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SYNTHESIS AND PRELIMINARY EVALUATION OF (S)-2-(4'-CARBOXYCUBYL)GLYCINE, A NEW SELECTIVE mGluR1 ANTAGONIST

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Abstract: The synthesis and the pharmacological evaluation at metabotropic glutamate receptors of *S*-2-(4'-carboxy-cubyl)glycine (ACUDA, **9**) are described. *S*-2-(4'-Carboxy-cubyl)glycine is a structurally novel group I selective antagonist for mGluR1 subtype.

Introduction. The modulation of the G-protein coupled metabotropic glutamate receptor family (mGluR) is gaining importance among the new strategies aimed at reducing neuronal damage in neurological disorders.[1] Molecular cloning studies have revealed that this receptor family consists of at least eight subtypes (mGluR1 to mGluR8) grouped in the PLC-coupled group I (mGluR1 and mGluR5) and the negatively cAMP-coupled group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8) according to amino acid sequence, pharmacology and second messenger cascade.[2] Although more than ten years have elapsed since the discovery of the mGluR family by Nicoletti[3a] and Sladeczek,[3b] subtype selective agonists and antagonists have only recently been disclosed.[4]

As a part of a major effort aimed at the discovery and development of novel agents for glutamatergic pathway, we have recently directed our attention to the discovery of group I subtype selective ligands. Transgenic mice lacking mGluR1 have recently been introduced.[5] The intervention of compensatory events occurring in mGluR knock-out animals during development, however, has led to the generation of conflicting data and the availability of selective ligands, is still needed for the understanding of the functional role and the therapeutic potential of group I mGluRs.

The class of carboxyphenylglycines (CPGs, Chart I, **1**-**6**), first reported by Watkins,[6] has played an important role in the characterization of group I mGluRs. While CPGs of the first generation such as S-4CPG (**1**), S-4C3HPG (**2**) and S-M4CPG (**3**) exhibited mixed agonist and / or antagonist activity at both group I and II mGluRs, group selective CPG derivatives such as the group I agonist 3,5-DHPG (**4**), the mGluR1-subtype selective antagonist (+)-2MCPG (LY367385, **5**)[7a] and the mGluR5-subtype selective agonist 2CHPG (**6**)[7b] have recently become available.

In the framework of a research project directed to the design and synthesis of mGluRs ligands, we have addressed ourselves to the task of further defining the S.A.R profile of CPG derivatives. All the CPGs are characterized by the amino acid moiety and the distal carboxylate kept in a co-linear disposition, at a fixed distance, by the aromatic ring, a feature shared by all the so far known group I mGluR antagonists. While extensive structure-activity relationships have been performed on the effect of different substituents on the aromatic ring, less attention has been paid to other two features of CPGs, namely the importance on mGluR activity of the spatial arrangement of the amino acid moiety relative to the phenyl ring, as defined by the torsional angle between the Cα-C'1 bond and the role played by the phenyl ring. Thus, we have first designed

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and synthesized a series of rigid CPG analogs, among which (±)-1-aminoindan-1,5-dicarboxylic acid (AIDA, **7**) was shown to be a selective group I antagonist with no activity at either group II or group III mGluRs.[8] AIDA (**7**), a widely employed tool for the characterization of group I receptors,[9] encodes two low energy conformations ($\tau_{C9\text{-}C1\text{-}C2\text{-}C7}$ = -20° and +20°) resulting from the interconversion of the two envelop extremes of the cyclopentane ring. Only one of these two conformations, the one endowed with $\tau_{C9\text{-}C1\text{-}C2\text{-}C7}$ = 20°, mimics a low energy conformation of 4CPG (**1**), thus demonstrating that conformational tuning around Cα-C1 bond is needed for achieving group-selectivity.

