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Synthesis and Biological Evaluation of 2-(3′-(1*H*-Tetrazol-5-yl) bicyclo[1.1.1]pent-1-yl)glycine (*S*-TBPG), a Novel mGlu1 Receptor Antagonist

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Abstract—The design and synthesis of 2-(3′-(1*H*-tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl)glycine (*S*-TBPG), a novel mGluR1 antagonist is reported. *S*-TBPG is characterized by the bioisosteric replacement of the distal carboxy group of 2-(3′-carboxybicyclo [1.1.1]pent-1-yl)glycine (*S*-CBPG) by a tetrazolyl moiety. Despite a moderate reduction in potency, *S*-TBPG is a selective mGluR1 antagonist (69 μM), with no activity at other mGluR subtypes. The interesting biological profile of *S*-TBPG, coupled with its peculiar chemical structure, is discussed in terms of the structure–activity relationship (SAR) of mGluR1 antagonists.

Introduction

Metabotropic glutamate (mGlu) receptors constitute a heterogeneous family of G-protein coupled receptors activated by synaptically released L-glutamic acid (**1**).[1] At least eight genes encode for molecularly diverse receptor subtypes that, when functionally expressed in heterologous systems, couple to a variety of effector systems. On the basis of the deduced sequence homology and the transduction mechanisms, the eight mGlu receptor subtypes so far cloned have been classified into three groups. Group I includes mGlu1 and mGlu5 which are positively coupled to phospholipase C (PLC); group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7, and mGlu8) are both negatively coupled to adenylyl cyclase (AC) but are endowed with different pharmacology, different neuronal and/or glial localization and share little sequence homology with each other.

The molecular and functional diversity of the mGlu family has fostered the search for potent and subtype selective agonists and antagonists which may be of help in proving the physiopathological role of individual subtypes en route towards clinically useful drugs. Recent reports, in particular, point out an inherent role for mGlu1 subtype in the induction and in the progression of the post-ischemic neuronal damage,[2] as well as the notion that mGlu1 antagonists might be clinically employed as neuroprotective agents following ischemia and oxygen/glucose deprivation.[3]

The search for selective mGlu1 antagonists, as well as for other subtype selective antagonists, has been for many years a knotty problem. The class of carboxyphenylglycines (CPGs, **2**–**5**, Chart 1), first reported by Watkins in 1993,[4] has provided the source for mGlu receptor antagonists. CPGs have played a fundamental role in the study of mGlu receptors since they were the first antagonists with selectivity over glutamate ionotropic receptors; nonetheless, CPGs of the first generation were generally endowed with low potency and poor selectivity. Several research laboratories have since then addressed the problem of improving the potency and the selectivity of the CPG class of compounds. As a result, CPG derivatives such as **4**, endowed with low to sub-micromolar range potency and group or subtype selectivity, are now available.[5]

In this frame, we focused our attention on the task of defining the structure–activity relationship (SAR) for the class of carboxyphenylglycines with the aim of disclosing the structural features responsible for selective

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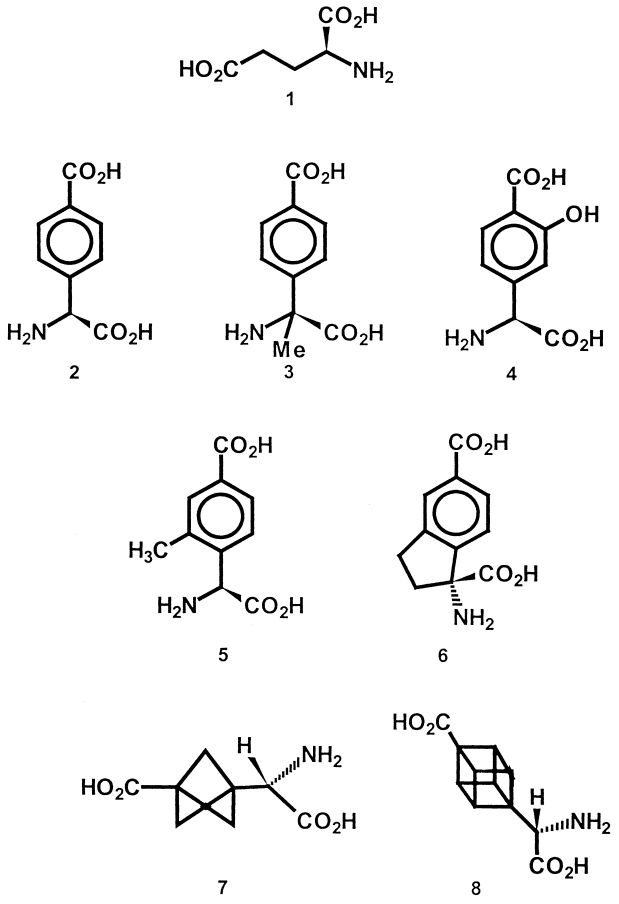


