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Synthesis and pharmacology of 3-hydroxy-Δ^2-isoxazoline-cyclopentane analogues of glutamic acid

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Abstract

The synthesis and pharmacology of two potential glutamic acid receptor ligands are described. Preparation of the bicyclic 3-hydroxy-Δ^2-isoxazoline-cyclopentane derivatives (±)-**7** and (±)-**8** was accomplished via 1,3-dipolar cycloaddition of bromonitrile oxide to suitably protected 1-amino-cyclopent-3-enecarboxylic acids. Their structure was established using a combination of ^{1}H NMR spectroscopy and molecular mechanics calculations carried out on the intermediate cycloadducts (±)-**11** and (±)-**12**. Amino acid derivatives (±)-**7** and (±)-**8** were assayed at ionotropic and metabotropic glutamic acid receptor subtypes and their activity compared with that of *trans*-ACPD and *cis*-ACPD. The results show that the replacement of the ω-carboxylic group of the model compounds with the 3-hydroxy-Δ^2-isoxazoline moiety abolishes or reduces drastically the activity at the metabotropic glutamate receptors. Conversely, on passing from *cis*-ACPD to derivative (±)-**8**, the agonist activity at NMDA receptors is almost unaffected.

Keywords: 1,3-Dipolar cycloaddition; Isoxazoline–cyclopentane amino acids; Ionotropic glutamic acid receptors; Metabotropic glutamic acid receptors; NMDA agonist

1. Introduction

The involvement of glutamatergic synapses in the processes underlying memory and learning is now well established [1]. As a consequence, the role of excitatory amino acid (EAA) receptors, i.e. glutamate receptors, in different neurological and psychiatric disorders has been the subject of strong research investigations [2,3]. Numerous efforts have been devoted to the design of EAA receptor-specific ligands or modulators as pharmacological tools and/or potential therapeutic agents [4]. (*S*)-Glutamic acid, which is the main EAA in the central nervous system, acts through disparate receptors which can be categorized into two distinct families: ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) [1,4–6]. The iGluRs, which mediate fast excitatory responses via ligand gated ion channels, have been classified pharmacologically into three subtypes: *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) and kainic acid (KA) receptors [4–6]. On the other hand, mGluRs, which play a modulatory role in synaptic transmission, are coupled to G-proteins and transduce signals through the production of second messengers [1]. Molecular cloning of the mGluRs has revealed eight different receptor proteins (mGluR1-8) that have been classified into three groups (I–III) based on primary amino acid sequence homology, agonist pharmacology and signal transduction mechanisms [7]. The receptors (mGluR1,5) belonging to the group I subfamily are coupled to phospholipase C and stimulate the release of diacyl glycerol (DAG) and inositol tris-phosphate (IP_3), whereas group II (mGluR2,3) and group III (mGluR4,6,7,8) receptors are negatively coupled to adenylyl cyclase activity.

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In order to better characterize the roles played by mGluRs in physiological processes, numerous efforts have been devoted to identify novel, high affinity ligands that are family and subtype specific [8]. The first agonist highly selective for mGlu versus iGlu receptors was the conformationally constrained glutamate analogue *trans*-ACPD (**1a**) [9] (Fig. 1). Later on, it was established that the mGluRs agonist activity of **1a** resides exclusively in its (1*S*,3*R*)-enantiomer (**1b**) [10]. For this reason, stereoisomer **1b** is widely employed in the study of these receptors both in vitro and in vivo. However, this ligand is rather nonselective, since it displays a similar potency across all mGluR subtypes, except for mGluR7, where no agonist activity was noted. Interestingly, (1*S*,3*S*)-ACPD (**2**, Fig. 1), the *cis* stereoisomer of **1b**, showed a significant selectivity for mGluR2,3 (group II) compared to its effects at group I and III receptor subtypes [11]. Unfortunately, derivative **2** possesses additional pharmacological activity, including agonist activity at NMDA receptors [12,13]. Taking advantage of the results obtained with the ACPD stereoisomers, a number of analogues and conformationally locked derivatives were prepared and tested with the goal to uncover the molecular determinants needed for a selective interaction with the mGluRs when compared to iGluRs and, hopefully, among mGluR subtypes. On this line, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylic acid [(2*R*,4*R*)-APDC, **3**] [14], the bicyclic derivative (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740, **4**) [15] and its heteroatom analogues (**5** and **6**) [16] (Fig. 1) have been prepared and their pharmacological profile defined. All the ligands proved to be agonists highly potent and selective for mGluR2,3 (group II), supporting the hypothesis that glutamate interacts with these receptors in a fully extended conformation.