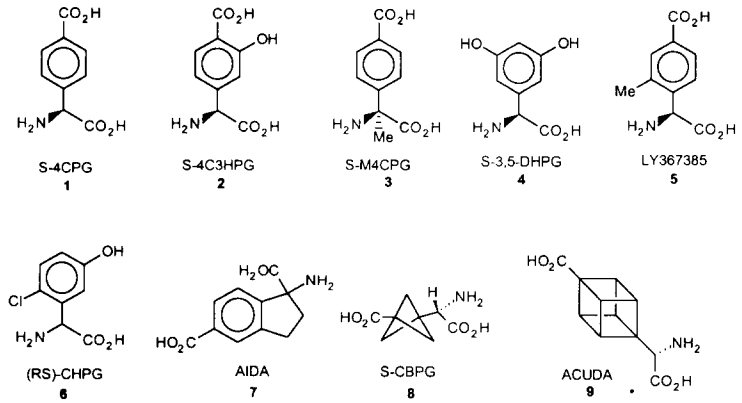
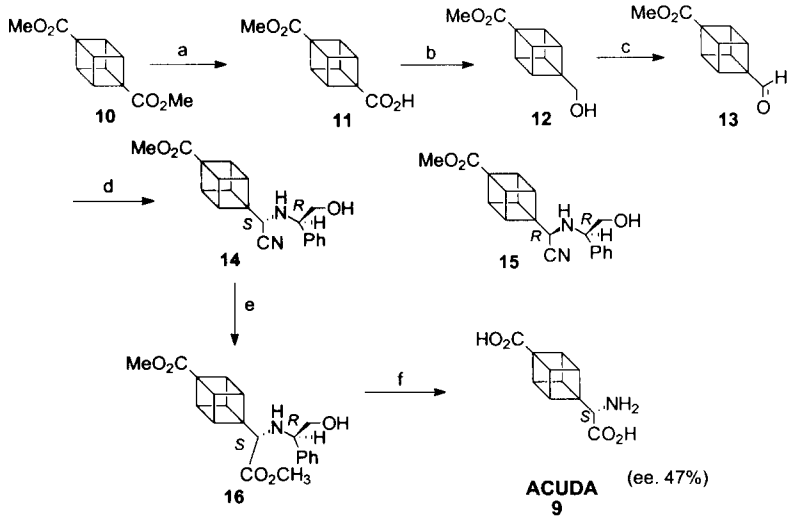


Chart I

The second aim was to ascertain the role played by the phenyl ring in the CPG class of derivatives. Indeed, while the co-linearity between pharmacophoric points provided by the aromatic ring is an invariable feature of all the CPGs, no reports have specifically dealt with the function of the phenyl ring, i.e. whether it is involved or not in direct interaction with the receptor site. In order to clarify this point, we have designed and synthesized (S)-2-(3'-carboxy-[1.1.1]bicyclopentyl)glycine (S-CBPG, **8**),[10] in which the co-linearity between pharmacophoric points is kept by the propellane moiety, endowed with a different stereoelectronic profile that the phenyl ring. The good activity of S-CBPG (**8**) as mGluR1 antagonist and mGluR5 partial agonist indicated that the phenyl ring is not necessary for group I activity provided that a suitable spacer is able to keep the amino acidic moiety and the distal carboxylate in a co-linear arrangement. In light of the activity of S-CBPG (**8**) we have decided to exploit the possibility of modifying the non-aromatic spacer on route towards structurally diverse mGluR ligands. The cubane moiety, larger in size than the propellane and still able to keep the pharmacophoric points in a co-linear disposition at the correct distance, was chosen as a well suited scaffold for this purpose (Fig.3). The synthesis of (*S*)-2-(4'-carboxycubyl)glycine (ACUDA, **9**) as well as its preliminary biological evaluation at mGluR subtype are described herein.

Chemistry The synthesis of the (*S*)-2-(4'-carboxycubyl)glycine (**9**) was undertaken as reported in Scheme 1. Thus, the commercially available dimethyl-1,4-cubane-dicarboxylate (**10**) was selectively deprotected under mild conditions (THF-MeOH 18:1, NaOH 1 eq. rt) [11] to give the corresponding monoester **11** (89% yield) which was then transformed into the mixed anhydride and subsequently reduced with $NaBH_4$ to the alcohol **12**

