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Design, synthesis and biological evaluation of novel bicyclo[1.1.1]pentane-based ω-acidic amino acids as glutamate receptors ligands

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ABSTRACT

A novel series of bicyclo[1.1.1]pentane-based ω-acidic amino acids, including (2*S*)- and (2*R*)-3-(3′-carboxybicyclo[1.1.1]pentyl)alanines (**8** and **9**), (2*S*)- and (2*R*)-2-(3′-carboxymethylbicyclo[1.1.1]pentyl)glycines (**10** and **11**), and (2*S*)- and (2*R*)-3-(3′-phosphonomethylbicyclo[1.1.1]pentyl)glycines (**12** and **13**), were synthesized and evaluated as glutamate receptor ligands. Among them, (2*R*)-3-(3′-phosphonomethylbicyclo[1.1.1]pentyl)glycine (**13**) showed relatively high affinity and selectivity at the NMDA receptor. The results are also discussed in light of pharmacophoric modelling studies of NMDA agonists and antagonists.

1. Introduction

Bicyclo[1.1.1]pentane is a strained, yet stable cage largely utilized, in virtue of its symmetry, as a valuable unit in the construction of oligomers with predetermined shape, and polymers and dendrimers with novel properties.[1] The unique properties of the bicyclo[1.1.1]pentane moiety made it an useful scaffold in the rational design of small molecules as tool compounds in drug discovery.[2] In particular, the ability of the bicyclo[1.1.1]pentane moiety to keep bridgehead substituents in a coplanar, linear disposition has been exploited by us for the design of (2*S*)-2-(3′-carboxybicyclo[1.1.1]pentyl)glycine (*S*-CBPG, **1**),[3] a competitive antagonist of the subtype 1 of metabotropic glutamate receptors (mGluR1) also endowed with partial agonist activity on mGluR5 subtype. The idea behind the design of *S*-CBPG (**1**) was the observation that the class of 4-carboxyphenylglycines (CPGs), firstly reported as group I mGluRs competitive antagonists,[4] founded its pharmacological profile on the co-linearity imparted by the phenyl ring to the two pharmacophoric groups, namely the ω-carboxylate and the glycine moiety. The ability of *S*-CBPG (**1**) to act as group I antagonist suggested that the phenyl ring of CPGs acted as a spacer between pharmacophoric groups and was not involved in specific interactions with the receptor. It should be noted, however, that *S*-CBPG (**1**) is also characterized by a different distance between the pharmacophoric groups with respect to 4-CPG (**2**) (5.6 Å vs 6.6 Å, respectively). With the aim of evaluating the effect of lengthening the distance between the two pharmacophoric groups in *S*-CBPG (**1**), we carried out the synthesis of (2*S*)-2-(3′-(1*H*-tetrazol-5-yl)bicyclo[1.1.1]pentyl)glycine (*S*-TBPG, **3**),[5] which proved 2.5-fold less potent as mGluR1 antagonist than the parent compound **1**, and inactive at mGluR5 subtype. These data indicated that, although the introduction of the tetrazole ring in place of the ω-carboxylate moiety increased the pharmacophore distance up to the 'standard' one of 4-CPG (**2**), the substitution was detrimental for the activity and this could be ascribed to the lower acidity of the tetrazole ring ($pK_a = 4.9$) with respect to the carboxylate group ($pK_a = 4.2$) or to the different hydrogen bonding geometry.

To further investigate the role played by the distal acidic moiety in the class of bicyclopentane derivatives, we recently reported the synthesis of (2*S*)-2-(3′-phosphonobicyclo[1.1.1]pentyl)glycine (*S*-PBPG, **5**), the ω-phosphonic acid analog of *S*-CBPG (**1**).[6] The isosteric replacement of the ω-carboxylic moiety of L-Glutamic acid (L-Glu) or glutamate analogs with the ω-phosphonic moiety has indeed represented a productive strategy to obtain different classes of acidic amino acids endowed with specific activities at

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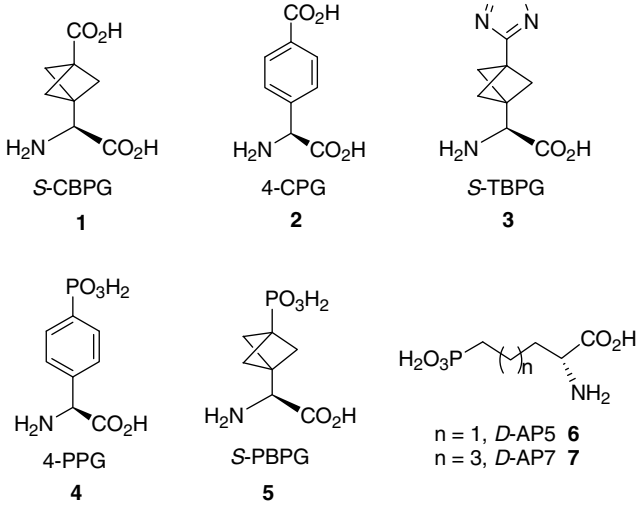
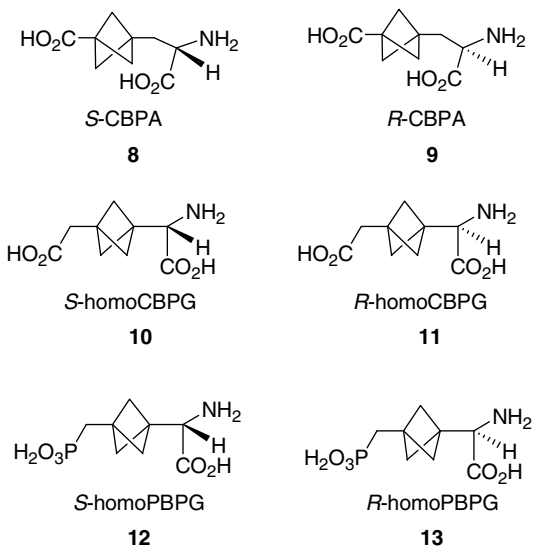
ionotropic- and metabotropic glutamate receptors. Thus, ω-phosphono amino acids characterized by an appropriate intermediate chain and by the D-configuration at the amino acidic centre, such as D-AP5 (**6**) and D-AP7 (**7**) have represented the first generation of potent and selective NMDA competitive antagonists,[7] whereas 4-PPG (**4**), the ω-phosphonate analog of 4-CPG (**2**), turned out to be a potent and selective group III mGluRs agonist.[8] Accordingly, *S*-PBPG (**5**), characterized by the bioisosteric replacement of the phenyl ring of 4-PPG (**4**) by the bicyclo[1.1.1]pentane nucleus showed moderate potency and selectivity at mGluR4.[6]



As a continuation of our work in the design and synthesis of 3′-substituted-2-(bicyclo[1.1.1]pentyl)glycine derivatives and in search for new excitatory amino acid (EAA) receptor ligands to be used as pharmacological tools for the characterization of this vast family of receptors, we report herein the synthesis and the preliminary biological results of (2*S*)- and (2*R*)-3-(3′-carboxybicyclo[1.1.1]pentyl)alanines (*S*-CBPA, **8** and *R*-CBPA, **9**), (2*S*)- and (2*R*)-2-(3′-carboxymethylbicyclo[1.1.1]pentyl)glycines (*S*-homoCBPG, **10** and *R*-homoCBPG, **11**), where the carboxylate group is maintained as distal acidic moiety and the increase of the distance between the pharmacophoric groups is obtained by homologation of the carbon backbone. The preparation and the pharmacological evaluation of (2*S*)- and (2*R*)-3-(3′-phosphonomethylbicyclo[1.1.1]pentyl)glycines (*S*-homoPBPG, **12** and *R*-homoPBPG, **13**) are also reported.