Chart 1.

antagonism towards individual mGlu receptor subtypes (Chart 2). Thus, our approach was to dissect the structure of 4-carboxyphenylglycine (4-CPG, **2**) into two main features and to investigate their respective role in affecting potency and selectivity as group I mGlu receptor antagonists. The first structural feature we took into account was the rotatable bond between the aromatic ring of 4-CPG (**2**) and the glycine moiety. The rigidification of such bonds into a five-membered ring yielded AIDA (**6**), a conformationally constrained analogue endowed with a lower potency than the parent compound 4-CPG (**2**) but with an increased selectivity, with no activity at group II, group III and mGlu5 receptor subtypes.[6]

The second structural feature to be investigated was the role played by the aromatic ring of CPGs. Indeed, we thought that the aromatic ring may either serve as a spacer between pharmacophoric groups or be involved in more specific interactions. Thus, we designed and synthesized *S*-CBPG (**7**),[7] where the propellane moiety adequately substitutes for the aromatic ring in keeping the pharmacophore groups in a coplanar, linear disposition but is endowed with a very different physicochemical profile. The good activity of **7** as mGlu1 receptor antagonist was the confirmation that the aromatic ring is not strictly necessary for the activity. *S*-CBPG (**7**) is, however, significantly shorter than 4-CPG (**2**) and we wondered whether this reduction in length could have affected the potency of *S*-CBPG (**7**). Thus, we reported the synthesis of ACUDA (**8**),[8] where the unusual cubyl moiety is able to keep the pharmacophoric groups still in a coplanar disposition as the aromatic ring or the propellane moiety. Interestingly, ACUDA (**8**) has exactly the same length as 4-CPG (**2**). When tested as mGlu ligand, however, **8** was shown to be only a weak mGlu1 antagonist. The lost of affinity of **8** with respect to 4-CPG (**2**) or *S*-CBPG (**7**) must therefore be ascribed to the increase in the volume of the spacer. It should be noted, however, that *S*-CBPG (**7**) and ACUDA (**8**) are characterized by different distances between pharmacophoric groups. Indeed, since *S*-CBPG (**7**) is significantly shorter than ACUDA (**8**) and 4-CPG (**2**) itself, the SAR study requires the effect of the distance between pharmacophoric groups to be investigated in the case of propellane derivatives. Thus, we have envisaged the synthesis of a novel propellane derivative, 2-(3′-(1*H*-tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl)glycine (*S*-TBPG, **9**), characterized by the substitution of the distal carboxylate by a tetrazole moiety. Indeed, the tetrazole

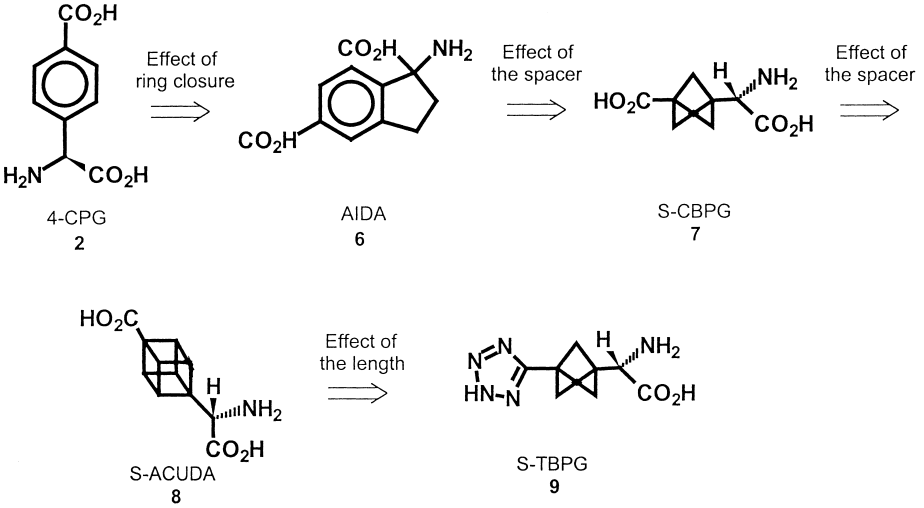


Chart 2.

ring is a suitable bioisoster in terms of acidity and hydrogen bond capability but it is significantly longer than the carboxylate group. The synthesis and the preliminary biological evaluation of **9** are reported herein.

