Communications to the Editor

1-Aminoindan-1,5-dicarboxylic Acid: A Novel Antagonist at Phospholipase C-Linked Metabotropic Glutamate Receptors

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Introduction. L-Glutamic acid (L-Glu) is the major neurotransmitter at excitatory synapses in the central nervous system (CNS) and is considered to be involved in events leading to neuronal plasticity and in those involved in neuronal death.[1,2] Synaptically released L-Glu acts on ionotropic and metabotropic classes of excitatory amino acid (EAA) glutamate receptors. Ionotropic EAA receptors belong to the ligand-gated ion channels superfamily, while the metabotropic glutamate receptors (mGluRs) are coupled to cyclic AMP (cAMP) as well as to inositol triphosphate/diacylglycerol (IP_3/DG) effector systems through GTP binding proteins.[3,4] The characterization of mGluR subtypes has been accomplished by functional expression of individual mGluR subtypes in model oocyte systems and has been facilitated by the availability of the selective agonist 1-aminocyclopentane-1,3-dicarboxylic acid [(1*S*,3*R*)-ACPD]. To date, eight metabotropic glutamate receptor subtypes (mGluR1 to mGluR8) have been sequenced and functionally characterized. They may be classified into three distinct subgroups according to their sequence homology, transduction mechanism, and agonist selectivity.[3] Group I includes mGluR1 and mGluR5 which are coupled to the phosphoinositide/Ca^{2+} cascade and has a high affinity for quisqualate. (1*S*,3*S*)-*trans*-Azetidinedicarboxylic acid (*t*-ADA)[5] selectively activates mGluR5. Group II contains mGluR2 and mGluR3 subtypes which are highly sensitive to (1*S*,3*R*)-ACPD and are coupled to inhibition of cAMP formation. (2*S*,3*S*,4*S*)-α-(Carboxycyclopropyl)glycine (CCG I)[6] and (2*S*,1′*R*,2′*R*,3′*R*)-2-(-2,3-dicarboxycyclopropyl)glycine (DCG IV)[7] are potent and selective agonists of mGluR2 subtype. Finally, group III contains mGluR4, mGluR6, mGluR7, and the newly reported[8] mGluR8 receptor subtypes which are activated by L-2-amino-4-phosphonobutyrate (L-AP4)[9] and are coupled to inhibition of cAMP formation.

The physiological role played by mGluRs is still largely unclear, although there is evidence that mGluRs are involved in the induction of long-term potentiation (LTP),[10] depotentiation,[11] and long-term depression (LTD)[12] of synaptic transmission in hippocampus. Most of the experiments performed so far for assessing the physiological role of mGluR subtypes have relied on indirect approaches or on the recently available technique of gene knock out.[13] Indeed, only few mGluR antagonists have so far been described. The α-methyl derivative of L-AP4 (MAP4) selectively antagonizes the presynaptic depressant action of L-AP4 while the α-methyl derivative of (2*S*,3*S*,4*S*)-CCG I has been shown to antagonize the (1*S*,3*R*)-ACPD-induced presynaptic depression.[14] (Carboxyphenyl)glycines, first reported as metabotropic agonists and antagonists by Watkins and co-workers in 1992, represent the most interesting class of compounds so far described.[15] In particular, (*S*)-(4-carboxyphenyl)glycine (*S*-4CPG, **1**, Chart 1)[16] and (*S*)-(4-carboxy-3-hydroxyphenyl)glycine (*S*-4C3HPG, **2**)[17] have been found to act as antagonists at mGluR1 and as agonists at mGluR2 receptor subtypes; (*S*)-(3-hydroxyphenyl)glycine (*S*-3HPG, **3**)[18] is a mGluR1 agonist and (±)-α-methyl-(4-carboxyphenyl)glycine [(±)-α-M4CPG, **4**][16] is an antagonist at both mGluR1 and mGluR2 receptor subtypes.

As a part of a project directed to the design of new and selective mGluR modulators,[19] we report now the synthesis and preliminary biological evaluation of (±)-1-aminoindandicarboxylates **5**–**7**, in which the (carboxyphenyl)glycine moiety is inserted in a constrained framework.