2. Results and discussion

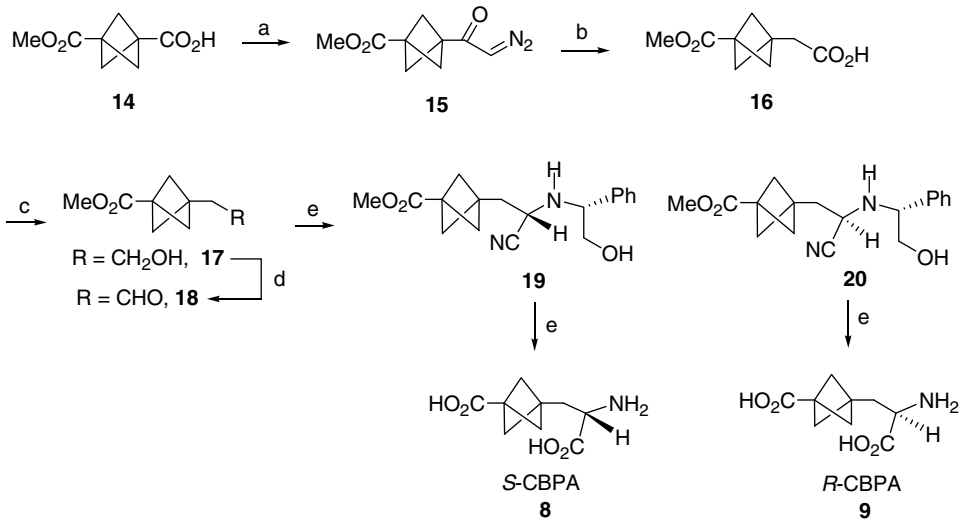
The synthesis of *S*-CBPA (**8**) and *R*-CBPA (**9**) involved 3-(methoxycarbonyl)bicyclo[1.1.1]pentane-1-carboxylic acid (**14**), synthesized according to the Michl's procedure,[9] as the starting material (Scheme 1). The one-carbon homologation of the carboxylic moiety of **14** was accomplished by a modified Arndt–Eister reaction[10] involving the formation of α-diazomethyl ketone **15**, obtained by the treatment of the intermediate oxyanhydride with trimethylsilyldiazomethane,[11] then submitted to Wolff rearrangement in aqueous THF in the presence of silver benzoate as catalyst to give the homologated carboxylic acid **16** (88%). 3-(Methoxycarbonyl)bicyclo[1.1.1]pentylacetic acid (**16**), thus obtained, was converted into the corresponding alcohol **17** by borane reduction (90%). Dess–Martin oxidation of the primary alcoholic function of **17** led in 76% yield to the corresponding aldehyde **18** which was submitted to the condensation with *R*-α-phenylglycinol.[12] The subsequent addition of trimethylsilyl cyanide to the Schiff base thus formed, afforded the mixture of the two expected α-amino nitriles, as a major (2*S*)- and a minor (2*R*)-component (85:15 by HPLC).[13] Medium pressure liquid chromatography of the reaction mixture allowed us to obtain **19** and **20**, in 35% and 15% yields, respectively. The α-amino nitrile **19** was submitted to oxidative cleavage of the chiral auxiliary, acidic hydrolysis and ion exchange resin chromatography to afford *S*-CBPA (**8**) in 73% yield. By an analogous procedure, starting from the minor α-amino nitrile **20**, *R*-CBPA (**9**) was obtained in 85% yield.

The carboxylic acid **16** was also the starting material for the synthesis of homoCBPG enantiomers **10** and **11**, characterized by the insertion of one methylene unit between the ω-carboxylate moiety and the bicyclopentane core (Scheme 2). Thus, treatment of **16** with di-*tert*-butyl dicarbonate in the presence of DMAP afforded in high yield the mixed diester **21**, which was converted by alkaline hydrolysis into the monoester **22** (87%). Also in this case the transformation of the carboxylic function of **22** into the corresponding aldehyde **24** was made by a two step-sequence involving the initial reduction of **22** to the primary alcohol **23** (81%), then reoxidized with Dess–Martin periodinane (76%).

Starting from the aldehyde **24**, the preparation of the title amino acids **10** and **11** was achieved following the synthetic protocol above described for the synthesis of *S*-CBPA (**8**) and *R*-CBPA (**9**). Thus, diastereoselective Strecker reaction, cleavage of the chiral auxiliary and final hydrolysis of the α-amino nitriles **25** and **26**, thus obtained, provided *S*-homoCBPG (**10**) and *R*-homoCBPG (**11**), respectively.

The synthesis of the ω-phosphonic acid analogs, *S*-homoPBPG (**12**) and *R*-homoPBPG (**13**), was performed starting from methyl 3-(hydroxymethyl)bicyclo[1.1.1]pentane-1-carboxylate (**27,** Scheme 3).[3a] Thus, the treatment with bromine and triphenylphosphine, in the presence of imidazole, transformed **27** into the corresponding bromide **28** (60%), then converted by conventional Arbuzov reaction conditions into methyl 3-(diethoxyphosphoryl)bicyclo[1.1.1]pentane-1-carboxylate (**29**) in 50% yield. DIBAH reduction of the carboxylic ester function of **29** in THF at −60 °C, led us to obtain the corresponding aldehyde **30** in 53% yield. Starting from **30**, according to the three-step sequence already described, consisting of diastereoselective Strecker reaction, cleavage of the chiral auxiliary and final hydrolysis of the two α-amino nitriles **31** and **32**, thus obtained, the preparation of the title compounds *S*-homoPBPG (**12**) and *R*-homoPBPG (**13**), was completed.

The biological profiles of the synthesized compounds **8–13** were examined at ionotropic glutamate receptors (iGluRs), NMDA, AMPA and kainate, and recombinant mGluRs subtypes (mGluR1a, mGluR2, mGluR4 and mGluR5) in binding experiments performed as described previously.[8,14] As shown in Table 1, *S*-homoCBPG (**10**)

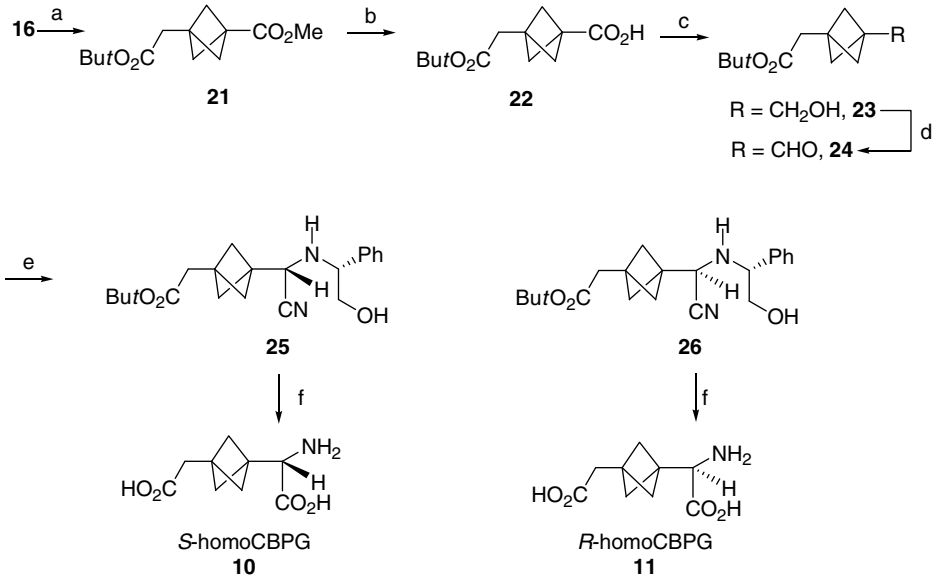


Scheme 1. Reagents and conditions: (a) i—ClCOOEt, Et_3N, THF, −15 °C, 30'; ii—$TMSCHN_2$, CH_3CN, 0 °C, 24 h, 74%; (b) AgOCOPh, Et_3N, THF–H_2O 4:1, ultrasound, 30', 88%; (c) 1 M BH_3 in THF, THF, −15 °C, 6 h, 90%; (d) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 1 h, 76%; (e) i—*R*-(−)-α-phenylglycinol, MeOH, rt, 8h; ii—TMSCN, 0 °C then rt, 24 h; iii—mplc, 35% (**19**), 15% (**20**); (f) i—$Pb(OAc)_4$, CH_2Cl_2–MeOH, 0 °C, 1 h; ii—6 N HCl, reflux, 24h; iii—Dowex 50WX2-200, 73% (**8**), 85% (**9**).