Chemistry

The synthesis of *S*- and *R*-TBPG (**9** and **10**, respectively), reported in Scheme 1, involves the diacid **11** as a key intermediate.

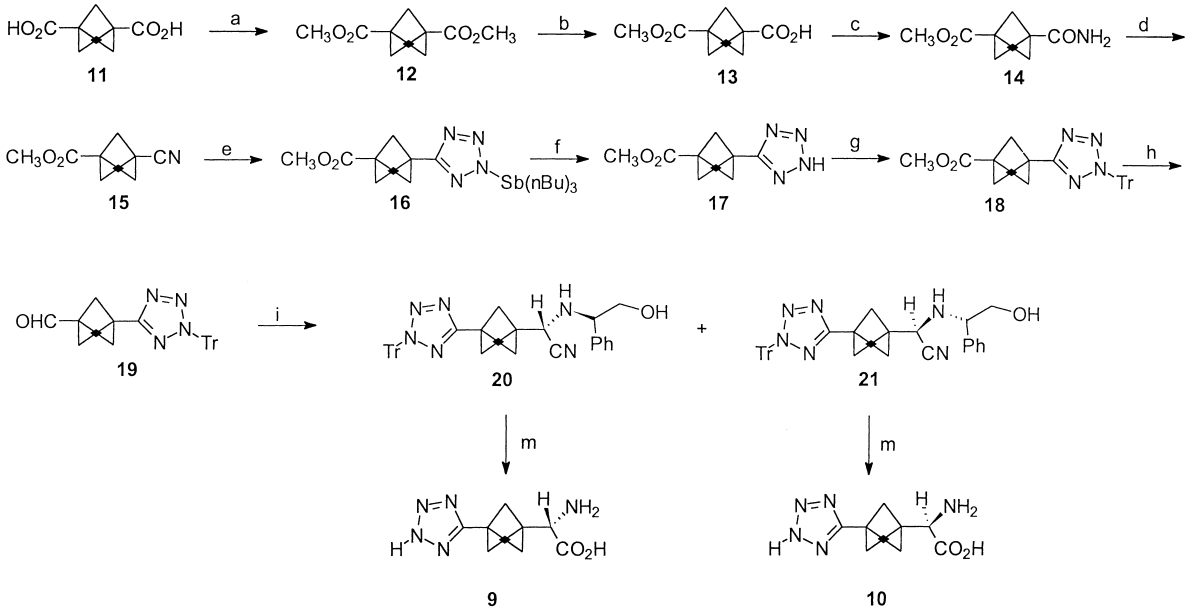
Briefly, **11** was prepared basically as described by Michl[9] and previously reported by us.[7] Esterification of **11** via the corresponding acyl chloride afforded the diester **12**, which was converted into the half ester **13** under mild alkaline conditions (86%).[10] Methyl-3-carbamoylbicyclo[1.1.1]pentane-1-carboxylate (**14**) was accessed from the monoester **13** through the corresponding acyl chloride and dehydration of **14** by $SOCl_2$ gave the nitrile **15** (94%).[10] This compound was converted to the 1-tributyltin-substituted tetrazole **16** using tri-*n*-butyltin azide (*n*-$BuSnN_3$) in xylene (98%).[11] The Sn–N bond was readily cleaved by treatment with gaseous hydrogen chloride in MeOH to give the corresponding free tetrazole **17**, which was then protected as trityl derivative[12] using trityl bromide and Et_3N (64%). DIBAH reduction of **18** yielded the aldehyde **19**,[13] which was then reacted with (*R*)-α-phenylglycinol in MeOH followed by treatment of the resulting Shiff base with TMSCN to give a 2.5:1 (^1H NMR) mixture of the two expected aminonitriles **20** and **21**, which were separated by flash chromatography in 28 and 10% yields, respectively. According to the known inductive effect of chiral α-phenylglycinol when employed in diastereoselective Strecker reaction, the more abundant aminonitrile has the newly formed chiral center opposite in sign to that of the α-phenylglycinol employed.[14] Thus, aminonitrile **20** can be attributed with an *S*-configuration at the α-aminonitrilic center. The two aminonitriles **20** and **21** were submitted to oxidative cleavage with lead tetraacetate, acidic (HCl) hydrolysis and ion exchange chromatography to afford (*S*)-2-(3′-(1*H*-tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl)glycine (**9**, *S*-TBPG) and (*R*)-2-(3′-(1*H*-tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl)glycine (**10**, *R*-TBPG) in 75 and 70% yield, starting from the respective aminonitrile derivatives.

