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SYNTHESIS OF CONFORMATIONALLY-CONSTRAINED STEREOSPECIFIC ANALOGS OF GLUTAMIC ACID AS ANTAGONISTS OF METABOTROPIC RECEPTORS

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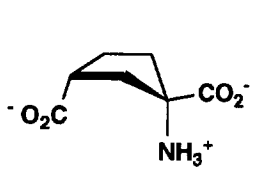
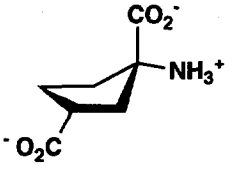
Abstract: Rigid analogs of ACPD have been synthesized to mimick different potential conformations of ACPD in aqueous solution. One of them, (±)-ABHD-I is a competitive antagonist at mGluR1a receptor with a K_B value of 300 μM.

Glutamic acid is a major excitatory amino acid and neurotransmitter in the central nervous system. It mediates fast synaptic transmission in the brain and probably has some role in neurodegenerative disorders. It is generally admitted that L-glutamic acid, a flexible molecule, displays different bioactive conformations depending on each type of glutamate receptors.[1] Five classes[2] have been identified and named according to their selective agonists: NMDA (N-methyl-D-aspartic acid), AMPA (L-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid, KA (kainic acid), ACPD (1S,3R-1-amino-cyclopentane-1,3-dicarboxylic acid), and L-AP4 (L-2-amino-4-phosphonobutyric acid).

The ACPD-receptor family is represented by several G-protein coupled receptors (metabotropic glutamate receptors or mGluR)[3,4] which mediate a variety of transduction mechanisms, including stimulation of phosphoinositide hydrolysis (mGluR1, mGluR5), or inhibition of adenylyl-cyclase (mGluR2, mGluR3). 1S,3R-ACPD, one of the few specific ligands of these glutamate receptor subtypes, has been considered as one of the possible conformationally-rigid analogs of glutamic acid.[5-7] However, in a recent study by NMR and molecular dynamics, it has been shown[8] that both *cis*- and *trans*-isomers of ACPD are rather flexible, and can adopt various envelop (E) conformations which have been classified in four types (I-IV), according to their α-amino/γ-carboxy groups (d_1) and α-carboxy/γ-carboxy groups (d_2) distances. The most populated conformation (E_1) of 1S,3R-ACPD, in aqueous solution at pH 7.0 seems to be quite different from what is generally accepted as the "extended" bioactive conformation of ACPD (^1E) for metabotropic receptors.[1,9]



E_1



^1E

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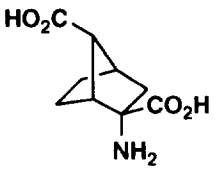
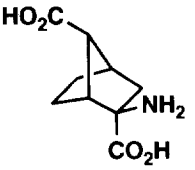
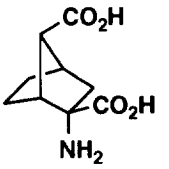
It can be speculated that 1S,3R-ACPD binds to the receptor in a definite conformation in a first stage, and that a different folding is then induced, due to interactions with the protein receptor residues. Moreover, if some flexibility is needed for agonist activity, as generally accepted for NMDA receptors,[10,11] and as can be found in the ACPD molecule, more rigid analogs of the recognized conformations should behave as high-affinity antagonists. We have thus undertaken the synthesis of fully conformationally-locked analogs, related to *cis*- or *trans*-ACPD, and derived from a norbornane structure, such as the isomeric amino-bicyclo[2.2.1]heptane dicarboxylic acids **ABHD I** to **VIII** (only one enantiomer of each is represented).



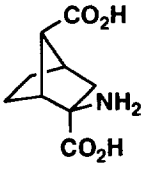
ABHD I



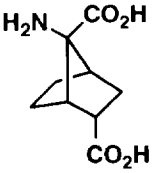
ABHD II



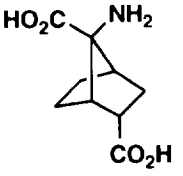
ABHD III



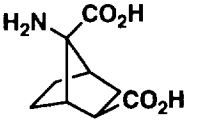
ABHD IV



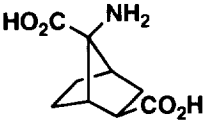
ABHD V



ABHD VI



ABHD VII

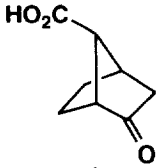


ABHD VIII

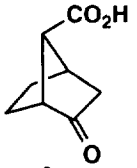
Chemical syntheses

The methodology used to obtain these amino acid analogs was based on the classical hydantoin formation (Bucherer-Bergs' reaction)[12,13] from adequate keto-carboxylic acids, in order to obtain simultaneously, as racemic mixtures, all (or most of) the stereoisomers expected from the combination of functional groups positions. The amino acids were then obtained by acidic or alkaline hydrolysis of the spirohydantoins and separated by ion exchange chromatography (see experimental procedure). Their structure was deduced from NMR spectra and reciprocal correlations, making use of the characteristic acid-catalyzed epimerization of the carboxyl-bearing carbon atom, previously described in γ-alkyl glutamic acid analogs.[14-16]