As an extension of previous investigations in this field [17] we designed the 3-hydroxy-Δ^2-isoxazoline-cyclopentane derivatives (±)-**7** and (±)-**8** (Fig. 1) as structural analogues of ACPD where the 3-hydroxyisoxazoline moiety could mimic the 3-carboxylic group of ACPD. It is noteworthy that such a replacement successfully provided ligands with a GABA-ergic activity, i.e. GABA versus dihydromuscimol [18]. This paper deals with the synthesis of the two stereoisomers (±)-**7** and (±)-**8** and the evaluation of their activity and selectivity at both iGlu and mGlu receptors.

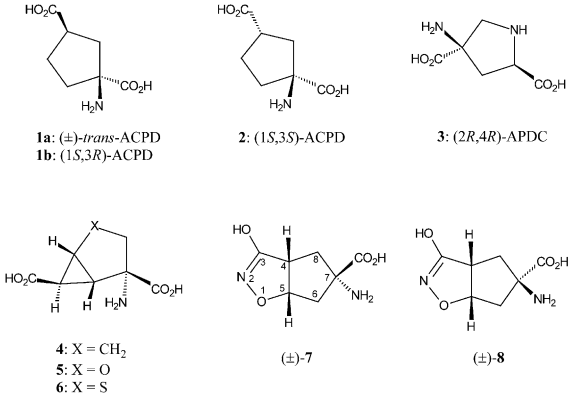


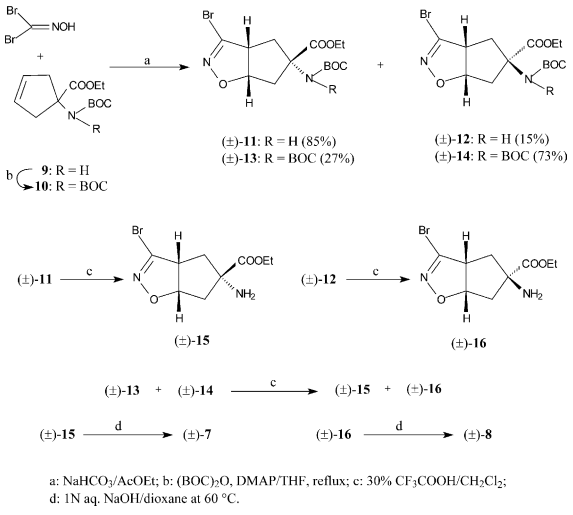
Fig. 1. Chemical structure of model and target compounds.

2. Chemistry

The synthesis of target compounds (±)-**7** and (±)-**8** was accomplished according to the reaction sequence illustrated in Scheme 1. Bromonitrile oxide, generated in situ by treatment of dibromoformaldoxime with a base, was reacted with suitably protected 1-amino-cyclopent-3-enecarboxylates **9** and **10** (Scheme 1). Dipolarophile **9** was prepared according to the procedure reported in the literature [19], whereas **10** was synthesized by refluxing a THF solution of **9** with excess di-*tert*-butyl dicarbonate in the presence of 4-(dimethylamino)pyridine.