Pharmacological Evaluation

The effect of *S*-TBPG (**9**) was examined on HEK 293 cells transiently expressing mGluR1a, mGluR5a, mGluR2 or mGluR4a. The same read out of receptor activation was used for any mGluR subtypes. The assay consisted of measuring the IP accumulated in the cells upon agonist treatment. Though group I mGluRs (mGluR1a, R5a) naturally coupled to phospholipase C, the group II and III (mGluR2 and R4a, respectively) do not. The coupling of these latter mGluRs to PLC was made possible by co-expressing these receptors with the chimeric G-protein α subunit in which the carboxyl terminal 9 residues of Gq were replaced by those of Gi2.[15,16] We have previously reported that the pharmacological profiles of mGluR2 and mGluR4 determined using this assay were identical to those determined by measuring the inhibition of cAMP formation.[16]

Discussion

As shown in Figure 1, L-glutamic acid at 1 mM stimulated IP formation in cells expressing mGluR1a or R5a alone, or mGluR2 or R4a with Gqi9. When applied at a concentration of 1000 μM S-TBPG (**9**) did not significantly stimulate IP formation in cells expressing



Scheme 1. (a) (i) $SOCl_2$ reflux; (ii) MeOH, reflux; (b) THF, NaOH/CH_3OH; (c) (i) $C_2O_2Cl_2$, Et_2O; (ii) NH_3, CH_2Cl_2; (d) $SOCl_2$; (e) (*n*-$Bu)_3SnN_3$, xylene, 110°; (f) HCl (g)/CH_3OH; (g) TrBr, $CHCl_3$/DMF; (h) DIBAH, toluene, −78 °C; (i) (i) (*R*)-2-phenylglycinol, MeOH, rt; (ii) TMSCN, 0 °C, then rt; (iii) flash chromatography; (m) (i) $Pb(OAc)_4$, CH_2Cl_2:MeOH (1:1, v/v), 0 °C; (ii) 6 N HCl, reflux; (iii) Dowex 50X2-200, 10% Py.

mGluR1a, R5a, R2, or R4a. To test a possible antagonistic effect of *S*-TBPG (**9**) on rat mGluRs, cells expressing these receptors were stimulated with a Glu concentration around its EC_{50} value (1 μM for mGluR1a, 5 μM for mGluR5a, 20 μM for mGluR2, and 30 μM for mGluR4a) (Fig. 2).

S-TBPG (**9**) was applied at a concentration of 1000 μM 5 min before and during the stimulation with Glu. No inhibition of the Glu-effect was observed in cells expressing mGluR5a, R2, or R4a. However, a strong inhibition of the Glu effect (98.6±5.8% inhibition; n = 3) was observed with 1000 μM *S*-TBPG (**9**), with an IC_{50} of 68.9±10.7 μM, as determined by inhibiting the Glu (1 μM)-induced stimulation of mGluR1a by increasing concentrations of *S*-TBPG (**9**).

These data indicate *S*-TBPG (**9**) as a novel, moderately potent but subtype selective mGluR1 antagonist. Although more potent and selective mGlu1 antagonists