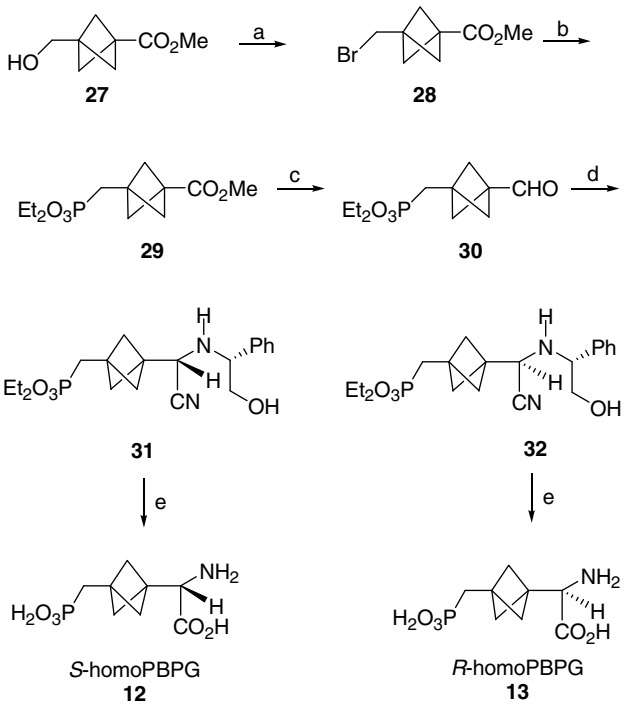
was a mGluR1 ligand with moderate affinity (*Ki* = 59 μM). It should be noticed that compound **10** was also able to bind NMDA and mGluR5, albeit with 2- and 5-fold lower affinities than mGluR1, respectively.

The most interesting results are related to the affinity of the compounds **8–13** at the NMDA receptor. In particular, among the ω-carboxylate derivatives **8–11**, *R*-CBPA (**9**) and *R*-homoCBPG (**11**) displayed low micromolar affinities at this receptor subtype and a very good selectivity, being completely inactive at the other iGluRs and mGluRs. Both the ω-phosphonate derivatives, **12** and **13**, showed higher affinities at the NMDA receptor, than the *R*-ω-carboxylate ones, still maintaining high degrees of selectivity. In particular, *R*-homoPBPG (**13**) resulted the most interesting compound of the series, showing a K_i of 0.73 μM at the NMDA receptor and inactive at the other iGluRs and mGluR subtypes tested.

To preliminary asses their functional interaction with the NMDA receptor, the compounds were tested in a [^{3}H]MK801 binding assay to rat cortical membranes measuring the effects on potentiation of binding by glutamate/glycine.[15] In agreement with previous observations, glutamate and glycine stimulated [^{3}H]MK801 binding 3–400% of basal levels. In this assay, compounds **8–13** showed no agonist activity at concentrations up to 1 mM. In contrast, the NMDA antagonist 2(*R*)-CPP (**33**), when tested in the presence of 30 μM glutamate and 1 μM glycine, inhibited [^{3}H]MK801 binding with affinity of 103 ± 10 nM. In this assay, only compounds **12** and **13** showed moderate inhibition of [^{3}H]MK801 binding at higher μM concentrations (data not shown). While these preliminary data suggest that compounds **8–13** have no agonist activity at the NMDA, they indicate that compounds **12** and **13** may be antagonists at this receptor.



Scheme 2. Reagents and conditions: (a) Boc_2O, *t*BuOH, DMAP, rt, 7 h, 88%; (b) $NaOH_{aq}$, MeOH, rt, 72 h, 87%; (c) 1 M BH_3 in THF, THF, −15 °C, 6 h, 81%; (d) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 1 h, 76%; (e) i—*R*-(−)-α-phenylglycinol, MeOH, rt, 8 h; ii—TMSCN, 0 °C then rt, 24 h; iii—mplc, 30% (**25**), 15% (**26**); (f) i—$Pb(OAc)_4$, CH_2Cl_2–MeOH, 0 °C, 1 h; ii—6 N HCl, reflux, 24 h; iii—Dowex 50WX2-200, 80% (**10**), 50% (**11**).

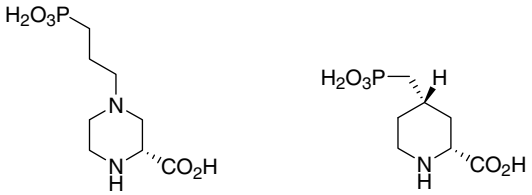


Scheme 3. Reagents and conditions: (a) Br_2, Ph_3P, imidazole, CH_2Cl_2, rt, 48 h, 60%; (b) $(EtO)_3P$, 110 °C, 72 h, 50%; (c) 1.5 M DIBAH in toluene, toluene, −60 °C, 18 h, 53%; (d) i—*R*-(−)-α-phenylglycinol, MeOH, rt, 3 h; ii—TMSCN, 0 °C then rt, 12 h; iii—mplc, 19% (**31**), 13% (**32**); (e) i—$Pb(OAc)_4$, CH_2Cl_2–MeOH, 0 °C, 1 h; ii—6 N HCl, reflux, 24 h; iii—Dowex 50WX2-200, 65% (**12**), 50% (**13**).

To further support these observations, we tested the abilities of compounds **8**–**13** to fit either a pharmacophoric model of NMDA agonists (Model A, Fig. 1a) or a pharmacophoric model of NMDA antagonists (Model B, Fig. 1b).

Briefly, NMDA receptors are obligate heteromers composed of the glycine-binding NR1, glutamate-binding NR2 and glycine-binding NR3 subunits.[16] Their activation requires the binding of both L-Glu and the co-agonist glycine, plus the relief of the magnesium block by membrane depolarization.[17,18] According to the accepted notion concerning the activation mechanism of iGluRs,[19] the ligand binding domain (LBD) of the receptor is composed of two lobes consisting of part of the amino-terminal domain (segment S1) and the second extracellular loop (segment S2) of the protein. A folded bioactive conformation of L-Glu and synthetic agonists interacts at both lobes of the LBD promoting the stabilization of the functionally active closed conformation of the receptor, as observed in the crystal structure of the NR2 subunit (pdb code: 2A5S).[20] Conversely, an extended bioactive conformation of the antagonists is proposed to bind at one lobe of the LBD stabilizing the functionally inactive open conformation of the receptor, though a crystallographic study of the NR2 subunit with antagonists is not available yet.

Thus, while the pharmacophoric model of NMDA agonists (Model A) was constructed using a structure-based approach and the experimental folded bioactive conformation of L-Glu as observed in the crystal structure of NR2 (pdb code: 2A5S),[20] the pharmacophoric model of antagonists (Model B) was built using a rigid-analog approach and the global minimum conformation of the NMDA competitive antagonist CGS19755 (**34**, Fig. 2).[21]



Model A was composed of five pharmacophoric points that capture the interactions observed in the crystal complex between L-Glu and the NR2A subunit of NMDA receptor (pdb code: 2a5s). In particular, they comprise two hydrogen bond donors located on the α-amino group ($D1_A$, $D2_A$), which are involved in the interactions with the residues Glu387, Ser486 and Thr488; one negatively charged feature located on the α-carboxylic group ($N1_A$) which interacts with the residue Arg493; and finally, two hydrogen bond acceptor located on one oxygen of the γ-carboxylic group ($A1_A$, $A2_A$) describing the hydrogen bond formation with residues Ser664 and Thr665.