(64% yield). Oxidation of the primary alcoholic function by PCC gave the key aldehyde **13** which was used without further purification in the next step. A diastereoselective Streker synthesis involving the condensation of the crude aldehyde **13** with optically active R-(-)-α-phenylglycinol [12] (MeOH, rt, Argon atm, 4h) followed by nucleophilic addition of a cyanide ion to the Schiff base (TMSCN, 0°C, then rt, 12h) afforded the two aminonitriles (2*S*)-**14** and (2*R*)-**15**. Separation of the two aminonitriles by flash chromatography (light petroleum-AcOEt 7:3) afforded the more abundant diastereoisomer **14** (42% yield from **12**), which was assigned with a *S* configuration at the α-aminonitrile chiral center according to the known chiral induction of *R*-α-phenylglycinol.[13] Oxidation of **14** with lead(IV)tetraacetate followed by acidic hydrolysis (HCl 6N, 12 h, 80 °C) was first tried. Under this conditions, however, the yield of formation of the corresponding amino acid **9** was very low (6%). Thus, **14** was transformed into the corresponding aminoester **16** (MeOH, HCl (g)., 0°C) which was then submitted to lead(IV)tetraacetate oxidation. Mild acidic hydrolysis (HCl 3N-Dioxane 5:1, 60°C, 12h), followed by ion-exchange chromatography (Dowex 50WX2-200, 10% Py, 62% yield) afforded the (S)- 2-(4'-carboxycubyl)glycine (**9**) (ee. 47%, determined by HPLC on a chiral stationary phase: Crownpak CR (+) 150x4mm, H_2O /$HClO_4$ pH 2.07, 0.6 mL / min) with 14% overall yield from **10**. [14]



a) NaOH, THF/MeOH 18:1; 89% b) i. iBuOCOCl, N-Me-morpholine, THF, -10°C.
ii. $NaBH_4$, H_2O, rt, 3h; 64% c) PCC, CH_2Cl_2, rt, 15h d) i. R-(-)-phenylglycinol, MeOH, rt, 4.30 h
ii. TMSCN, 0°C then rt, 12 h iii. flash chromatography (light petroleum-AcOEt 7:3).
e) HCl (g), MeOH, 0°C, 78%. f) i. $Pb(OAc)_4$,CH_2Cl_2/MeOH 2:1; 0°C ii. HCl 3N / Dioxane 5:1, 60°C 12h
iii. Dowex 50WX2-200, 10% Py.

Scheme I

Biological Method and Results Culture and transfection of HEK 293 cells. HEK 293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Cergy Pontoise, France) supplemented with 10% fetal calf serum and transfected by electroporation as previously described.[15] 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 mGluR4 (5 µg) and 10 million cells. To allow mGluR2 and mGluR4 to activate PLC, these receptors were co-expressed with the chimeric G-protein Gqi9 as previously described.[15]

Determination of inositol phosphates (IP) accumulation. Determination of IP 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 the indicated concentration of agonist. The basal IP formation was determined after 30 min incubation in the presence of 10 mM LiCl and the Glu degrading enzyme glutamate pyruvate transaminase (1U/ml) and 2 mM pyruvate to avoid the possible action of Glu released from the cells. Results are expressed as the amount of IP produced over the radioactivity present in the membranes. The dose-response curves were fitted using the equation $y = [(y_{max} - y_{min}) / (1 + (x / EC_{50})^n)] + y_{min}$ and the kaleidagraph program. The KB value was determined either by constructing a Shild plot, or by using the Cheng and Prussof equation: $EC_{50}'=EC_{50}(1+I/KB)$, where EC_{50} is the EC_{50} value of the agonist, and EC_{50}' is the EC_{50} value of the agonist determined in the presence of a concentration I of antagonist.

The effect of ACUDA (**9**) was examined on HEK 293 cells transiently expressing mGluR1b, mGluR5a,

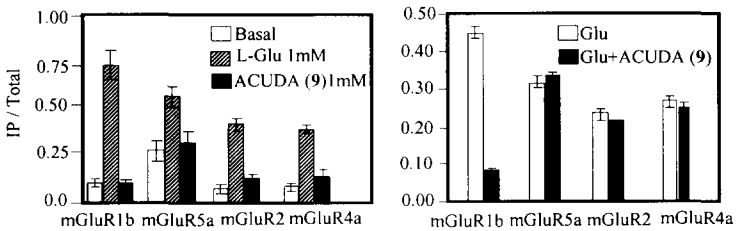


Figure 1. Agonist (left) and antagonist (right) effetct of ACUDA (**9**) on mGluR1b, mGluR2, mGluR5a, mGluR4a