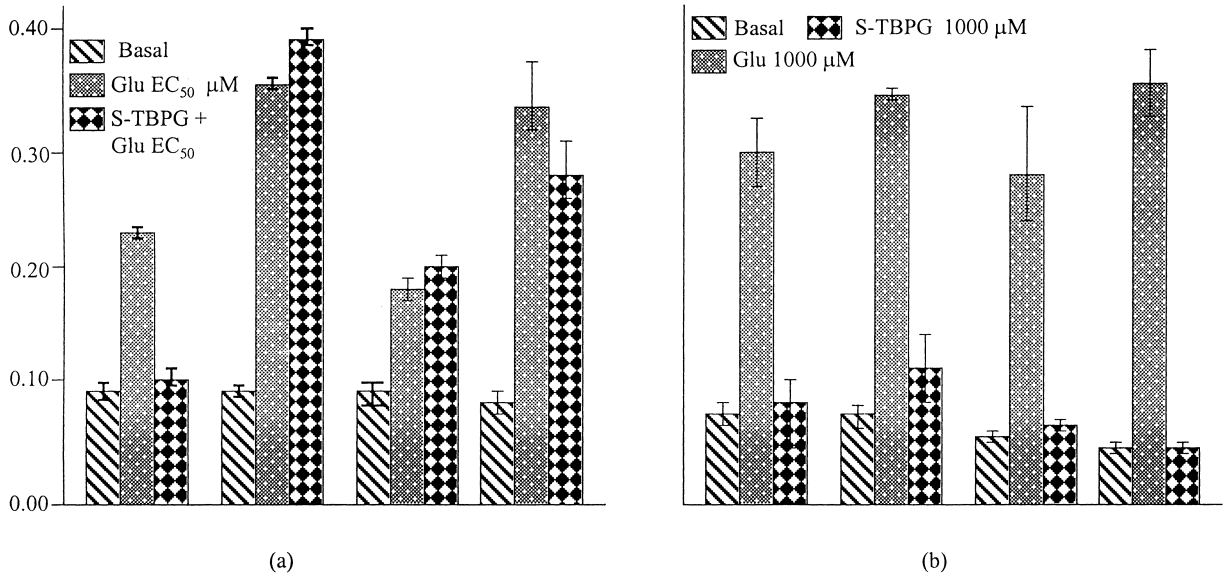


Figure 1. (a) *S*-TBPG inhibits L-Glu-induced stimulation of mGluR1a. Cells expressing the different receptors were incubated with *S*-TBPG and then stimulated with L-Glu (concentration around the EC_{50} of L-Glu on each receptor). Only the cells expressing mGluR1a did not show any increase in the production of IP upon Glu stimulation, thus indicating that *S*-TBPG acted as an antagonist at this receptor. (b) *S*-TBPG has no agonist activity on mGluR1a, R5a, R2 and R4a. Cells expressing the mGluR1a or mGluR5a, or mGluR2 or R4a with Gqi9 were stimulated with L-Glu (1000 μM) or *S*-TBPG (1000 μM). No significant effect on any of these receptors was observed for *S*-TBPG, while Glu stimulated each receptor 3.5- to 7.5-fold in three experiments.

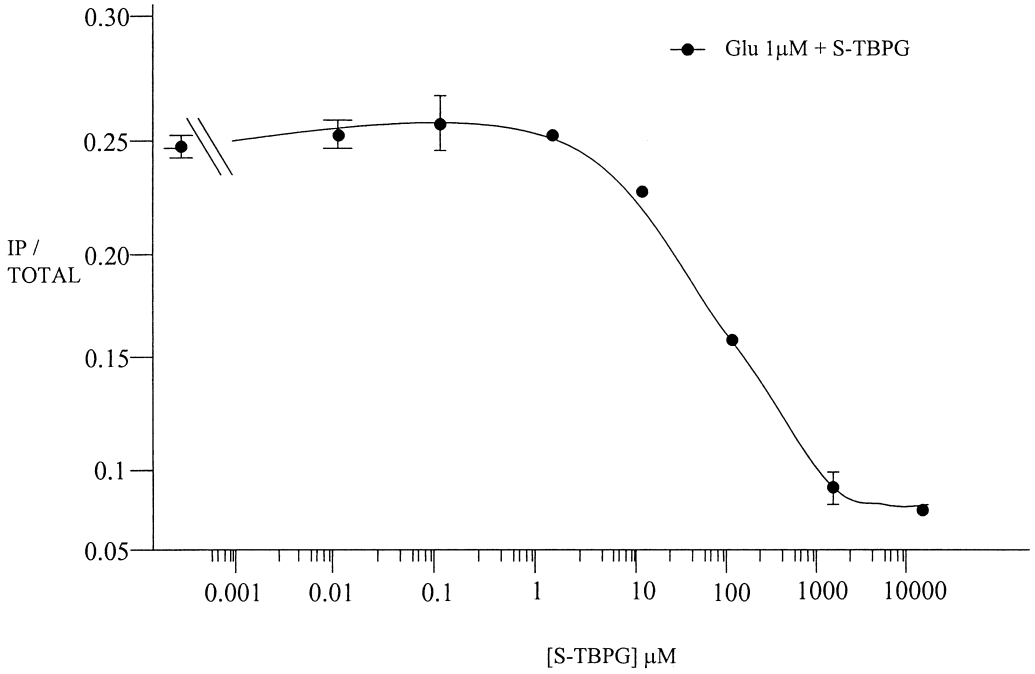


Figure 2. The IC_{50} of *S*-TBPG on mGluR1a was estimated by inhibiting Glu (1 μM)-induced stimulation of mGluR1a expressing cells by increasing concentrations of *S*-TBPG.

have recently appeared in the literature, the activity of *S*-TBPG (**9**) deserves some consideration in relation to the SAR for mGlu1 antagonists. In particular, *S*-TBPG (**9**) was designed with the aim of evaluating the effect of lengthening the distance between pharmacophoric groups in *S*-CBPG (**7**) analogues. The introduction of the tetrazole ring in place of the carboxylate moiety increases this pharmacophore distance of about 1 Å (Fig. 3).