Model B consisted of four points: one hydrogen bond donor located on the α-amino group of the compounds (D_B); three negatively charged groups of which one ($N1_B$) was located on the α-carboxylic group and the others ($N2_B$, $N3_B$) on the two oxygen atoms of the phosphonic acid moiety. This model is in agreement with the previously reported pharmacophoric model of competitive antagonists,[22] and is consistent with the interaction pattern described for the NMDA competitive antagonists on the basis of a mutagenesis study.[23] In particular, the latter suggests Arg493, Ser486, Glu387 and Thr488 as interacting with the amino acidic moiety of the compounds, and Lys459, Lys462 making salt bridges with the double negatively charged phosphonic group.

In Table 2, the number of features matched and the relative fitting score of compounds **8**–**13** on both models is reported. It is worth noting that the considerable length between the α-amino acidic moiety and the distal acidic group of compounds **8**-**13**, typical of NMDA antagonists, hampers the fitting of the bicyclo[1.1.1]pentylalanine and -glycine moieties on the agonist pharmacophoric model (Model A), yielding a null score of fitness.

Conversely, a nice correlation (Fig. 3, $r^2 = 0.85$) is observed between the binding (pK_i) of compounds **8**–**13** and their fitness scores on Model B. In particular, the best score obtained for

Table 1
Binding affinities values expressed as K_i (μM), for *S*-CBPA (**8**), *R*-CBPA (**9**), *S*-homoCBPG (**10**), *R*-homoCBPG (**11**), *S*-homoPBPG (**12**) and *R*-homoPBPG (**13**)

Compound	NMDA	AMPA	KA	mGluR1a	mGluR5	mGluR2	mGluR4
S-CBPA (**8**)	>300	>300	>300	>1000	>300	>1000	>1000
R-CBPA (**9**)	29 ± 4	>300	>300	210 ± 30	336 ± 22	>1000	>1000
S-homoCBPG (**10**)	124 ± 32	>300	>300	59 ± 12	293 ± 71	>1000	>1000
R-homoCBPG (**11**)	53 ± 3	>300	>300	>1000	>300	>1000	>1000
S-homoPBPG (**12**)	4.6 ± 0.8	>300	>300	>300	>300	nt	>1000
R-homoPBPG (**13**)	0.73 ± 0.12	>300	>300	>1000	>1000	nt	>1000
L-Glu	0.29 ± 0.09	0.17 ± 0.01	0.092 ± 0.028	0.57 ± 0.08	1.16 ± 0.03	6.4 ± 0.5	1.4 ± 0.2
L-AP4	>300	>1000	>1000	>1000	>1000	>1000	0.16 ± 0.02

Data are means ± SEM of 4–6 individual experiments performed in triplicate. For further details, see Section 4. nt, not tested.

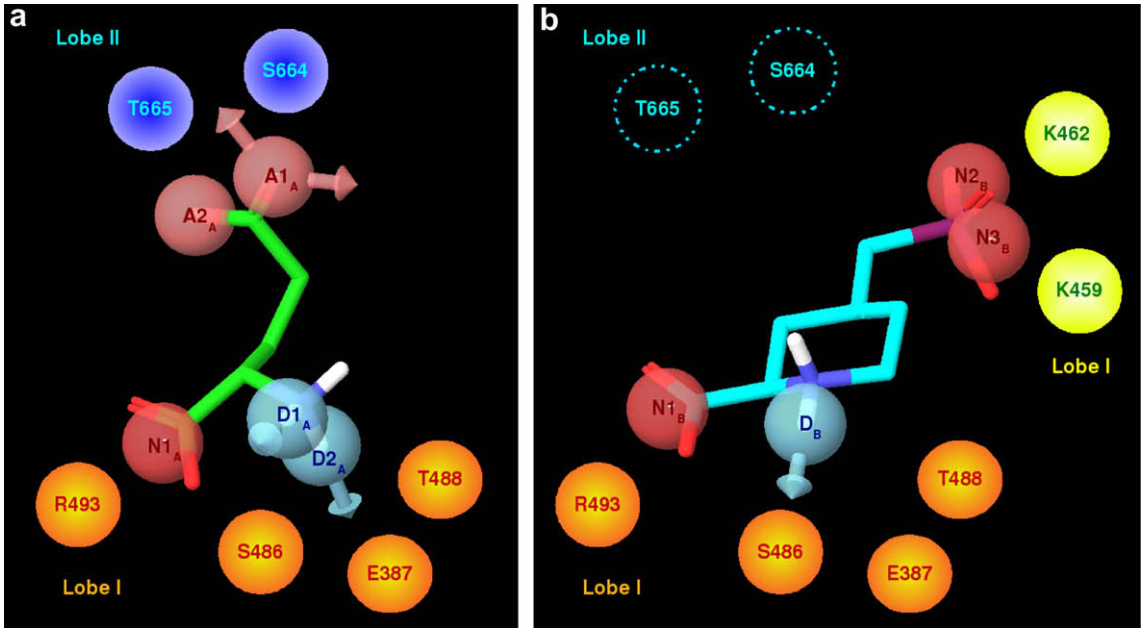


Figure 1. The pharmacophoric model of NMDA agonists (a) and NMDA antagonists (b). A, hydrogen bonding acceptor site; D, hydrogen bonding donor site; N, negatively charged group; P, positively charged group. The putative position of interacting residues as observed into the crystal structure of NR2A-NMDA (pdb code: 2A5S) is highlighted with orange spheres (residues from lobe I), blue spheres (residues from lobe II) and yellow spheres (residues from lobe I).

compounds **12** and **13** in fitting all features of Model B, is in agreement with the lower K_i values shown by these compounds at the NMDA receptor. On this basis, an antagonistic pharmacological profile of **12** and **13** at the NMDA receptor may be suggested also in agreement with the above preliminary data, obtained using [^{3}H]MK801 binding as a surrogate functional assay.

3. Conclusion

Novel competitive NMDA modulators belonging to the class of bicyclo[1.1.1]pentane-based ω-acidic amino acids have been presented. *S*-homoPBPG (**12**) and *R*-homoPBPG (**13**), characterized by the presence of the phosphonic acid moiety in the ω-position, have been shown to have high affinity and selectivity at the NMDA receptor. In particular, *R*-homoPBPG (**13**) displaying an inhibitory constant in the low micromolar range has the highest affinity in the series.

Although further experimental data, including electrophysiology experiments, are required to fully profile the properties of these compounds, [^{3}H]MK801 binding experiments and pharmacophoric studies suggest a competitive antagonist mode of action for these compounds at the NMDA receptor.

Finally, *R*-homoPBPG (**13**) represents a relatively potent and selective lead compound for the NMDA receptor and may be an useful template for generating pharmacological tools to be employed in the further characterization of this class of glutamate receptors.

4. Experimental

All reagents were analytical-grade and purchased from Sigma–Aldrich (Milan–Italy). Flash chromatography was performed on Merck silica gel (0.040–0.063 mm). ^{1}H NMR and ^{13}C NMR spectra were registered on Bruker AC 400 with $CDCl_3$, as solvent, unless

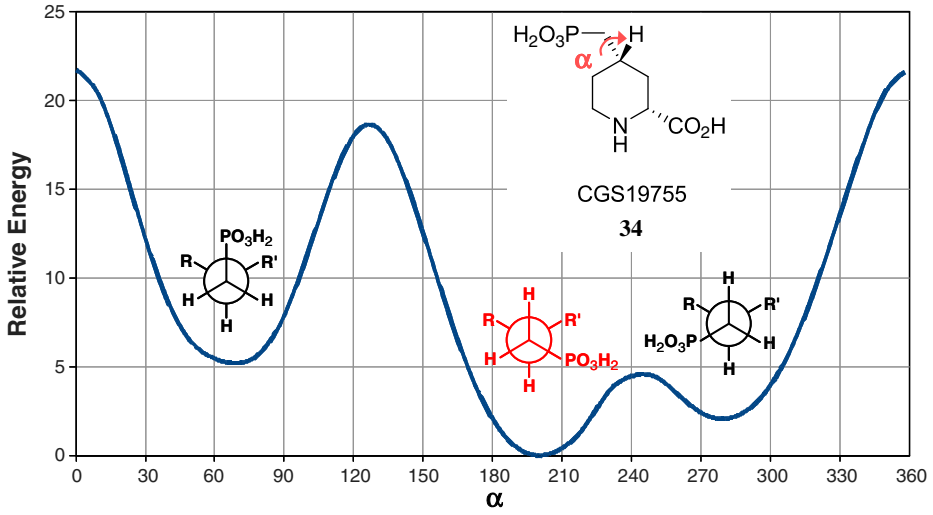


Figure 2. Conformational landscape of CGS19755 (**34**) with its local (black colour coded) and global (red colour coded) minimum conformations.