The pericyclic reaction yielded a mixture of stereoisomers (±)-**11** and (±)-**12** in a 85:15 ratio (Scheme 1). It is worth pointing out that the 1,3-dipolar cycloaddition gave cycloadduct (±)-**11** as the major stereoisomer. As shown in Fig. 2, the hydrogen bonding between the carbamate group and the oxygen of the nitrile oxide stabilizes the transition state leading to (±)-**11**. Such an effect has already been observed in 1,3-dipolar cycloadditions of nitrile oxides with alkenes bearing hydroxy, amido and carbamate groups [20–23]. This hypothesis is confirmed by the outcome of the cycloaddition of bromonitrile oxide to dipolarophile **10**. In this case the lack of hydrogen bond in the related transition state reversed the ratio between the stereoisomers [(±)-**13** and (±)-**14** ratio 27:73].

The cycloadducts (±)-**11** and (±)-**12** were separated by silica gel column chromatography whereas the



Scheme 1.

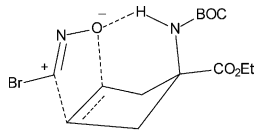


Fig. 2. Representation of the transition state leading to (±)-**11**.

mixture of (±)-**13** and (±)-**14** turned out to be inseparable. Treatment of the latter mixture with excess trifluoroacetic acid afforded the corresponding primary amines (±)-**15** and (±)-**16**, which were separated by a silica gel column chromatography. The final amino acids (±)-**7** and (±)-**8** were obtained by reacting intermediate amino esters [(±)-**15** and (±)-**16**, respectively] with a 1 N sodium hydroxide/dioxane mixture at 60 °C followed by a cationic exchange column chromatography.

The relative stereochemistry of the synthesized compounds was preliminarily assigned on the basis of the ratio among cycloadducts (±)-**11** and (±)-**12**, taking into account the stabilized transition state described above. The relative configuration was subsequently confirmed by the use of a combination of molecular mechanics calculations and ^{1}H NMR spectroscopy, carried out on cycloadducts (±)-**11** and (±)-**12**. The preferred conformations of the two diastereoisomers were determined by full geometry optimizations carried out with the MM+ force field implemented in the HyperChem program [24]. All the degrees of conformational freedom were examined, with particular attention to the rotation around the acyclic single bonds and to the pseudorotation of the cyclopentane ring. The conformational freedom of the two substituents at C-7 is relatively high, whereas the pseudorotation of the cyclopentane ring is strongly reduced by the presence of the isoxazoline ring. As a matter of fact, only the two envelope conformations ^{7}E and E$_7$ are allowed. Thus, the conformations of each stereoisomer can be grouped in two families, both characterized by a similar geometry of their bicyclic moiety. In Fig. 3 is depicted a representative conformation for each family. The percentage contribution of each conformation to the overall population, determined through the application of the Boltzmann equation on the values of the relative energy, showed that the conformational profile of the two families is significantly different. While compound (±)-**11** presents a large preference for the ^{7}E geometry, the ^{7}E and E$_7$ envelope conformations are equally populated in their stereoisomer (±)-**12**.

The analysis of the ^{1}H NMR spectra of (±)-**11** and (±)-**12**, recorded in deuteriated benzene, allowed the measurement of the coupling constants for all vicinal hydrogen atoms (Table 1, experimental values). For each conformer located in the modeling study, the vicinal coupling constants were also calculated with the electronegativity-modified Karplus relationship [25] and weight averaged on the basis of its population percentage (Table 1, theoretical values). The close agreement of the experimental with the calculated values confirmed the assignment to be (4*R*,5*S*,7*S*/4*S*,5*R*,7*R*) and (4*R*,5*S*,7*R*/4*S*,5*R*,7*S*) for compounds (±)-**11** and (±)-**12**, respectively.