When compared with the activity of *S*-CBPG (**7**), *S*-TBPG (**9**) is about 2.5-fold less potent as mGlu1 antagonist and does not show any partial agonism at mGlu5. These data indicate that the substitution of the carboxylate moiety by the tetrazole ring is detrimental for the activity. However, since *S*-TBPG (**9**) has the same length as 4-CPG (**2**), the decrease in the activity should be mainly ascribed to the lower acidity of the tetrazole ring with respect to the carboxylate moiety. Hence, it can be concluded that the effect of increasing the length between pharmacophore groups up to the 'standard' distance of CPGs is somehow compensated by the lower pK_a. This observation allows us to complete the SARs for this class of mGlu1 antagonists. The propellane nucleus is well accepted by the active site and can be considered as a bioisoster of the phenyl ring. The increase in the size of the spacer, as in the case of ACUDA (**8**), is detrimental for the activity, thus suggesting the existence of a region of limited space in the active site. The distance between pharmacophore groups seems not to be an essential issue. Indeed, the optimal distance between the distal acidic group and the α-aminoacidic moiety, owned by CPGs, can be lowered as in the case of *S*-CBPG (**7**) providing that the acidity of the distal moiety is kept.

In conclusion, we have reported *S*-TBPG (**9**) as a novel, subtype selective, mGlu1 antagonist.

S-TBPG (**9**) has allowed us to extend the structure–activity relationship for mGlu1 antagonists and represents a new example of an mGlu1 antagonist with no effect at mGlu5 receptors to be utilized as a pharmacological tool to study the role of mGlu1 subtypes in physiopathological conditions.

Experimental

Chemistry

General methods. Methanol and toluene were distilled over magnesium and sodium, respectively. All reactions were carried out under an argon atmosphere. Melting points were determined by the capillary method on a Büchi 535 electrothermal apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were taken on a Bruker AC 200 spectrometer. Proton chemical shifts are reported in ppm downfield from tetramethylsilane, except with D_2O which was also used as an internal standard. Carbon chemical shifts are reported in ppm using acetone as internal standard. The abbreviations used are as follows: s, singlet; dd, double doublet; m, multiplet. Flash chromatography was performed on Merck silica gel (0.040–0.063 mm). Specific rotations were recorded on a Jasco Dip-360 digital polarimeter.

Methyl-3-(1-tri-*n*-butylstannyltetrazol-5-yl)bicyclo[1.1.1]-pentane-1-carboxylate (16). Tri-*n*-butyltin azide (19.9 g, 0.06 mol) was added to a solution of the nitrile **15** (4.5 g, 0.03 mol) in xylene (30 mL). The resulting mixture was heated at 110 °C under argon. After 60 h the solvent was evaporated off, acetonitrile was added (50 mL) and the solution was washed with hexane (8×20 mL). The acetonitrile phase was dried (Na_2SO_4) and concentrated to yield the desired tetrazole **16** (13.9 g, 95%): ^{1}H NMR ($CDCl_3$) δ 1.00–1.60 (27H, m, 3×*n*-Bu); 2.50 (6H, s, 3×CH_2); 3.65 (3H, s, CO_2CH_3); ^{13}C NMR (CDCl3) δ 13.2, 17.1, 26.6, 27.7, 33.5, 38.8, 51.3, 53.9, 160.1, 169.5.

Methyl 3-(1*H*-tetrazol-5-yl)bicyclo[1.1.1]pentane-1-carboxylate (17). A solution of **16** (10.7 g, 0.02 mol) in dry MeOH (300 mL) was treated with anhydrous hydrogen chloride. After 3 h the solvent was evaporated off and the residue was triturated with hexane to give the free tetrazole **17** (3.74 g, 88%): ^{1}H NMR ($CDCl_3$) δ 2.65 (6H, s, 3×CH_2), 3.70 (3H, s, CO_2CH_3), 9.15 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 31.4, 39.5, 52.1, 54.2, 154.6, 169.4.