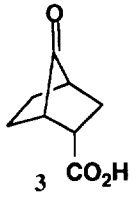
A prerequisite for such syntheses was a convenient preparation of the corresponding carboxy-substituted norbornanones **1**-**4**.



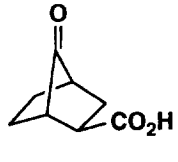
1



2

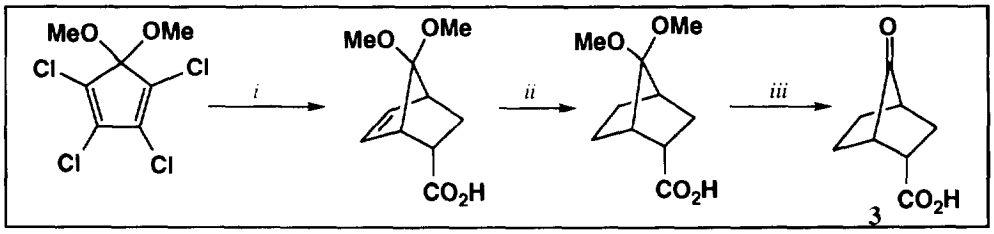


3



4

The ketoacids **1** and **2**[17-19] were prepared according to the literature. The ketoacid **3** was obtained in 30% yield according to Scheme 1, starting from a Diels-Alder reaction of 2,3,4,5-tetrachloro-1,1-dimethoxy-cyclopentadiene with methyl acrylate, from a known procedure used for the synthesis of bicyclo[2.2.1]hept-2-en-7-one.[20,21] This procedure was stereospecific, as previously shown,[22] and thus did not allow us to obtain the diastereomeric ketoacid **4**. However, it was thought that an epimerization of the carboxylic bearing position, at the subsequent amino acid level, would be feasible. The ketoacid **3**[23] was found relatively unstable and was used in the following reaction as a crude product.



Scheme 1

(*i*) CH_2=CH-CO_2Me; KOH, MeOH-H_2O; Na, EtOH;[24] (*ii*) H_2, Pd/C (8 h, rt); (*iii*) H_2SO_4 (5%) (48 h, rt)

Isomeric hydantoins **5a** and **5b** were obtained (Scheme 2) from the ketoacid **1** in a 9:1 ratio, and characterized by ^{1}H-NMR. Acidic or alkaline hydrolysis of the mixture afforded diastereomeric **ABHD I** and **ABHD II** in the same 9:1 ratio.