3. Results and discussion

The two stereomeric amino acids (±)-**7** and (±)-**8** were assayed in vitro by means of receptor binding techniques, second messenger assays, and the rat cortical wedge preparation. The receptor affinity of the derivatives for NMDA, AMPA, and KA receptors was determined by using the radioligands [^{3}H]CPP, [^{3}H]AMPA, and [^{3}H]KA, respectively [26–28]. The activity of the same compounds was also evaluated at mGluR1/mGluR5, mGluR2, and mGluR4a, expressed in CHO cells, as representatives for group I, II, and III metabotropic receptors, respectively [29]. Compound (±)-**7**, tested up to 0.1 mM in binding assays at iGluRs and up to 1.0 mM in functional assays at mGluRs as well as in electrophysiological tests at iGluRs, showed no significant affinity or activity neither as an agonist nor as an antagonist. On the contrary, compound (±)-**8** possessed an affinity for NMDA receptors, a result confirmed in the electrophysiological test where it showed an activity roughly three times weaker than that of NMDA itself (Table 2). Furthermore, compound (±)-**8** behaved as a weak agonist at mGluR2; when tested at 1 mM it reached 25–50% of the maximal activity observed with a full agonist.

A comparison of the results reported for racemic *trans*-ACPD (**1a**) [30] as well for its eutomer [(1*S*,3*R*)-ACPD, **1b**] [31] with those collected for the structurally related (±)-**7** (Table 3) indicates that the replacement of the ω-carboxylic group with the 3-hydroxy-Δ^2-isoxazoline moiety is non-productive since it abolishes the activity at all mGlu receptor subtypes. Conversely, the same analysis carried out on homochiral *cis*-ACPD [(1*S*,3*S*)-ACPD, **2**] [30] versus (±)-**8** evidences that, while the activity at mGluR2 is significantly reduced, i.e. 13 μM versus 25–50% at 1 mM (Table 3), the activity at NMDA remains almost unaffected, i.e. **2** and (±)-**8** are 2.6 [13] and 3.0 times less potent than NMDA, respectively. It has to be pointed out that the comparison has been made between the eutomer of *cis*-ACPD [(1*S*,3*S*)-ACPD, **2**] and racemic (±)-**8**. We previously reported that the 3-hydroxy-2-isoxazoline moiety possesses acidity comparable to that of the ω-carboxylic group of potent GluR ligands [32]. Thus, the lack of activity at mGlu receptors of (±)-**7** and (±)-**8** could not be ascribed to such a parameter but, reasonably, to the reduced conformational mobility of both the cyclopentane ring and the ω-acidic function.