Table 2
Scores of compounds (**8**–**13**) in the pharmacophoric Models A and B

Compound	NMDA activity		Model A		Model B	
	K_i (μM)	pK_i	No of matched sites/No of sites	Fitness score	No of matched sites/No of sites	Fitness score
S-CBPA (**8**)	300	−2.48	0/5	Null	3/4	1.886
R-CBPA (**9**)	29	−1.46	0/5	Null	3/4	1.892
S-HomoCBPG (**10**)	124	−2.09	0/5	Null	3/4	1.790
R-HomoCBPG (**11**)	53	−1.72	0/5	Null	3/4	1.816
S-HomoPBPG (**12**)	4.6	−0.66	0/5	Null	4/4	2.172
R-HomoPBPG (**13**)	0.73	0.14	0/5	Null	4/4	2.334

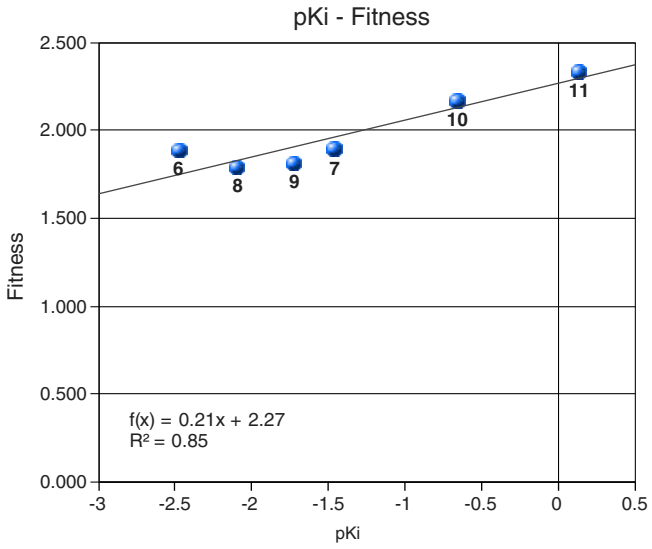


Figure 3. Correlation plot between the fitness score of Model B (*y* axis) and the affinities of compounds **8**–**13** at the NMDA receptor (*x* axis).

otherwise indicated. Chemical shifts are reported in ppm. The abbreviations are as follows: s, singlet; d, doublet; dd, double doublet; m, multiplet. Optical rotations were recorded on a Jasco Dip-360 digital polarimeter. GC analyses and MS spectra were carried out with an HP 6850 gas chromatography equipped with an HP 5975 Mass Selective Detector. ESIMS analysis was carried out on a Finnigan LCQ Deca ion trap instrument. Microanalyses were carried out on a Carlo Erba 1106 elemental analyzer. The analytical HPLC analyses were carried out on a Shimadzu (Kyoto, Japan) Workstation Class LC-10 equipped with a CBM-10A system controller, two LC-10 AD high-pressure binary gradient delivery systems, an SPD-10A variable wavelength UV/Vis detector, and a Rheodyne 7725i injector (Rheodyne Inc, Cotati, CA, USA) with a 20 μL stainless steel loop. The optical purity of the final compounds 8–**13** were determined by ligand-exchange chromatography.[24]

4.1. Diazomethyl ketone (15)

Ethyl chloroformate (0.52 g, 3.98 mmol) was added dropwise to a magnetically stirred solution of **14** (1.06 g, 3.62 mmol) and triethylamine (0.40 g, 3.00 mmol) in dry THF (10 mL) kept at −15 °C under vigorous stirring in an argon atmosphere. After 30 min the solid was filtered off and a solution of (trimethylsilyl)diazomethane (1.0 g, 1.0 mmol) in dry acetonitrile (40 mL) was directly added to the solution kept at 0 °C. The resulting mixture was stirred at 0 °C for 30 min and then stored at −4 °C for 24 h. After evaporation, diethyl ether (80 mL) was added and the mixture was washed with 0.5 M aqueous citric acid (30 mL), 5% $NaHCO_3$ solution (30 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated to afford a residue that was submitted to flash chromatography. Elution with hexane/AcOEt (80:20) gave **15** (0.520 g, 74%) as a pale-yellow oil; IR (film) 2384.6, 2128.2, 1739, 1632.4 cm^{-1}; ^1H NMR: δ = 2.27 (s, 6H, 3× CH_2), 3.64 (s, 3H, CO_2CH_3), 4.17 ppm (s, 1H, $COCHN_2$); ^{13}C NMR: δ = 30.1, 37.9, 38.3, 52.3, 53.2, 170.2, 175.2 ppm.

4.2. 2-(3-Methoxycarbonylbicyclo[1.1.1]pentane)acetic acid (16)

A solution of silver benzoate (0.47 g, 2.05 mmol) in Et_3N (6.0 mL) was added dropwise to a magnetically stirred solution of **15** (2.0 g, 10.25 mmol) in THF/water (4:1, 60 mL) and the resulting mixture was stirred at room temperature for 30 min under irradiation with ultrasounds. The solvent was partially removed in vacuo and the residue was diluted with water (20 mL) and extracted with AcOEt (3× 10 mL). The aqueous phase was then extracted with 5% $NaHCO_3$ solution (3× 30 mL). The combined basic extracts were acidified until pH 2 and extracted with AcOEt (4× 50 ml). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated in vacuo to afford **16** (0.980 g, 88%); ^1H NMR: δ = 2.18 (s, 6H, 3× CH_2), 2.61 (s, 2H, 2-CH_2), 3.72 ppm (s, 3H, s, CO_2CH_3); ^{13}C NMR: δ = 35.9, 37.2, 38.3, 51.1, 52.3, 170.2, 177.2 ppm; ESIMS *m/z* 183.2 [M−1].

4.3. Methyl 3-(2′-hydroxyethyl)bicyclo[1.1.1]pentane-1-carboxylate (17)

Borane tetrahydrofuran complex solution (1.0 M in tetrahydrofuran, 3.0 mL) was added dropwise to a cold (−15 °C), magnetically stirred solution of **16** (0.57 g, 3.11 mmol) in dry THF (3.0 mL) and the resulting mixture was stirred at −15 °C for 6 h. The reaction mixture was then neutralized with diluted acetic acid (2 mL) and the organic solvent partially removed in vacuo. The residue was diluted with 5% $NaHCO_3$ solution and the solution extracted with ether (4× 15 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give **17** (0.47 g, 90%); ^1H NMR: δ = 1.82 (t, *J* = 6.4 Hz, 2H, 1′-CH_2), 2.10 (s, 6H, 3× CH_2), 3.70 (s, 3H, CO_2CH_3), 3.72 ppm (t, *J* = 6.4 Hz 2H, 2′-CH_2); ^{13}C NMR: δ = 34.1, 38.0, 38.1, 51.8, 52.3, 60.8, 170.2 ppm; ESIMS *m/z* 171.2 [M+1].