Chemistry. The preparation of (±)-1-aminoindan-1,4-dicarboxylic acid (**5**, UPF 531) (Scheme 1) was achieved by utilizing the known[20] 4-indancarboxylic acid (**8**) as starting material. Treatment of **8** with diazomethane followed by Jones oxidation of the corresponding ester **9** gave the ketone **10** (11%), which was then submitted to Bucherer–Bergs reaction to give the corresponding hydantoin **11** (37%).[21] Alkaline hydrolysis with barium hydroxide[22] followed by ion exchange resin chromatography (Dowex 1 × 8-200, AcO^- form) afforded (±)-1-aminoindan-1,4-dicarboxylic acid (**5**) (31%).[23] For the preparation of (±)-1-aminoindandicarboxylic acids **6** (UPF 523) and **7** (UPF 524), 5-indancarboxylic acid (**12**)[24] was selected as starting material (Scheme 2). Esterification of **12** with diazomethane followed by Jones oxidation of the corresponding ester **13** afforded a mixture of 5- and 6-carbomethoxyindanones **14** and **15** (26 and 30%, respectively).[25] The two ketones **14** and **15** were separated by medium-pressure chromatography and submitted to Bucherer–Bergs reaction at 120 °C to give the corresponding hydantoins **16** and **17** in 58% and 43% yield, respectively.[21] Alkaline hydrolysis of **16** and **17** with barium hydroxide at 120 °C for 3 h followed by ion exchange resin chromatography (Dowex 50WX2-200) and reversed-phase medium-pressure chromatography afforded (±)-1-aminoindan-1,5-dicarboxylic acid (**6**) and (±)-1-aminoindan-1,6-dicarboxylic acid (**7**) in 43 and 30% yield, respectively.[23] Several attempts to separate the enantiomers constituting **6** have so far been unsuccessful.

Biological Results and Discussion. The metabotropic glutamate receptor activity profile of rigidified compounds **5**–**7** was evaluated through functional as-

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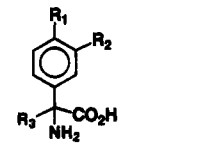
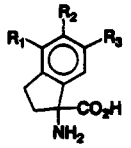
Chart 1

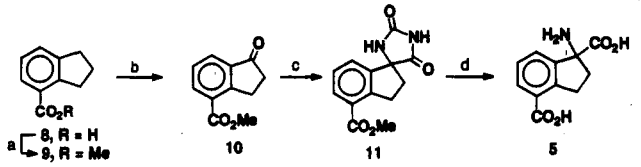


1, $R_1 = CO_2H$, $R_2 = R_3 = H$
2, $R_1 = CO_2H$, $R_2 = OH$, $R_3 = H$
3, $R_1 = H$, $R_2 = OH$, $R_3 = H$
4, $R_1 = CO_2H$, $R_2 = H$, $R_3 = Me$



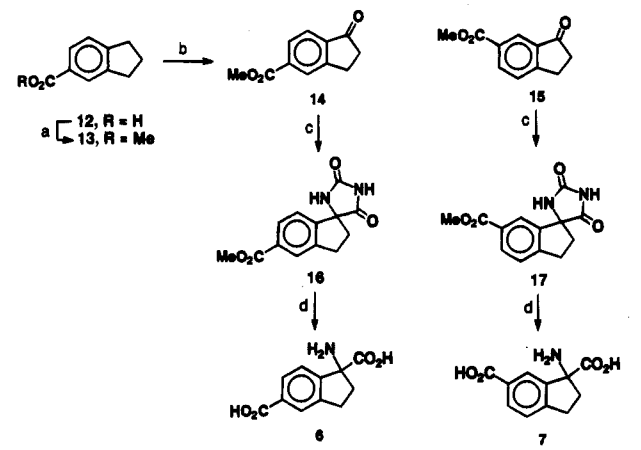
5, $R_1 = CO_2H$, $R_2 = R_3 = H$
6, $R_1 = H$, $R_2 = CO_2H$, $R_3 = H$
7, $R_1 = R_2 = H$, $R_3 = CO_2H$

Scheme 1[a]



[a] (a) CH_2N_2, Et_2O; (b) CrO_3, AcOH; (c) NaCN, $(NH_4)_2CO_3$, DMF, H_2O; (d) (i) $Ba(OH)_2{\cdot}8H_2O$; (ii) Dowex 1×8-200, AcO^- form, 0.3 N AcOH.