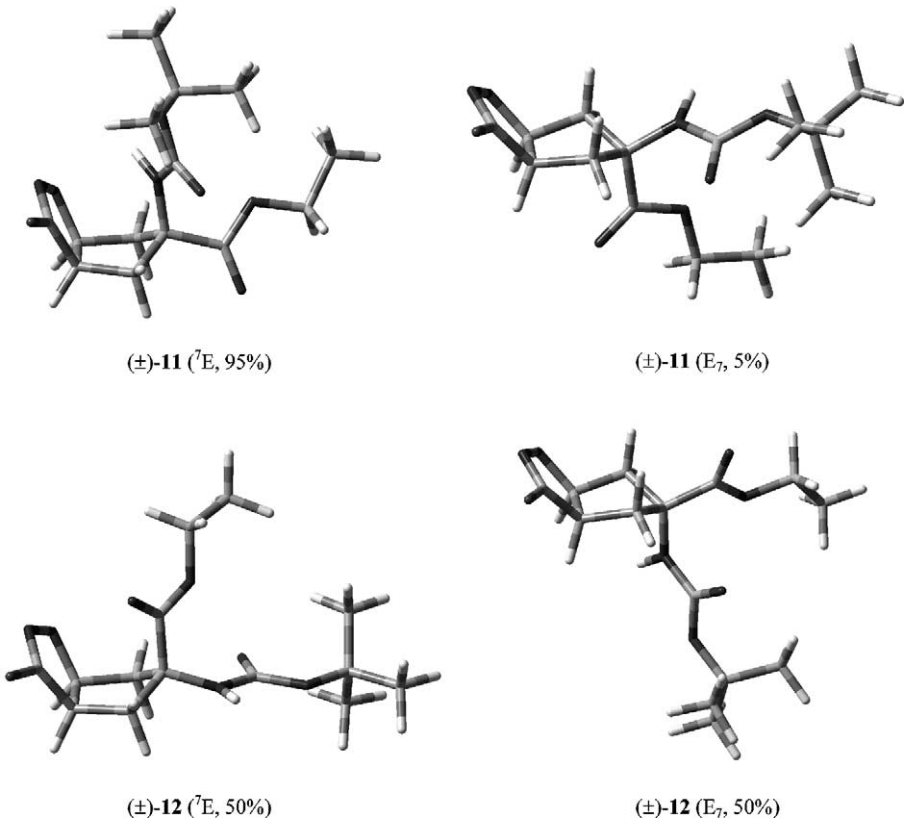


Fig. 3. 3D-plot of representative conformations (^{7}E and E$_7$) of diastereoisomers (±)-**11** and (±)-**12**. The calculated population percentages for each conformation are given in parentheses.

Table 1
Experimental ^{1}H NMR vicinal coupling constants of compounds (±)-**11** and (±)-**12** in comparison with the calculated values (Hz)

	$J_{5,6a}$	$J_{5,6b}$	$J_{4,5}$	$J_{4,8a}$	$J_{4,8b}$
(±)-**11** (experimental)	2.0	6.2	9.0	9.0	2.0
(±)-**11** (theoretical)	1.4	6.7	9.1	8.2	1.5
(±)-**12** (experimental)	5.0	7.0	9.8	9.8	5.2
(±)-**12** (theoretical)	4.5	7.5	9.0	9.0	5.4

Table 2
Receptor binding and in vitro electrophysiology (values ± SEM, $n = 3–4$)

Comp.	Receptor binding			Electropharmacology
	[^{3}H]AMPA	[^{3}H]KAIN IC$_{50}$ (μM)	[^{3}H]CPP	EC$_{50}$ (μM)
(±)-**7**	> 100	> 100	> 100	> 1000
(±)-**8**	> 100	> 100	28[11–73]	34.0[31.9–36.3]
AMPA [a]	0.040 ± 0.014	> 100	> 100	3.5 ± 0.2 [b]
KA [b]	4.0 ± 1.2	0.007 ± 0.002	> 100	25 ± 3
NMDA	> 100	> 100		11 ± 3 [c]

[a] AMPA (5 μM) is fully antagonized by 5 μM NBQX.
[b] KA (10 μM) is not antagonized by 5 μM NBQX (< 20% reduction) but fully antagonized by 20 μM NBQX (> 80% reduction).
[c] Antagonized by 10 μM CPP.

In summary, the results of the present investigation show that the structural modification made on model compounds *trans*-ACPD and *cis*-ACPD is non-productive as far as the activity at mGlu receptors is concerned, whereas the activity profile of (±)-**8** at NMDA receptors remains unaffected.

4. Experimental

4.1. Material and methods

Dibromoformaldoxime [33] and ethyl *N*-(*tert*-butoxycarbonyl)-1-amino-cyclopent-3-enecarboxylate **9** [19] were prepared according to literature procedures. ^{1}H NMR spectra were recorded with a Bruker AC-E 200 (200 MHz) spectrometer in $CDCl_3$, D_2O or C_6D_6 solution at 20 °C: the signal assignments are the result of a combination of 1D and 2D COSY. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in hertz. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution or with ninhydrin. Melting points were determined on a Büchi apparatus and are

Table 3
Potency and subtype selectivity at cloned mGlu receptors expressed in CHO cells

Comp.	EC_{50} (μM)			
	mGluR1	mGluR5	mGluR2	mGluR4
Glu [a]	4.9	3.1	0.29	9.8
1a [a]	15	23	2	~ 800
1b [b]	42	15	5	60[c]
2 [a]	> 300	> 300	13	50
3 [b]	100	200	0.3	110 [c]
(±)-**7**	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$
(±)-**8**	$> 10^3$	$> 10^3$	25–50% [d]	$> 10^3$

[a] Ref. [30].
[b] Ref. [31].
[c] Tested at mGluR6.
[d] Activity of 1 mM (±)-**8** relative to a maximal response.

uncorrected. Microanalyses of new compounds agreed with theoretical value ±0.3%.