4.4. Methyl 3-(2-oxoethyl)bicyclo[1.1.1]pentane-1-carboxylate (18)

A solution of **17** (0.46 g, 2.64 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a cooled (0 °C), magnetically stirred solution of Dess–Martin periodinane (1.15 g, 2.64 mmol) in dry CH_2Cl_2 (10 mL). After 30 min at 0 °C, the solution was allowed to warm to room temperature and 2 N NaOH solution (12 mL) was added. The aqueous phase was then extracted with ether (2× 50 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue that was submitted to flash chromatography. Elution with hexane/*tert*-butylmethylether (80:20) afforded **18** (0.340 g, 76%); ^1H NMR: δ = 2.18 (s, 6H, 3× CH_2), 2.62 (s, 2H,

1′-CH_2), 3.72 (s, 3H, CO_2CH_3), 9.72 ppm (s, 1H, CHO); ^{13}C NMR: δ = 35.1, 38.3, 45.8, 51.8 52.3, 170.2, 200.1 ppm.

4.5. Methyl 3-(*tert*-butoxycarbonylmethyl)bicyclo[1.1.1]pentane-1-carboxylate (21)

4-(Dimethylamino)pyridine (0.08 g, 0.64 mmol) and di-*tert*-butyl dicarbonate (0.95 g, 4.35 mmol) were added to a magnetically stirred solution of **16** (0.40 g, 2.13 mmol) in dry *tert*-butanol (10 mL). After 7 h, the solvent was partially removed in vacuo, the residue was diluted with ether (20 mL) and the solution washed with H_2O (10 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated in vacuo to give a residue that was submitted to flash chromatography. Elution with hexane/AcOEt (90:10) afforded **21** (0.45 g, 88%); ^{1}H NMR: δ = 1.40 (s, 9H, $C(CH_3)_3$), 2.05 (s, 6H, 3× CH_2), 2.40 (s, 2H, $CH_2CO_2C(CH_3)_3$), 3.65 ppm (s, 3H, CO_2CH_3); ^{13}C NMR: δ = 28.2, 35.9, 38.2, 38.4, 51.1, 52.3, 78.84, 170.2 ppm.

4.6. 3-(*tert*-Butoxycarbonylmethyl)bicyclo[1.1.1]pentane-1-carboxylic acid (22)

A solution of sodium hydroxide (0.072 g, 1.8 mmol) in water (2.0 mL) was added dropwise to a magnetically stirred solution of 21 (0.43 g, 1.77 mmol) in MeOH (4.0 mL). After 72 h, the solvent was partially removed, the residue diluted with water (15 mL) and extracted with ether (3× 10 mL). The aqueous layer was acidified to pH 3 with 10% citric acid solution and extracted with ethyl acetate (4× 20 ml). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent removed in vacuo to afford 22 (0.35 g, 87%); ^{1}H NMR: δ = 1.40 (s, 9H, $C(CH_3)_3$), 2.05 (s, 6H, 3× CH_2), 2.42 ppm (2H, $CH_2CO_2C(CH_3)_3$); ^{13}C NMR: δ = 25.8, 35.9, 36.15, 36.53, 50.5,78.8, 168.31, 173.6 ppm; ESIMS *m/z* 225.0 [M−1].

4.7. *tert*-Butyl (3-(hydroxymethyl)bicyclo[1.1.1]pentyl)acetate (23)

Borane tetrahydrofuran complex solution (1.0 M in THF, 2.0 mL) was added dropwise to a cold (−15 °C), magnetically stirred solution of **22** (0.45 g, 1.99 mmol) in dry THF (3.0 mL) and the resulting mixture was stirred at −15 °C for 6 h. The reaction mixture was then neutralized with diluted acetic acid (2 mL) and the organic solvent partially removed in vacuo. The residue was diluted with 5% $NaHCO_3$ solution and the solution extracted with ether (4× 15 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give **23** (0.34 g, 81%); ^{1}H NMR: δ = 1.41 (s, 9H, $C(CH_3)_3$), 1.67 (s, 6H, 3x CH_2), 2.42 (s, 2H, $CH_2CO_2C(CH_3)_3$), 3.63 ppm (s, 2H, CH_2OH); ^{13}C NMR: δ = 26.2, 34.5, 37.2, 38.4, 47.7, 61.2, 78.64, 168.9 ppm.

4.8. *tert*-Butyl (3-formylbicyclo[1.1.1]pentane)acetate (24)

A solution of **23** (0.32 g, 1.50 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a cooled (0 °C), magnetically stirred solution of Dess–Martin periodinane (0.65 g, 1.49 mmol) in dry CH_2Cl_2 (10 mL). After 30 min at 0 °C, the solution was allowed to warm to room temperature and 2 N NaOH solution (12 mL) was added. The aqueous phase was then extracted with ether (2× 50 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue that was submitted to flash chromatography. Elution with hexane/*tert*-butylmethylether (50:50) afforded **24** (0.24 g, 76%); ^{1}H NMR: δ = 1.41 (s, 9H, $C(CH_3)_3$), 2.02 (s, 6H, 3× CH_2), 2.42 (s, 2H, $CH_2CO_2C(CH_3)_3$), 9.54 (s, 1H, CHO); ^{13}C NMR: δ = 25.5, 34.8, 36.7, 42.5, 48.6, 78.64, 168.9, 196.6 ppm.

4.9. Methyl 3-(bromomethyl)bicyclo[1.1.1]pentane-1-carboxylate (28)

Bromine (6.0 g, 37.5 mmol) was added dropwise to a magnetically stirred solution of triphenylphosphine (23.7 g, 90 mmol) in dry CH_2Cl_2 (118 mL) kept at room temperature under an argon atmosphere. After 10 min, imidazole (3.57 g, 52 mmol) was added. A solution of **27** (3.3 g, 21 mmol) in dry CH_2Cl_2 (115 mL) was then added over the course of 20 min and the resulting mixture was stirred for 48 h at room temperature. The mixture was washed with water (2× 100 mL) and brine (2× 100 mL), dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to afford a residue that was submitted to flash chromatography. Elution with light petroleum/AcOEt (95:5) afforded **28** (2.8 g, 60%); ^{1}H NMR: δ = 1.96 (s, 6H, 3× CH_2), 3.37 (s, 2H, CH_2Br), 3.61 ppm (s, 3H, CO_2CH_3); ^{13}C NMR: δ = 32.50, 36.69, 38.98, 50.82, 51.22, 169.48 ppm; GC-MS *m/z* (relative intensity): 219 [M+](<1), 139 (26), 107 (27), 79 (100), 59 (20).

4.10. Methyl 3-[(diethoxyphosphoryl)methyl]bicyclo[1.1.1]-pentane-1-carboxylate (29)

A magnetically stirred mixture of **28** (2.7 g, 12.3 mmol) and triethylphosphite (25 mL) was heated at 110 °C for 72 h. Excess of triethylphosphite was distilled off and the residue purified by flash chromatography. Elution with AcOEt/light petroleum (80:20) afforded **29** (1.7 g, 50%); ^{1}H NMR: δ = 1.24 (t, J = 7. 0 Hz, 6H, 2× $PO_3CH_2CH_3$), 2.01 (m, 8H, 3× CH_2 and CH_2 $PO_3CH_2CH_3$), 3.59 (s, 3H, CO_2CH_3), 4.10 ppm (m, 4H, 2× $PO_3CH_2CH_3$); ^{13}C NMR: δ = 16.42 (d, J_{CP} = 6.1 Hz), 28.18 (d, J_{CP} = 139 Hz), 34.08 (d, J_{CP} = 8.0 Hz), 38.26, 51.55, 53.34 (d, J_{CP} = 7.7 Hz), 61.38 (d, J_{CP} = 6.3 Hz), 170.00 ppm. ^{31}P NMR (81 MHz): δ = 29.1 ppm; GC-MS *m/z* (relative intensity): 277 [M+](3), 245 (16), 216 (47), 188 (100), 160 (63), 139 (17), 107 (32), 79 (47).