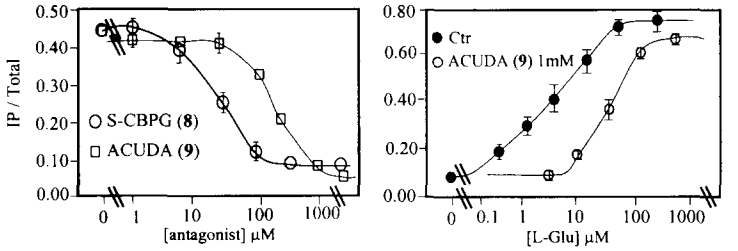


Figure 2. ACUDA (**9**) is a competitive mGluR1 antagonist, comparison with S-CBPG (**8**). (Left) Cells expressing mGluR1b were stimulated with 3μM Glu and the indicated concentration of ACUDA (**9**) (open squares) and S-CBPG(**8**) (open circles). (Right) Cells expressing mGluR1b were stimulated with the indicated concentration of L-Glu alone (black circles) or in the presence of 1mM ACUDA (**9**) (open circles).

mGluR2 or mGluR4a. In any case, the activation of the receptor was estimated by measuring the IP accumulated in the cells upon agonist treatment. The coupling of mGluR2 and mGluR4 to PLC was made possible by coexpressing these receptors with the chimeric G-protein α subunit Gqi9, in which the carboxyl terminal 9 residues were replaced by those of Gi2.[15,16] We previously reported that the pharmacological profiles of these two receptors determined using this assay were identical to those determined by measuring the inhibition of cAMP formation.[15]

ACUDA (**9**) did not stimulate IP formation in cells expressing mGluR1b or mGluR5a (Fig. 1, left). A small activation of mGluR2 and mGluR4a was observed with 1 mM ACUDA (**9**), the effect being equal to 16.2 ± 1.1 (n=7) % and 26.1 ± 8.8 (n=4) % of the maximal effect of Glu on mGluR2 and mGluR4a respectively (Fig. 1a). On mGluR2, this effect corresponds to the maximal effect of this drug, the EC_{50} being 54.9 ± 12.9 μM (n=4). In contrast, when applied at concentrations equal or lower than 300 μM, **9** was devoid of effect on mGluR4a-expressing cells. The possible antagonist action of ACUDA (**9**) was also examined. At a

concentration of 1 mM ACUDA (**9**) did not significantly inhibit the effect induced by Glu used at a concentration close to its EC_{50} value (1, 10 and 20 μM on mGluR5a, mGluR2 and mGluR4a respectively) on mGluR5a, mGluR2 or mGluR4a (Fig. 1, right). Moreover, in the presence of 1 mM ACUDA (**9**), no significant shift to the right of the Glu dose-response curve was observed with any of these receptors (data not shown). In contrast, 1 mM ACUDA (**9**) inhibited the effect of 3 μM Glu by 94.3 ± 0.9 % (n=5) (Fig. 1, right). This effect was dose-dependent with an IC_{50} of 232 ± 55 μM (n=4) and a Hill coefficient of 1.5 ± 0.3 (n=4) (Fig. 2, left). Under the same experimental conditions, the previously described mGluR1 antagonist (S)-CBPG (**8**) was found to be more potent than ACUDA (**9**), with an IC_{50} of 32 μM, in agreement with previous results.[10] Using the Cheng and Prussof equation, and an EC_{50} of 1.48 ± 0.4 μM (n=3, nH=0.96 ± 0.01) for Glu on mGluR1b, a KB value of 76 μM could be calculated for **9**. As expected for a competitive antagonist, the dose response curve of Glu was shifted to the right in the presence of a fixed concentration of **9** (1 mM) (Fig. 2, right), with an estimated KB value of 60 μM which is in agreement with the value determined with the inhibition curves.

Conclusion. The fully extended, co-planar arrangement between the α-amino acidic moiety and the distal carboxylate as provided by the phenyl ring in the CPG class of derivatives is generally accepted as a key feature of all the group I mGluR antagonists.[17] We have previously shown that this arrangement can be kept with no lost of activity by substituting the phenyl ring of CPGs with a non-aromatic spacer such as the propellane moiety.[6] In this paper, we report that significant mGluR1 antagonist activity is maintained when the cubane is used in the place of the propellane. The cubane moiety is larger in size than the propellane and, when functionalized, disposes the pharmacophoric points at a distance very similar to that of CPGs (Fig. 3).