4.1.1. Synthesis of ethyl N,N-(di-tert-butoxycarbonyl)-1-amino-cyclopent-3-enecarboxylate (*10*)

To a stirred solution of **9** [19] (1.25 g, 4.9 mmol) in THF (20 ml) was sequentially added 4-(dimethylamino)pyridine (60 mg, 0.49 mmol) and a THF solution (10 ml) of di-*tert*-butyl dicarbonate (1.6 g, 7.35 mmol). The mixture was refluxed for 6 days and di-*tert*-butyl dicarbonate (0.5 equiv.) was added every day. The disappearance of the starting material was monitored by TLC (petroleum ether/ethyl acetate 9:1). The solvent was evaporated at reduced pressure and the residue was column chromatographed on silica gel (petroleum ether/ethyl acetate 95:5) to give 1.48 g (yield: 85%) of derivative **10** as yellow oil.

Alkene **10**: R_F 0.49 (cyclohexane/ethyl acetate 9:1); ^{1}H NMR ($CDCl_3$): 1.30 (t, 3H, $J = 7.2$); 1.50 (s, 18H); 2.85 (d, 2H, $J = 16.3$); 3.09 (d, 2H, $J = 16.3$); 4.18 (q, 2H, $J = 7.2$); 5.64 (s, 2H).

4.1.2. 1,3-Dipolar cycloaddition of bromonitrile oxide to ethyl N-(tert-butoxycarbonyl)-1-amino-cyclopent-3-enecarboxylate (*9*)

To a solution of **9** (1.8 g, 7.06 mmol) in ethyl acetate (40 ml) was added dibromoformaldoxime (2.86 g, 14.12 mmol) and $NaHCO_3$ (5 g). The mixture was vigorously stirred for 3 days; the progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate 4:1). Water was added to the reaction mixture and the organic layer was separated and dried over anhydrous sodium sulfate. The crude material, obtained after evaporation of the solvent, was chromatographed on silica gel (petroleum ether/ethyl acetate 4:1) to give 2.12 g of (±)-**11** and 0.375 g of (±)-**12**. Overall yield: 94%.

Cycloadduct (±)-**11**: colorless needles from isopropyl ether, m.p. 125–127 °C; R_F 0.35 (cyclohexane/ethyl acetate 7:3); ^{1}H NMR (C_6D_6, 60 °C): 0.95 (t, 3H, $J =$ 7.3); 1.41 (s, 9H); 1.85 (dd, 1H; $J = 6.2$ and 15.0); 1.90 (ddd, 1H; $J = 2.0$, 2.0, 15.0); 2.30 (dd, 1H; $J = 9.0$ and 14.5); 2.70 (ddd, 1H; $J = 2.0$, 2.0, 14.5); 3.09 (ddd, 1H; $J = 2.0$, 9.0 and 9.0); 3.93 (q, 2H; $J = 7.3$); 4.51 (ddd, 1H; $J = 2.0$, 6.2 and 9.0); 4.80 (bs, 1H). *Anal.* ($C_{14}H_{21}BrN_2O_5$) C, H, N.

Cycloadduct (±)-**12**: colorless needles from isopropyl ether, m.p. 108.5–109.5 °C; R_F 0.39 (cyclohexane/ethyl acetate 7:3); ^{1}H NMR (C_6D_6, 60 °C): 0.96 (t, 3H, $J =$ 7.3); 1.39 (s, 9H); 2.15 (ddd, 1H; $J = 1.0$, 7.0 and 14.0); 2.18 (ddd, 1H; $J = 1.0$, 9.8 and 14.0); 2.35 (dd, 1H; $J =$ 5.0 and 14.0); 2.55 (dd, 1H; $J = 5.2$ and 14.0); 3.28 (ddd, 1H; $J = 5.2$, 9.8 and 9.8); 3.89 (q, 2H; $J = 7.3$); 4.80 (ddd, 1H; $J = 5.0$, 7.0 and 9.8); 4.91 (bs, 1H). *Anal.* ($C_{14}H_{21}BrN_2O_5$) C, H, N.