Methyl 3-(1-Trityltetrazol-5-yl)bicyclo[1.1.1]pentane-1-carboxylate (18). A solution of EtN_3 (4.6 g, 45 mmol) in $CHCl_3$:DMF (24 mL, 2:1) was dropped over a period of 45 min on to a vigorously stirred solution of **17** (4.4 g,

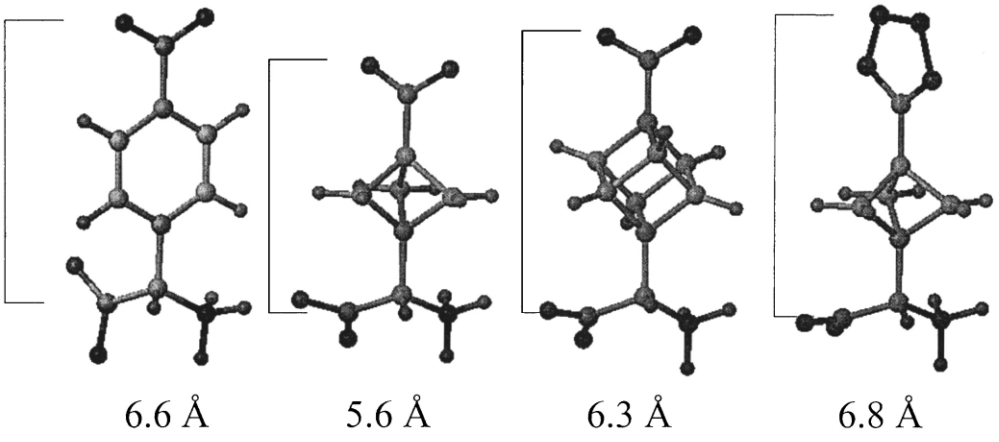


Figure 3. Comparison between 4-CPG (**2**), *S*-CBPG (**7**), *S*-ACUDA (**8**) and *S*-TBPG (**9**) in terms of distances between pharmacophoric groups.

23 mmol) and trityl bromide (8.2 g, 25 mmol) in $CHCl_3$:DMF (115 mL, 2:1). After 1.5 h the reaction mixture was neutralized with 2 N HCl and the solvent was evaporated off. The residue was taken up in Et_2O (300 mL) and the organic phase was washed with H_2O (4×40 mL), dried (Na_2SO_4) and evaporated to give a semisolid product which was purified by flash chromatography. Elution with petroleum ether:EtOAc (90:10) gave **18** (6.3 g, 64%): mp 184–187 °C; ^{1}H NMR ($CDCl_3$) δ 2.50 (6H, s, 3×CH_2), 3.70 (3H, s, CO_2CH_3), 7.0–7.50 (15H, s, aromatics).

3-(1-Trityltetrazol-5-yl)bicyclo[1.1.1]pentane-1-carboxaldehyde (19). A −78 °C solution of DIBAL-H (1.5 M in toluene, 8.70 mL) was dropped over 30 min to a −78 °C solution of **18** (5.8 g, 13.0 mmol) in dry toluene (150 mL). After 30 min the reaction was quenched with methanol (20 mL) and saturated NH_4Cl (100 mL) and then allowed to warm up to room temperature. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (3×40 mL). The combined organic phases were washed with water (2×50 mL), dried (Na_2SO_4), evaporated and purified by flash chromatography. Elution with petroleum ether:EtOAc (80:20) gave **19** (3.5 g, 64%): mp 159–163 °C; ^{1}H NMR ($CDCl_3$) δ 2.45 (6H, s, 3×CH_2), 6.90–7.40 (15H, s, aromatics), 9.55 (1H, s, CHO); ^{13}C NMR (CDCl3) δ 34.6, 45.1, 52.8, 83.1, 127.7, 128.3, 130.2, 141.3, 162.9, 197.9.

(2*S*)- and (2*R*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[3′-(1-trityl-1*H*-tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl]glycinonitriles (20 and 21). (*R*)-α-Phenylglycinol (1.0 g, 7.4 mmol) was added to a solution of the aldehyde **1** (3.0 g, 7.4 mmol) in dry MeOH (150 mL) and the resulting mixture was stirred at room temperature for 3 h. After cooling to 0 °C, TMSCN (1.5 g, 14.8 mmol) was added. After 12 h, the solvent was evaporated off and the residue was purified by flash chromatography. Elution with petroleum ether:EtOAc (80:20) gave **20** (0.38 g, 28%): mp 72–75 °C; ^{1}H NMR ($CDCl_3$) δ 2.20 (6H, s, 3×CH_2), 3.40 (1H, s, CHCN), 3.40–3.55 (1H, m, CHPh), 3.70 (1H, dd, J = 3.8 and 9.3 Hz, CH_aOH), 4.05 (1H, dd, J = 3.8 and 9.3 Hz, CH_bOH), 7.00–7.50 (20H, s, aromatics); ^{13}C NMR (CDCl3) δ 33.6, 40.2, 48.9, 51.2, 63.0, 67.2, 83.2, 118.1, 127.8, 128.3, 128.8, 130.3, 138.4, 141.3, 163.0; $[\alpha]_{20}^{D}$ −36.0 (*c* 1, MeOH). Subsequent elution with the same solvent gave **21** (0.14 g, 10%): mp 75–78 °C; ^{1}H NMR ($CDCl_3$) δ 2.25 (6H, s, 3×CH_2), 3.50–3.80 (2H, m, CHPh and CH_aOH), 3.80 (1H, s, CHCN), 3.95 (1H, dd, J = 4.3 and 7.5 Hz, CH_bOH), 6.90–7.40 (20H, s, aromatics); ^{13}C NMR ($CDCl_3$) δ 33.6, 40.6, 49.5, 51.4, 63.2, 66.5, 83.1, 118.1, 127.4, 127.7, 128.2, 128.8, 130.2, 139.4, 141.3, 162.8; $[\alpha]_{20}^{D}$ −16.0 (*c* 1, MeOH).