Scheme 2[a]



[a] (a) CH_2N_2, Et_2O; (b) CrO_3, AcOH, H_2O; (c) KCN, $(NH_4)_2CO_3$, DMF, H_2O; (d) (i) $Ba(OH)_2{\cdot}8H_2O$; (ii) Dowex, 50×2-200, 10% py; (iii) RP-8 mpc.

says and binding experiments. In particular, compounds **5**–**7** were not able to displace at 100 μM [^{3}H]-ligand binding from AMPA, KA, and NMDA ionotropic glutamate receptors in rat cortical membranes.[26] The functional activity of compounds **5**–**7** at metabotropic glutamate receptor subtypes was evaluated by measuring second messenger formation in baby hamster kidney (BHK) cell lines stably expressing individual mGluR subtypes which are representative of group I (mGluR1α), group II (mGluR2), or group III (mGluR4) mGluRs.[27] Compound **6** inhibited glutamate-stimulated phosphoinositide (PI) hydrolysis in BHK cells expressing mGluR1α in a dose-dependent manner as shown in Figure 1. The concentration of **6** inhibiting half of the maximal response by this compound was 7 ± 2 μM (Table 1). No effects on basal PI hydrolysis was observed by compound **6** at concentrations ranging from 1 to 100 μM, suggesting that **6** is not a partial agonist at mGluR1α. However, further studies are necessary in order to establish whether the antagonism of mGluR1α-mediated PI hydrolysis is competitive or not. The incomplete antagonism of mGluR1α-mediated PI hydrolysis by **6** may be related to limitations in the solubility of the compound or to an interaction between **6** and L-Glu uptake carrier(s).[28] Compounds **5** and **7** did not show any agonist or

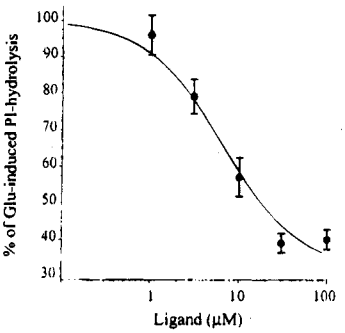


Figure 1. Antagonism of glutamate-induced PI hydrolysis in mGluR1 α expressing cells by **6**. The cells were exposed to the antagonist 5 min before the addition of 10 μM glutamate. Data are the mean of three experiments done in triplicate and are expressed as percentage over glutamate-induced PI hydrolysis with basal levels of PI hydrolysis subtracted. **6** did not increase basal levels of PI hydrolysis at 100 μM.

Table 1. Functional Activities of (±)-1-Aminoindandicarboxylic Acids **5**–**7** and Representative (Carboxyphenyl)glycines at Metabotropic Glutamate Receptor Subtypes

	IC_{50}[a]		
compound	mGluR1α PI hydrolysis	mGluR2 cAMP	mGluR4 cAMP
5 (UPF 531)	NE[b]	NE[b]	NE[b]
6 (UPF 523)	7 ± 2	NE[b]	NE[b]
7 (UPF 524)	NE[b]	NE[b]	NE[b]
1 ((*S*)-4CPG)	65 ± 5[c]	577 ± 74[c]	>1000[c]
2 ((*RS*)-4C3HPG)	40 ± 3[c]	48 ± 5[c,d]	>1000[c]
4 ((*RS*)-α-M4CPG)	155 ± 38[c]	340 ± 59[c]	>1000[c]

[a] The values represent half-maximal concentrations (μM) for inhibition of functional responses in BHK cells. [b] Not effective at the highest concentration tested (100 μM). [c] Data are from ref 27. [d] (*RS*-4C3HPG) is an agonist at mGluR2.

antagonist activity at mGluR1α when tested in concentrations up to 100 μM (Table 1). When measuring forskolin-stimulated cAMP formation in BHK cells expressing mGluR2 or mGluR4, compounds **5**, **6**, or **7** were not able to inhibit cAMP formation (agonist effect) or to reverse the inhibitory effects of 50 μM glutamate (antagonist effect) (Table 1).

Thus, **6** is a potent antagonist of group I mGluRs, having no effects on group II or group III mGluRs or on ionotropic glutamate receptors at the higher concentration tested (100 μM). While further data are necessary to establish its precise pharmacological profile, **6** can be anticipated as a potent and selective group I antagonist.