4.1.3. 1,3-Dipolar cycloaddition of bromonitrile oxide to *10*

To a solution of **10** (1.48 g, 4.17 mmol) in ethyl acetate (40 ml) was added dibromoformaldoxime (1.69 g, 8.34 mmol) and $NaHCO_3$ (3 g). The mixture was vigorously stirred for 3 days; during this time a further equivalent of dibromoformaldoxime was added to the mixture. The progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate 9:1). Water was added to the reaction mixture and the organic layer was separated and dried over anhydrous sodium sulfate. The crude material, obtained after evaporation of the solvent, was chromatographed on silica gel (petroleum ether/ethyl acetate 9:1) to give an inseparable mixture of (±)-**13** and (±)-**14** (1.67 g, 84% yield).

4.1.4. Synthesis of amino esters (±)-*15* and (±)-*16*

The mixture of (±)-**13** and (±)-**14** (1.67 g, 3.5 mmol) was treated with a 30% dichloromethane solution of trifluoroacetic acid (8.9 ml) at 0 °C. The solution was stirred at room temperature until disappearance of the starting material (3 h). The volatiles were removed under vacuum and the residue was treated with a 10% potassium carbonate solution (20 ml) and extracted with ethyl acetate (3 × 10 ml). The pooled organic extracts were dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by a silica gel column chromatography (petroleum ether/ethyl acetate 1:1) to give 0.225 g of (±)-**15** and 0.609 g of (±)-**16** as yellowish oils in 86% overall yield.

Compound (±)-**15**: R_F 0.76 (ethyl acetate); ^{1}H NMR ($CDCl_3$) 1.28 (t, 3H, $J = 7.3$); 1.68 (bs, 2H); 2.11 (ddd, 1H, $J = 1.5$, 1.5, 13.9); 2.20 (ddd, 1H, $J = 1.5$, 1.5, 15.0); 2.33 (dd, 1H, $J = 9.2$, 13.9); 2.53 (dd, 1H, $J = 7.0$, 15.0); 3.86 (ddd, 1H, $J = 1.5$; 9.2; 9.2); 4.19 (q, 2H, $J = 7.3$); 5.28 (ddd, 1H, $J = 1.5$, 7.0, 9.2). *Anal.* ($C_9H_{13}BrN_2O_3$) C, H, N.

Compound (±)-**16**: R_F 0.38 (ethyl acetate); ^{1}H NMR ($CDCl_3$) 1.28 (t, 3H, $J = 7.3$); 1.60 (bs, 2H); 2.05 (ddd,

1H, $J = 2.2, 9.5, 13.0$); 2.23 (ddd, 1H, $J = 2.2, 7.0, 13.9$); 2.35 (dd, 1H, $J = 7.0, 13.0$); 2.37 (dd, 1H, $J = 5.9, 13.9$); 3.99 (ddd, 1H, $J = 7.0, 9.5, 9.5$); 4.17 (q, 2H, $J = 7.3$); 5.34 (ddd, 1H, $J = 5.9, 7.0, 9.5$). *Anal.* ($C_9H_{13}BrN_2O_3$) C, H, N.

The same treatment with a 30% dichloromethane solution of trifluoroacetic acid, carried out on cycloadducts (±)-**11** and (±)-**12**, gave the above-reported amino esters in comparable yields.