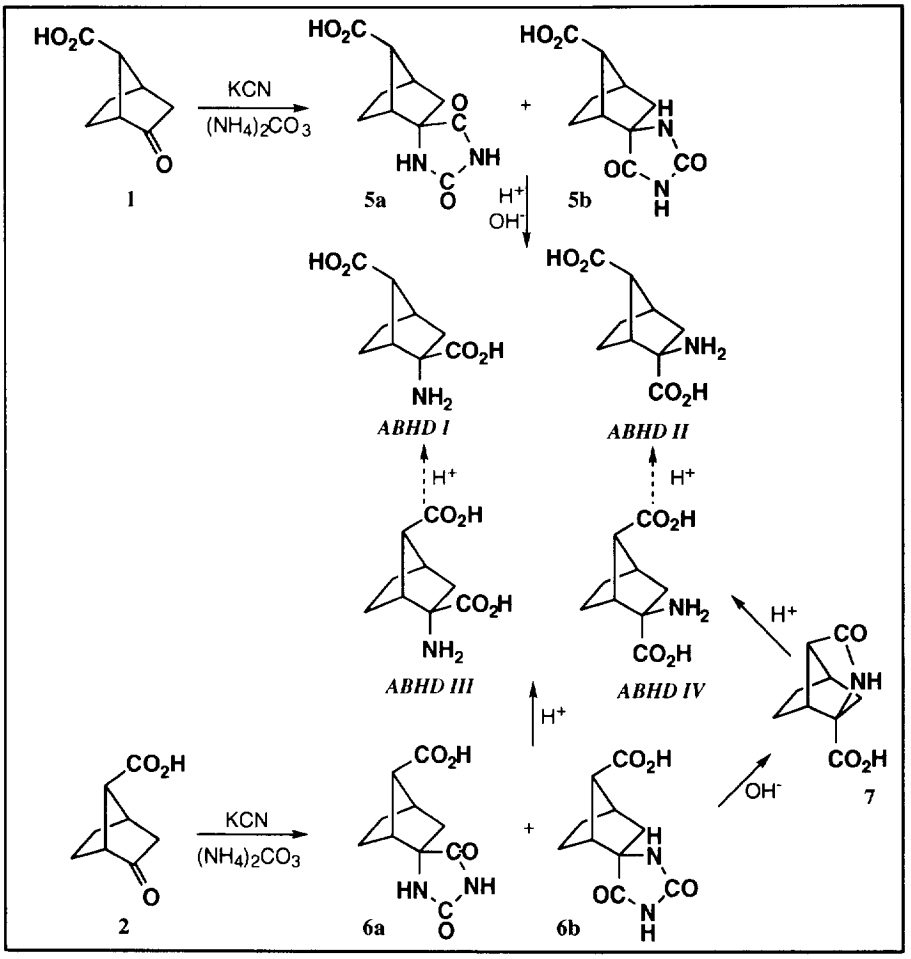
From the ketoacid **2** was obtained a mixture of hydantoins **6a** and **6b** (5:95), the acidic hydrolysis of which afforded mainly **ABHD IV** and small amounts of **ABHD I** and **ABHD II** (90:5:5). The latter amino acids resulted from an acidic epimerisation at C-7 of **ABHD III** and **ABHD IV**, respectively. Alkaline hydrolysis of the **6a+6b** mixture afforded mainly the lactam **7** and a small amount of **ABHD III** (95:5), which could not be isolated and spontaneously epimerized to **ABHD I**. The lactam **7** was characterized as its methyl ester [25] by ^{1}H and ^{13}C-NMR, mass spectrometry and IR spectra. The crude alkaline hydrolysis mixture could also be converted to **ABHD IV** by mild acidic hydrolysis (HCl 2N, 8 h, 100 °C), together with small amounts of **ABHD I** and **ABHD II**, in the same 90:5:5 ratio that was obtained by direct acidic hydrolysis of hydantoins **6**. The structure assignment of the lactam and the latter conversion establish the relative stereochemistry of this set of four analogs.

The ketoacid **3** gave a 1:1 mixture of hydantoins **8a** and **8b** (Scheme 3) which were completely destroyed by HCl hydrolysis. Alkaline hydrolysis afforded **ABHD V** and **ABHD VI**. Their structure assignment was obtained from NOE measurements, after derivatization to the corresponding N-Boc-dimethyl esters: a significant NOE effect between the C<u>H</u>-CO_2Me hydrogen and the N<u>H</u>- group could be observed with the derivative of **ABHD VI**; no effect was observed with the **ABHD V** derivative.

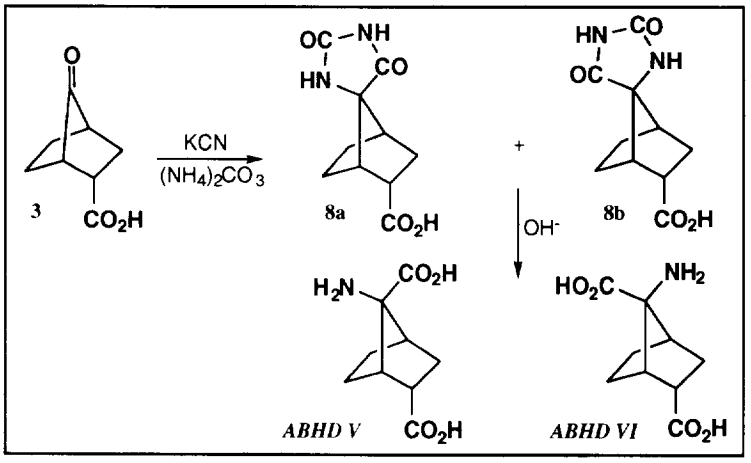
Typical experimental procedure: A solution of ketoacid (0.01 mol) in 30 ml of EtOH-water (1:1), $(NH_4)_2CO_3$ (0.044 mol) and potassium cyanide (0.012 mol) was heated with magnetic stirring at 55-60 °C (5 h for **1** and **3**, 48 h for **2**). The temperature was then raised to 90 °C during 1 h to eliminate excess ammonium carbonate. After evaporation of EtOH, the reaction mixture was acidified with HCl (for **1** and **2**) or H_2SO_4 (**3**) and evaporated to dryness. The residue was treated with EtOH and the solution filtered to eliminate salts. After evaporation of the solvent, crude crystalline hydantoins were obtained and used without further purification (yield: about 90%). The quantitative hydrolysis of hydantoins into the corresponding amino acids was performed in a tightly closed bottle, either in acidic (6N HCl solution, 5 days, 120 °C) or in alkaline conditions (2N $Ba(OH)_2$ solution, 48 h, 120 °C). The amino acid mixture resulting from hydrolysis was first purified by adsorption on a column of Dowex 50 (H^+) followed by elution with 0.5 N NH_4OH, then separated by anion exchange chromatography on an AG-1X4 (AcO^-) column eluted with increasing concentrations of AcOH. The amino acids were then characterized by ^{1}H and ^{13}C-NMR,[26] and GC-MS after derivatization.[27]