Since **6** is more rigid than the corresponding phenylglycine derivatives, it can provide valuable informations on the conformational requirements essential to mGluR group I subtype selectivity. Thus, a comparison of the potential energy surfaces of the representative (carboxyphenyl)glycine derivatives 1 and **4** along with the pseudotorsional angle values of **6** is reported in Figure 2. As expected, the amino acidic side chain can freely rotate in both **1** and **4** and this can be responsible for their poor mGluR subtype selectivity. On the other hand, **6** exhibits only two low-energy conformations, corresponding to the envelope extremes of the 5-membered ring. The improved potency of **6** over **1** and **4** can therefore be attributed to an optimal disposition of the amino acidic moiety relative to the phenyl carboxylate. These preliminary observations can be instrumental in the design of new, conformationally constrained, (carboxyphenyl)glycine analogs.

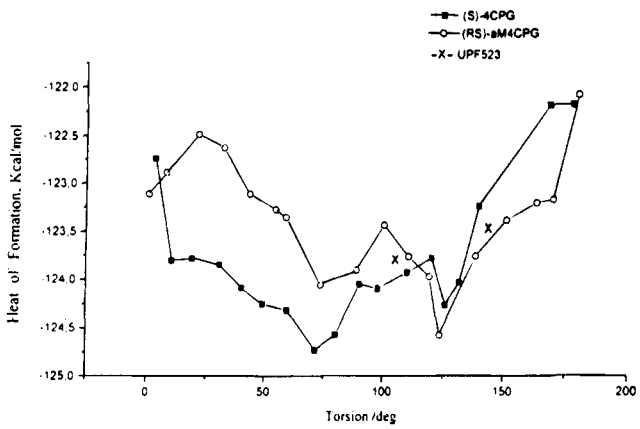


Figure 2. Conformational energy (kcal/mol) of *S*-4CPG (**1**), *(RS)*-α-M4CPG (**4**) and **6** as a function of the dihedral angle between the phenyl ring and the α-carbon. The energy was computed by the Mopac semiempirical method at the AM1 level.

In conclusion, we report the synthesis and preliminary biological profile of (±)-1-aminoindandicarboxylates **5**–**7**, constrained (carboxyphenyl)glycine analogs. Among them, (±)-1-aminoindan-1,5-dicarboxylic acid (**6**) has proved to be a truly selective and potent ligand for group I glutamate metabotropic receptor subtypes, useful for the determination of their hitherto ill-defined role. The exploitation of this series of compounds for molecular modeling studies and as source of additional antagonists of mGluR receptors with improved potency and selectivity is currently underway.

Supporting Information Available: Experimental data for **5**–**7**, **9**–**11**, and **13**–**17** (4 pages). Ordering information is given on any current masthead page.

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(23) Selected spectroscopic data for compounds **5**–**7**. **5**: ^{1}H-NMR (D_2O + HCl) δ 2.27 (1H, m, 2-CHa), 2.72 (1H, m, 2-CHb), 3.32 (2H, t, J = 7.2 Hz, 3-CH_2), 7.30 (1H, 2d, J = 7.7 Hz, 6-CH), 7.48 (1H, d, J = 7.7 Hz, 7CH), 7.87 (1H, d, J = 7.7 Hz, 5-CH); ^{13}C-NMR (D_2O + CD_3OD) δ 31.50, 34.72, 68.39, 123.08, 126.73, 128.23, 132.60, 139.38, 145.21, 147.12, 169.57, 172.93. **6**: ^{1}H-NMR (D_2O) δ 2.20 (1H, m, 2-CHa), 2.50 (1H, m, CHb), 2.95 (2H, t, J = 7.8 Hz, 3-CH2), 7.05 (1H, d, J = 7.8 Hz, 7-CH), 7.55 (1H, d, J = 7.8 Hz, 6-CH), 7.60 (1H, s, 4-CH); ^{13}C-NMR (D_2O) δ 30.42, 35.50, 68.70, 123.00, 127.20, 129.50, 132.50, 142.90, 146.00, 169.90, 172.70. **7**: ^{1}H-NMR (D_2O) δ 2.28 (1H, m, 2-CHa), 2.72 (1H, m, 2-CHb), 3.08 (2H, t, J = 6 Hz, 3-CH_2) 7.35 (1H, d, J = 8 Hz, 4-CH), 7.82 (1H, s, 7-CH), 7.85 (1H, d, J = 8 Hz, 5-CH); ^{13}C-NMR (D_2O) δ 30.30, 35.00, 68.54, 124.67, 125.93, 129.26, 131.90, 138.55, 151.10, 169.60, 173.32.

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