4.1.5. Synthesis of amino acid (±)-7

To a solution of (±)-**15** (0.55 g, 2.0 mmol) in dioxane (10 ml) was added 1N NaOH (8 ml). The reaction mixture was stirred at room temperature for 2 h then heated at 60 °C for 9 h. At the disappearance of the substrate the solvents were removed under vacuum, the residue was taken up with 2N HCl and the solution submitted to a cation exchange chromatography, using Amberlite IRA 120 plus. The acidic solution was slowly eluted onto the resin, and then the column was washed with water until the pH was neutral. The compound was then eluted off the resin with 10% aqueous pyridine, and the product-containing fractions (detected with ninhydrin on a TLC plate) were combined and concentrated under vacuum. The residue was crystallized from water/methanol, filtered, washed sequentially with methanol and ethyl ether and dried in vacuo at 50 °C to give amino acid (±)-**7** as white prisms (0.152 g, 41% yield).

Amino acid (±)-**7**: R_F 0.27 (*n*-butanol/water/acetic acid 4:2:1); decomposes in the range 230–280 °C; ^{1}H NMR (D_2O): 2.17 (dd, 1H, $J = 5.0$ and 15.0); 2.24 (dd, 1H, $J = 2.5$ and 14.0); 2.55 (dd, 1H, $J = 5.5$ and 15.0); 2.65 (dd, 1H, $J = 10.0$ and 14.0); 3.46 (m, 1H); 5.28 (m, 1H). *Anal.* ($C_7H_{10}N_2O_4 \cdot 1H_2O$) C, H, N.

The same treatment performed on amino ester (±)-**16** gave amino acid (±)-**8** in 42% yield.

Amino acid (±)-**8**: R_F 0.25 (*n*-butanol/water/acetic acid 4:2:1); decomposes in the range 210–260 °C; ^{1}H NMR (D_2O): 2.19 (dd, 1H, $J = 10.0$ and 14.5); 2.31 (dd, 1H, $J = 6.5$ and 15.0); 2.51 (dd, 1H, $J = 4.5$ and 15.0); 2.57 (dd, 1H, $J = 4.5$ and 14.5); 3.47 (m, 1H); 5.20 (m, 1H). *Anal.* ($C_7H_{10}N_2O_4$) C, H, N.

4.2. Modeling studies

Calculations on compounds (±)-**11** and (±)-**12** were performed with the HyperChem molecular modeling program [24] using the empirical MM+ force field approach. After building of the molecules, their allowed conformations were located using the conformational search module. From the values of the relative energy, the population percentage of each conformation was determined through the application of the Boltzmann equation. Finally, the vicinal coupling constants of each conformation were calculated with the electronegativity-modified Karplus relationship [25] and weight averaged on the basis of its population percentage.

4.3. Biological testing

4.3.1. Receptor binding

Affinity for NMDA, AMPA and KA receptors were determined using the ligands [^{3}H]CPP, [^{3}H]AMPA and [^{3}H]KA, respectively [26–28]. The membrane preparations used in all the receptor-binding experiments were prepared according to the method of Ranson and Stec [34].

4.3.2. In vitro electrophysiology

A rat cortical slice preparation for determination of EAA-evoked depolarizations described by Harrison and Simmonds [35] was used in a slightly modified version. Wedges (500 μM thick) of rat brain, containing cerebral cortex and corpus callosum, were placed through a grease barrier for electrical isolation with each part in contact with a DriRef-5SH (World Precision Instruments) electrode. The cortex and corpus callosum parts were constantly superfused with a Mg^{2+} free (and Ca^{2+} free for the corpus callosum) oxygenated Krebs buffer at room temperature. The test compounds were added to the cortex superfusion medium and the potential difference between the electrodes recorded on a chart recorder. Applications of agonists were made for 90 s at each concentration tested, typically at 15 min intervals. The sensitivity of agonist effects to CPP (10 μM) or NBQX (5 or 20 μM) was tested at agonist concentrations producing at least 50% of maximal responses. In experiments designed to detect antagonist effects the potential antagonist were applied alone for 90 s followed by co-application of agonists (NMDA, AMPA or KA) and the potential antagonist for another 90 s.

4.3.3. Metabotropic testing

Four metabotropic subtypes mGluR1, mGluR5, mGluR2 or mGluR4 were expressed in Chinese hamster ovary cell lines and used as representatives for group I, II and III metabotropic receptors, respectively [29].

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