Biological activity

Because 1S,3R-ACPD is the most potent and selective ACPD analog at mGluR1a,[28] the effects of both **ABHD-I** and **ABHD- IV** were first examined on cells transiently expressing mGluR1a. LLC-PK1 cells were transfected with a plasmid containing the cDNA of mGluR1a and incubated overnight in the presence of [^{3}H]-inositol as previously described.[28] Twenty four hours later, transfected cells were stimulated with the indicated compounds in the presence of 10 mM LiCl. The total amount of inositol phosphate (IP) produced in the cells was determined as previously described.[28] Neither **ABHD I** nor **ABHD IV** (at a 1mM concentration) stimulated IP formation in these cells, whereas 1S,3R-ACPD induced a two-fold increase in the IP formation (data not shown).



Scheme 2



Scheme 3

The 1S,3R-ACPD effect (EC_{50}= 9.3 ± 2 μM) was inhibited by either **ABHD I** or **ABHD IV**.[29] Because **ABHD I** was found to be more potent than **ABHD IV**, a complete analysis of its effect was undertaken. In the presence of 1 mM **ABHD I**, the dose response curve for 1S,3R-ACPD was shifted to the right, in agreement with a competitive inhibition (Figure 1a). Using the equation $EC_{50}(I) = EC_{50}$ (I+ [I]/K_B), where $EC_{50}(I)$ is the EC_{50} of the agonist determined in the presence of a concentration [I] of antagonist, a K_B value of 308 ± 25 μM (n=4) was calculated. Increasing the concentration of **ABHD I** induced a total inhibition of the IP production stimulated by 10 μM 1S,3R-ACPD, with an IC_{50} = 630 μM (Figure 1b). An estimated K_B value of 304 μM can be calculated according to $IC_{50} = K_B$ (1 + [agonist]/EC_{50}), in agreement with the above calculation. This K_B value (for a racemic compound) is in the same range as those reported for all known other competitive antagonists of mGluR1a.

The present results confirm that the lack of flexibility of glutamate analogs can promote antagonist activity at metabotropic glutamate receptor, suggesting that some flexibility is needed for agonist activity as previously noticed at NMDA receptors.[10,11] Further detailed biological investigations with (±)-**ABHD I** to **VIII** and their separated enantiomers, including potential activity on other mGluRs receptors, are in progress.