4.11. Diethyl (3-formylbicyclo[1.1.1]pent-1-yl)methylphosphonate (30)

Precooled (−78 °C) diisobutylaluminium hydride (4.5 mL, 6.75 mmol, 1.5 M in toluene) was added dropwise, within 20 min, to a cooled (−78 °C), magnetically stirred solution of **29** (1.7 g, 6.15 mmol) in dry toluene (68 mL) kept under an argon atmosphere. After the end of the addition, the reaction mixture was warmed to −55 °C. After 4 h, because the reaction was not complete, additional diisobutylaluminium hydride (2.3 mL, 3.37 mmol, 1.5 M in toluene) was added, and the mixture allowed to stir at −55 °C for 18 h. The reaction was quenched by the addition of methanol (8 mL). HCl (1 M, 30 mL) was then added and the mixture was extracted with AcOEt (4× 40 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to afford a residue that was submitted to flash chromatography. Elution with AcOEt/light petroleum (90:10) afforded **30** (0.80 g, 53%); ^{1}H NMR: δ = 1.24 (t, J = 7. 0 Hz, 6H, 2× $PO_3CH_2CH_3$), 2.01 (m, 8H, 3× CH_2 and CH_2 $PO_3CH_2CH_3$), 3.59 (s, 3H, CO_2CH_3), 4.01 (m, 4H, 2× $PO_3CH_2CH_3$), 9.46 ppm (s, 1H, CHO); ^{13}C NMR: δ = 16.45 (d, J_{CP} = 6.0 Hz), 28.50 (d, J_{CP} = 138.7 Hz), 35.08, 44.75, 52.16 (d, J_{CP} = 7.8 Hz), 61.53 (d, J_{CP} = 6.4 Hz), 198.43 ppm. ^{31}P NMR (81 MHz) δ = 28.9 ppm; GC-MS *m/z* (relative intensity): 245 [M+](3), 217 (25), 189 (26), 161 (68), 109 (58), 92 (80), 79 (100).

4.12. General procedure for the Strecker synthesis to give substituted α-amino nitriles

(*R*)-2-Phenylglycinol (1.0 mmol) was added to a solution of the aldehyde (1.0 mmol) in methanol (7 mL), and the resulting

solution was magnetically stirred at room temperature for 8 h. After cooling at 0 °C, TMSCN (2.0 mmol) was added and the resulting mixture was stirred for 12 h at room temperature. Evaporation of the solvent gave a residue that was submitted to medium pressure chromatography to afford the corresponding *N*-substituted α-amino nitriles.

4.13. *N*-[(*R*)-2-Phenylglycinyl]-α-amino nitrile (19)

Light petroleum/AcOEt (70:30); 35% yield; ^{1}H NMR: δ = 2.01 (m, 2H, 1-CH_2), 2.05 (s, 6H, 3× CH_2), 3.27 (t, J = 6.8 Hz, 1H, CHCN), 3.59 (t, J = 9.9 Hz, 1H, C*H*Ph), 3.70 (s, 3H, CO_2CH_3), 3.80 (dd, J = 4.1 and 9.6 Hz, 1H, CH_aOH), 4.11 (dd, J = 4.1 and 9.6 Hz, 1H, CH_bOH), 7.37 ppm (m, 5H, aromatics); ^{13}C NMR: δ = 35.5, 37.4, 38.2, 45.9, 52.0, 52.9, 63.4, 67.4, 120.1, 128.1, 128.4, 128.9, 138.6, 170.3 ppm; $[\alpha]^{D}_{20}$ = −107.0 (c 1, $CHCl_3$); ESIMS *m/z* 315.4 [M+1].

4.14. *N*-[(*R*)-2-Phenylglycinyl]-α-amino nitrile (20)

Light petroleum/AcOEt (70:30); 15% yield; ^{1}H NMR: δ = 2.01 (m, 2H, 1-CH_2), 2.05 (s, 6H, 3× CH_2), 3.75-3.8 (m, 2H, C*H*Ph and CH_aOH), 3.59 (t, J = 9.9 Hz, 1H, CHCN), 3.70 (s, 3H, CO_2CH_3), 3.80 (m, 1H, CH_bOH), 7.37 ppm (m, 5H, aromatics); ^{13}C NMR: δ = 35.5, 37.2, 38.4, 47.2, 52.0, 52.8, 63.2, 66.9, 120.5, 127.8, 128.2, 129.2, 140.1, 170.0 ppm; $[\alpha]^{D}_{20}$ = −31.6 (c 1, $CHCl_3$); ESIMS *m/z* 315.4 [M+1].

4.15. *N*-[(*R*)-2-Phenylglycinyl]-α-amino nitrile (25)

Light petroleum/EtOAc (70:30); 30% yield; ^{1}H NMR: δ = 1.48 (s, 9H, C$(CH_3)_3$), 1.83 (m, 6H, 3× CH_2), 2.48 (s, 2H, CH_2) 3.4 (s, 1H, CHCN), 3.59 (t, J = 10.3 Hz, 1H, C*H*Ph), 3.80 (dd, J = 4.1 and 10.1 Hz, 1H, CH_aOH), 4.35 (dd, J = 4.1 and 10.1 Hz, 1H, CH_bOH), 7.37 ppm (m, 5H, aromatics); ^{13}C NMR: δ = 26.2, 34.2, 36.6, 37.1, 47.1, 47.6, 61.1, 65.5, 78.6, 116.5, 125.6, 126.3, 126.9, 136.3, 168.4 ppm; $[\alpha]^{D}_{20}$ = −110.3 (c 1, $CHCl_3$); ESIMS *m/z* 357.5 [M+1].

4.16. *N*-[(*R*)-2-Phenylglycinyl]-α-amino nitrile (26)

Light petroleum/EtOAc(70:30); 15% yield; ^{1}H NMR: δ = 1.45 (s, 9H, C$(CH_3)_3$), 1.83 (m, 6H, 3× CH_2), 2.46 (s, 2H, CH_2), 3.75–3.8 (m, 2H, C*H*Ph and CH_aOH), 3.77 (s, 1H, CHCN), 3.9 (dd, J = 4.8 and 7.3 Hz, 1H, CH_aOH), 7.37 ppm (m, 5H, aromatics); ^{13}CNMR: δ = 25.4, 34.0, 35.6, 37.2, 47.8, 47.6, 61.1, 64.3, 78.7, 116.4, 125.6, 126.3, 126.9, 136.7, 168.14 ppm; $[\alpha]^{D}_{20}$ = −34.4 (c 1, $CHCl_3$); ESIMS *m/z* 357.5 [M+1].

4.17. *N*-[(*R*)-2-Phenylglycinyl]-α-amino nitrile (31)

EtOAc/MeOH (98:2); 19% yield; ^{1}H NMR: δ = 1.24 (t, J = 7. 0 Hz, 6H, 2× $PO_3CH_2CH_3$), 1.77 (s, 6H, 3× CH_2), 1.98 (d, J = 18.8 Hz, $CH_2PO_3CH_2CH_3$), 3.28 (s, 1H, CHCN), 3.47 (m, 1H, CH_aOH), 3.68 (dd, J = 3.6 and 10.8 Hz, 1H, CH_bOH), 4.00 (m, 5 H, 2× $PO_3CH_2CH_3$ and C*H*Ph), 7.25 ppm (m, 5H, aromatics); ^{13}C NMR: δ = 16.48 (d, J_{CP} = 6.0 Hz), 28.33 (d, J_{CP} = 138.5 Hz), 33.09 (d, J_{CP} = 7.9 Hz), 39.14, 48.94, 50.50 (d, J_{CP} = 7.6 Hz), 61.54 (d, J_{CP} = 6.4 Hz), 63.02, 67.34, 118.40, 127.57, 128.15, 128.80, 138.49 ppm; ^{31}P NMR (81 MHz): δ = 29.3 ppm; $[\alpha]^{20}_{D}$ = −68.8 (*c* 0.9, $CHCl_3$).