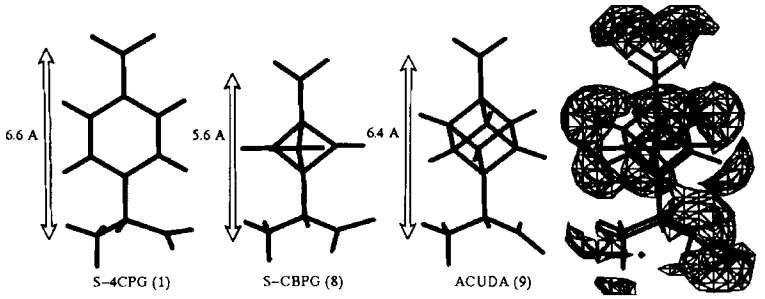


Figure 3. Comparison between pharmacophoric distances of S-4CPG (**1**), S-CBPG (**8**) and ACUDA (**9**). ACUDA (**9**) has a pharmacophoric distance very similar to S-4CPG (**1**). On the right the additional volume of ACUDA (**9**) with respect to S-CBPG (**8**) is shown.

The about nine fold of decrease of potency of ACUDA (**9**) with respect to S-CBPG (**8**) or 4CPG (**1**) must therefore be ascribed to the increase of volume of the non-aromatic spacer and we may conclude that the cubane moiety represents an upper limit for the steric accessibility of the mGluR1 receptor site. Interestingly, however, ACUDA (**9**) is completely devoid of any effect at mGluR5, whereas S-CBPG (**8**), under the same conditions, behaves as a partial mGluR5 agonist with an EC_{50} = 103 μM. This observation leads support to the notion that the mGluR5 receptor site is endowed with steric requirements more demanding than those of mGluR1, thus suggesting that mGluR1 / mGluR5 selectivity may be achieved by modulating the bulkiness of the spacer between pharmacophoric groups.

In summary, (*S*)-2-(4'-carboxycubyl)glycine (**9**), is a structurally novel group I antagonist with selectivity for the mGluR1 subtype. Albeit endowed with a relatively low potency, it can be considered a potentially useful

pharmacological tool for discriminating between mGluR1 and mGluR5.

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14. Analytical data for new compounds **12**, **14**, **15**, **9**: **12**: ^{1}H-NMR ($CDCl_3$) δ 2.45 (b, 1H, CH_2OH) 3.70 (s, 3H, CO_2CH_3), 3.75 (s, 2H, CH_2OH), 3.9 (m, 3H, 3',5',7'-CH), 4.15 (m, 3H, 2',6',8'-CH). **14**: ^{1}H-NMR ($CDCl_3$) δ 2.4 (bs, 1H, NH), 3.0 (bs, 1H, CH_2OH), 3.5 (s, 1H, CHCN), 3.55-3.80 (m, 6H, CO_2CH_3, CH_2OH, $CHCH_2OH$), 3.9-4.0 (m, 3H, 2',6',8'-CH), 4.0-4.1 (m, 3H, 3',5',7'-CH) 7.3-7.4 (m, 5H, aromatics) ^{13}C-NMR ($CDCl_3$) δ 44.4, 46.13, 49.7, 51.4, 56.3, 57.27, 62.8, 67.0, 117.6, 127.5, 128.1,138.3, 172. **15**: ^{1}H-NMR ($CDCl_3$) δ 2.7 (bs, 1H, CH_2OH), 3.3 (bs, 1H, NH), 3.5 (s, 1H, CHCN), 3.50-3.65 (dd, 2H, CH_2OH), 3.65-3.80 (m,7H, 2xCO_2CH_3, $CHCH_2OH$), 3.8-3.9 (m, 3H, 2',6',8'-CH), 4.0-4.1 (m, 3H, 3',5',7'-CH), 7.2-7.4 (m, 5H, aromatics). ^{13}C-NMR ($CDCl_3$) δ 44.7, 44.9, 46.0, 51.1, 51.3, 56.0, 58.5, 59.6, 62.9, 67.3, 127.1, 127.5, 128.3, 139.9, 172, 173.4. **9**: mp 250 °C (dec.) ^{1}H-NMR (D_2O + Py-D_5) δ: 3.75-3.9 (m, 6H, cubyl H). ^{13}C-NMR (D_2O + Py-D_5) δ: 43.25, 45.07, 54.8, 58.0, 60.6, 170.9, 180.3 $[\alpha]_D^{29}$: +10.18 (c 0.36, 10% Py)
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