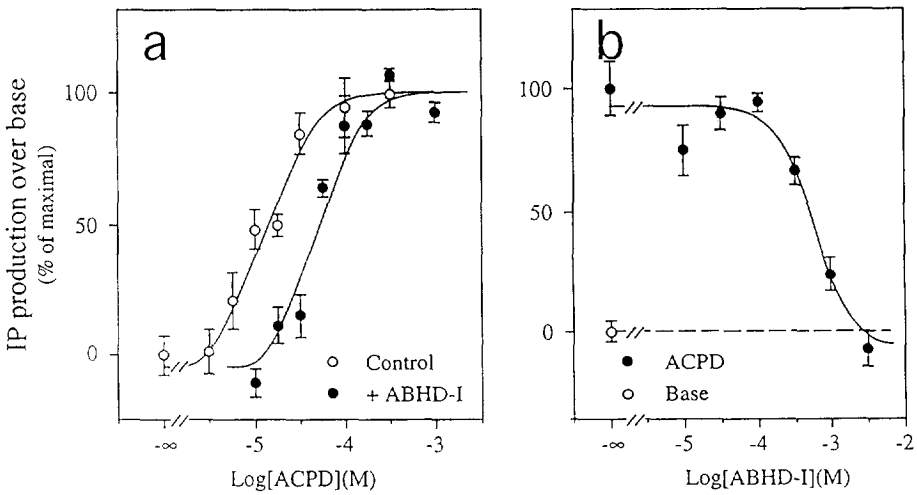


Figure 1: Inhibition of the 1S,3R-ACPD stimulation of IP formation by **ABHD I** in LLC-PK1 cells expressing mGluR1a. (a) After electroporation, cells were preincubated for 12 h with [^{3}H]-inositol, washed and incubated for 30 min in the presence of the indicated concentration of 1S,3R-ACPD, with (•), or without (o) 1 mM **ABHD I**. (b) Cells were not stimulated (o), or stimulated with 10 μM 1S,3R-ACPD (•), in the presence of the indicated concentration of **ABHD I**. Results are expressed as the percentage of the maximum IP production over base. Values are means ± s.e.m. of triplicate determinations from a typical experiment.

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References and Notes

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23. ^{1}H- and ^{13}C-NMR spectra were recorded at 250 MHz (δ in ppm). **3** ($CDCl_3$): ^{1}H-NMR: 1.55-2.35 (m,6H), 3.05-3.25 (m,2H), 4.10 (m,1H); ^{13}C-NMR: 18.99 (CH_2), 23.52 (CH_2), 26.73 (CH_2), 38.58 (CH), 38.70 (CH), 41.20 (CH), 178.39 (CO_2H), 213.76 (CO).
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25. The methyl ester of **7** was prepared by methylation of **7** (HCl in MeOH) or from **ABHD IV** ($SOCl_2$/MeOH).
26. **ABHD I** (D_2O, HCl-salt) ^{1}H-NMR: 1.45-1.72 (m,3H), 1.87-1.97 (m,2H), 2.46 (dm,1H), 2.66 (m,1H), 2.84 (m,1H), 3.57 (m,1H).^{13}C-NMR: 23.53 (CH_2), 28.12 (CH_2), 40.77 (CH), 41.87 (CH_2), 49.57 (CH), 56.06 (CH), 60.04 (quat.C), 176.52 (CO_2H), 178.31 (CO_2H)
ABHD II (D_2O, HCl-salt) ^{1}H-NMR: 1.44-1.53 (m,2H), 1.73-1.96 (m,3H), 2.37 (dm,1H), 2.75 (m,1H); 2.89 (m,1H), 3.18 (m,1H).^{13}C-NMR: 25.03 (CH_2), 27.44 (CH_2), 41.72 (CH), 41.81 (CH_2), 50.05 (CH), 55.67 (CH), 68.54 (quat.C), 174.91 (CO_2H), 177.94 (CO_2H).
ABHD IV (D_2O, HCl-salt) ^{1}H-NMR: 1.36-1.55 (m,2H), 1.70 (m,1H), 1.83 (dm,1H), 1.96 (dm,1H), 2.53 (d,1H), 2.83 (m,1H), 2.92 (m,1H), 2.99 (s,1H). ^{13}C-NMR: 27.75 (CH_2), 28.95 (CH_2), 40.54 (CH_2),, 44.93 (CH), 49.45 (CH), 58.62 (CH), 68.09 (quat.C), 175.16 (CO_2H), 182.10 (CO_2H).
ABHD V (D_2O, NH_4^+salt) ^{1}H-NMR: 1.55-1.83 (m,5H), 2.15 (m,1H), 2.42 (m,1H), 2.64 (m,1H), 3.12 (m,1H). ^{13}C-NMR: 24.73 (CH_2), 29.15 (CH_2), 34.01 (CH_2), 43.93 (CH), 47.37 (CH), 48.73 (CH), 76.96 (quat.C), 177.08 (CO_2H), 184.49 (CO_2H).
ABHD VI (D_2O, NH_4^+salt) ^{1}H-NMR: 1.47-1.53 (m,2H), 1.89-1.98 (m,3H), 2.03 (m,1H), 2.42 (m,1H), 2.64 (m,1H), 2.99 (m,1H).^{13}C-NMR: 25.96 (CH_2), 30.11 (CH_2), 32.56 (CH_2), 44.15 (CH), 47.27 (CH), 48.37 (CH), 77.78 (quat.C), 177.05 (CO_2H), 184.01 (CO_2H).
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29. Preliminary results with **ABHD V** and **ABHD VI** indicated no or marginal antagonist activity, respectively.

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