4.18. *N*-[(*R*)-2-Phenylglycinyl]-α-amino nitrile (32)

EtOAc/MeOH (97:3); 13% yield; ^{1}H NMR: δ = 1.24 (t, J = 7. 0 Hz, 6H, 2× $PO_3CH_2CH_3$), 1.81 (s, 6H, 3× CH_2), 1.95 (d, J = 18.8 Hz, $CH_2PO_3CH_2CH_3$), 3.46-3.69 (m, 3H, CHCN, CH_aOH and C*H*Ph), 3.89 (dd, J = 5.2 and 8.8 Hz, 1H, CH_bOH), 4.00 (m, 4 H, 2× $PO_3CH_2CH_3$), 7.26 ppm (m, 5H, aromatics); ^{13} C NMR: δ = 16.48 (d, J_{CP} = 6.0 Hz), 28.29 (d, J_{CP} = 138.8 Hz), 33.94 (d, J_{CP} = 7.8 Hz), 39.62, 49.58, 50.77 (d, J_{CP} = 7.6 Hz), 61.57 (d, J_{CP} = 6.0 Hz), 63.28, 66.46, 118.48, 127.48, 128.23, 128.81, 139.52 ppm. ^{31}P NMR (81 MHz): δ = 29.1 ppm; $[\alpha]^{20}_{D}$ = −24.0 (*c* 1.3, $CHCl_3$).

4.19. General procedure for the oxidative cleavage and hydrolysis of *N*-substituted α-amino nitriles

Lead(IV) acetate (0.45 mmol) was added to a cold (0 °C), magnetically stirred solution of the α-amino nitrile (0.38 mmol) in dry MeOH/CH_2Cl_2 (1:2, 10 mL). After 10 min, the cooling bath was removed, and stirring continued for 1 h. Water (5 mL) was then added, and the resulting mixture was filtered with the aid of Celite. After evaporation of the solvent, the residue was heated at 95 °C in 6 N HCl (10 mL) for 18–24 h. The reaction mixture was evaporated to dryness and the residue was submitted to ion-exchange resin chromatography (Dowex 50WX2-200).

4.20. (2*S*)-3-(3′-Carboxybicyclo[1.1.1]pentyl)alanine (*S*-CBPA, 8)

Pyridine (10%); 73% yield; ^{1}H NMR (D_2O): δ = 2.04 (s, 6H, 3× CH_2), 2.14 (m, 2H, 3-CH_2), 3.93 ppm (t, J = 6.8 Hz, 1H, 2-CH); ^{13}CNMR (D_2O): δ = 29.67, 33.53, 35.66, 49.77, 170.77, 172.70 ppm; 96.0% ee; $[\alpha]^{D}_{20}$ = +16.2 (c 1, D_2O); Anal. calcd for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 54.28; H, 6.57; N, 7.04.

4.21. (2*R*)-3-(3′-Carboxybicyclo[1.1.1]pentyl)alanine (*R*-CBPA, 9)

Pyridine (10%); 85% yield; 96.5% ee; $[\alpha]^{D}_{20}$ = −16.7 (c 1, D_2O); Anal. calcd for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 54.27; H, 6.59; N, 7.05.

4.22. (2*S*)-2-(3′-Carboxymethylbicyclo[1.1.1]pentyl)glycine (*S*-homoCBPG, 10)

Pyridine (10%); 80% yield; ^{1}H NMR (D_2O): δ = 1.80 (s, 6H, 3× CH_2), 2.50 (s, 2H, s, CH_2CO_2H); 3.93 ppm (s, 1H, 2-CH); ^{13}C NMR (D_2O): δ = 35.11, 36.78, 36.81, 50.44, 54.08, 170.89, 176.61 ppm; 96% ee; $[\alpha]^{D}_{20}$ = +14.5 (c 1, D_2O); Anal. calcd for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 54.28; H, 6.59; N, 7.06.

4.23. (2*R*)-2-(3′-Carboxymethylbicyclo[1.1.1]pentyl)glycine (*R*-homoCBPG, 11)

Pyridine (10%); 50% yield; 90% ee; $[\alpha]^{D}_{20}$ = −8.5 (c 1, D_2O); Anal. calcd for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 54.27; H, 6.60; N, 7.04.

4.24. (2*S*)-(3′-Phosphonomethylbicyclo[1.1.1]pentyl)glycine (*S*-homoPBPG, 12)

Pyridine (10%); 65% yield; ^{1}H NMR (D_2O): δ = 1.64 (s, 6H, 3× CH_2), 1.77 (d, J = 12.0 Hz, $CH_2PO_3H_2$), 3.73 ppm (s, 1H, 2-CH); ^{13}C NMR (D_2O): δ = 29.46 (d, J_{CP} = 131.4 Hz), 33.58 (d, J_{CP} = 7.0 Hz), 37.03, 50.66 (d, J_{CP} = 7.5 Hz), 54.18, 171.15 ppm. ^{31}P NMR (81 MHz) δ = 25.1 ppm; 96% ee; $[\alpha]^{20}_{D}$ = +9.5 (*c* 0.75, H_2O); Anal. calcd for $C_8H_{14}NO_5P$: C, 40.86; H, 6.00; N, 5.96. Found: C, 40.88; H, 6.03; N, 5.97.

4.25. (2*R*)-(3′-Phosphonomethylbicyclo[1.1.1]pentyl)glycine (*R*-homoPBPG, 13)

Pyridine (10%); 50% yield; 95% ee; $[\alpha]^{20}_{D}$ = −9.0 (*c* 0.75, H_2O); Anal. calcd for $C_8H_{14}NO_5P$: C, 40.86; H, 6.00; N, 5.96. Found: C, 40.87; H, 6.02; N, 5.97.

5. Molecular modelling

Pharmacophoric studies were performed using the program Phase v.2.0 as implemented in the software package Schrödinger suite 2006.[25] All ligands were constructed, prepared and energy refined with the program LigPrep v. 2.0,[26] assigning the correct chirality and considering all possible ionic states at pH 7 (±2). The conformational analysis of compound **34** was carried out using the coordinate scan module of MacroModel as implemented in the Schrödinger suite and the following setting: conformational window 360°; torsional angle increment 10°; forcefield OPLS 2005; gradient convergence threshold of energy minimization 0.05; implicit consideration of water solvent.

The pharmacophoric searches were carried out using Phase and its default parameters. The pharmacophoric model of NMDA agonists (Model A) was constructed using the structural information obtained from the inspection of the binding mode of L-Glu observed in the crystal structure of the NR2 subunit of the NMDA receptor (pdb code: 2A5S) and according to the interaction model proposed by Laube and coworkers.[23] Conversely, a rigid-analog approach based on the global minimum conformation of compound **34** was used for the construction of the pharmacophoric model of NMDA antagonists (Model B).[22]

For the evaluation of our compounds **8**–**13** on both agonist and antagonist pharmacophoric models, we used the program Phase with the following specific setting: the tolerance of matching a feature was set to 1.0 Å and 2.0 Å, respectively, for the agonist (Model A) and antagonist models (Model B); the minimum number of required features to be fitted by a compound was set to 4 of 5 and 3 of 4, respectively, for Model A and Model B. Furthermore, while in Model A two hydrogen bond acceptor features ($A1_A$ and $A2_A$, Fig. 1) and one negative charge feature ($N1_A$, Fig. 1) were defined as obligate matching, in Model B the α-aminoacid feature ($N1_B$ and D_B) was defined as obligate matching.

6. Pharmacology

Membranes from BHK cells expressing subtypes of mGluRs (mGluR1, mGluR2, mGluR4 and mGluR5) were used for receptor binding experiments as previously described.[8,14] [^{3}H]Quisqualate binding were used for mGluR1 and mGluR5, [^{3}H]LY341495 binding for mGluR2 and [^{3}H]L-AP4 binding for mGluR4. Affinities for native AMPA, kainate and NMDA receptors were determined using 5 nM [^{3}H]AMPA, 5 nM [^{3}H]kainic acid and 2 nM [^{3}H]CGP39653 ([^{3}H]D,L-(*E*)-2-amino-4-propyl-5-phosphono-3-pentenoic acid) as previously described.[8,14]

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