(*S*)-2-[3-(1*H*-Tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl]glycine (9). Lead (IV) acetate (635 mg, 1.44 mmol) was added to a 0 °C solution of the nitrile **20** (670 mg, 1.20 mmol) in anhydrous MeOH:CH_2Cl_2 (12 mL, 1:1). After 10 min phosphate buffer pH 7 was added (12 mL) and the resulting mixture was filtered with the aid of Celite. After evaporation of the solvent the residue was refluxed in 6 N HCl (20 mL) for 12 h. The reaction mixture was allowed to cool to room temperature and washed with diethyl ether (2×10 mL). The aqueous phase was evaporated to dryness and the residue was purified by ion exchange chromatography (Dowex 50WX2-200). Elution with 10% pyridine afforded **9** (189 mg, 75%): mp 282–284 °C; ^{1}H NMR (D_2O) δ 2.21 (6H, s, 3×CH_2), 3.65 (1H, s, CH); ^{13}C NMR (D_2O) δ 38.8, 51.2, 54.3, 154.4, 170.9; $[\alpha]_{20}^{D}$ +11.4 (*c* 1, H_2O).

(*R*)-2-[3-(1*H*-Tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl]glycine (10). Lead (IV) acetate (370 mg, 0.70 mmol) was added to a 0 °C solution of the nitrile **21** (386 mg, 0.70 mmol) in anhydrous MeOH–CH_2Cl_2 (8 mL, 1:1). After 10 min phosphate buffer pH 7 was added (12 mL) and the resulting mixture was filtered with the aid of Celite. After evaporation of the solvent the residue was refluxed in 6 N HCl (20 mL) for 12 h. The reaction mixture was allowed to cool to room temperature and washed with diethyl ether (2×10 mL). The aqueous phase was evaporated to dryness and the residue was purified by ion exchange chromatography (Dowex 50WX2-200). Elution with 10% pyridine afforded **10** (103 mg, 70%): mp 280–282 °C; ^{1}H NMR (D_2O) δ 2.21 (6H, s, 3×CH_2), 3.70 (1H, s, CH); ^{13}C NMR (D_2O) δ 38.6, 51.0, 54.1, 154.0, 170.7; $[\alpha]_{20}^{D}$ −15.3 (*c* 1, H_2O).

Biology

Culture and transfection of HEK 293 cells

HEK 293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Paris, France) supplemented with 10% fetal calf serum and transfected by electroporation as previously described.[16] Electroporation was carried out in a total volume of 300 μL with 10 μg carrier DNA, plasmid DNA containing mGluR1 (0.3 μg), mGluR2 (2 μg), or mGluR4a (5 μg) and 10 million cells.[17] To allow mGluR2 or mGluR4a to activate PLC, these receptors were co-expressed with the chimeric G-protein Gqi9 as previously described.[16,18] Because glutamate concentration in the cultured medium was found to profoundly affect its functioning, mGluR5a was co-expressed with the high affinity glutamate transporter EAAC1.[19]

Determination of inositol phosphate (IP) accumulation

Determination of inositol phosphate accumulation in transfected cells was performed after labeling the cells overnight with [^{3}H]-myo-inositol (23.4 Ci/mol, NEN, France). The stimulation was conducted for 30 min in a medium containing 10 mM LiCl and indicated concentration of antagonists or agonists. The basal IP formation was determined after 30 min incubation in the presence of 10 mM LiCl and the glutamate degrading enzyme glutamate pyruvate transaminase (1 U/mL) and 2 mM pyruvate to avoid the possible action of glutamate released from the cells. Results are expressed as the amount of IP produced over the radioactivity present in the membranes.

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