the experiment, the membranes were thawed and washed twice by centrifugation and resuspension with 50 ml/g of buffer C. [^{3}H]-MK-801 binding was assayed in a 0.5 ml final volume of buffer C. Aliquots of membranes (100–200 μg protein/assay) were pre-incubated at 25°C for 20 min with glutamate and/or glycine, ACPC or the new compounds. After pre-incubation, 4 nM [^{3}H]-MK-801 was added and incubation continued for 40 min. Incubation was terminated by vacuum filtration through Whatman GF/C filters presoaked in 0.05% polyethylenimine, followed by one wash with cold buffer C (3 ml/assay). Non-specific binding was determined in the

Table 4
In vitro receptor binding methods used in testing compounds

Receptor type	Radioligand (nM)	Buffer (pH)	Incubation conditions	Non-specific agent (μM)	Ref.
NMDA	^{3}H-CGP39653 (5.0)	Tris–HCl [a] (7.6)	25 min, 25°C	l-glutamic acid (100)	[23]
Glycine (strychnine- insensitive)	^{3}H-MDL105519 (2)	Tris–HCl (7.4)	30 min, 25°C	glycine (1000)	[24]
Quisqualate	^{3}H-AMPA (4.0)	Tris–HCl [b] (7.4)	60 min, 2°C	l-glutamic acid (1000)	[25]
Kainate	^{3}H-KA (4.0)	Tris–HCl (7.1)	60 min, 2°C	l-glutamic acid (600)	[26]

[a] 2.5 mM $CaCl_2$.
[b] Containing 100 mM KSCN.

presence of 20 μM MK-801, 20 μM 5,7-dichlorokynurenic acid (DCKA) and 50 μM D-2-amino-5-phosphonopentanoic acid (AP5).

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