Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 9, pp. 1195-1198, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00177-7

SYNTHESIS AND BIOLOGICAL ACTIVITY OF CYCLIC ANALOGUES OF MPPG AND MCPG AS METABOTROPIC GLUTAMATE RECEPTOR ANTAGONISTS

Dawei Ma,*[a] Hongqi Tian,[a] Hongbin Sun,[a] Alan P. Kozikowski,*[b] Sergey Pshenichkin,[c] Jarda T. Wroblewski[c]

[a]Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fengling Lu, Shanghai 200032, China, [b]Drug Discovery Laboratory, Georgetown University Medical Center, Institute for Cognitive & Computational Sciences, Drug Discovery Laboratory, 3970 Reservoir Road, N.W., Washington, DC 20007-2197, and [c]Department of Pharmacology, Georgetown University Medical Center, 3900 Reservoir Road, N. W., Washington, D.C. 20007

Abstract: The synthesis of two rigidified phenylglycine analogues is disclosed. The cyclic analogue **1** of (*R*,*S*)-α-methyl-4-phosphonophenylglycine (MPPG) is shown to be a particularly interesting pharmacological tool, for it is a group II selective mGluR antagonist that possesses an inverse agonist-like action.

The amino acid glutamate (Glu) plays a pivotal role in biological processes ranging from memory and learning to neuronal degeneration. This major excitatory amino acid (EAA) acts through disparate Glu receptors, which can be categorized into two distinct types, the so-called ionotropic glutamate receptors and the metabotropic glutamate receptors.[1] The ionotropic Glu receptors, or iGluRs, are associated with integral cation-specific ion channels and include the NMDA [*N*-methyl-D-aspartic acid], AMPA [2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid], and KA (kainic acid) subtypes. On the other hand, the metabotropic Glu receptors (mGluR) are coupled to cellular effectors through GTP-binding proteins. The mGluRs have been distinguished pharmacologically from the iGluRs by the use of the mGluR-selective agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid [(1*S*,3*R*)-ACPD] generally through measurements involving phosphoinositide hydrolysis or Ca^{2+} mobilization. To date the use of expression cloning techniques has led to the identification of eight mGluR subtypes which have been placed into three major categories based on their molecular structure, signal transduction mechanisms, and pharmacological properties. Group I mGluRs (mGluR1 and 5) are coupled to phosphoinositide (PI) hydrolysis, whereas group II (mGluR2 and 3) and group III (mGluR4, 6, 7, and 8) are negatively linked to adenylyl cyclase activity. The group I receptors are more sensitive to quisqualic acid than they are to ACPD, the group II receptors are more sensitive to ACPD than quisqualic acid, and the group III receptors are most sensitive to 2-amino-4-phosphonobutyric acid (L-AP4).[2]

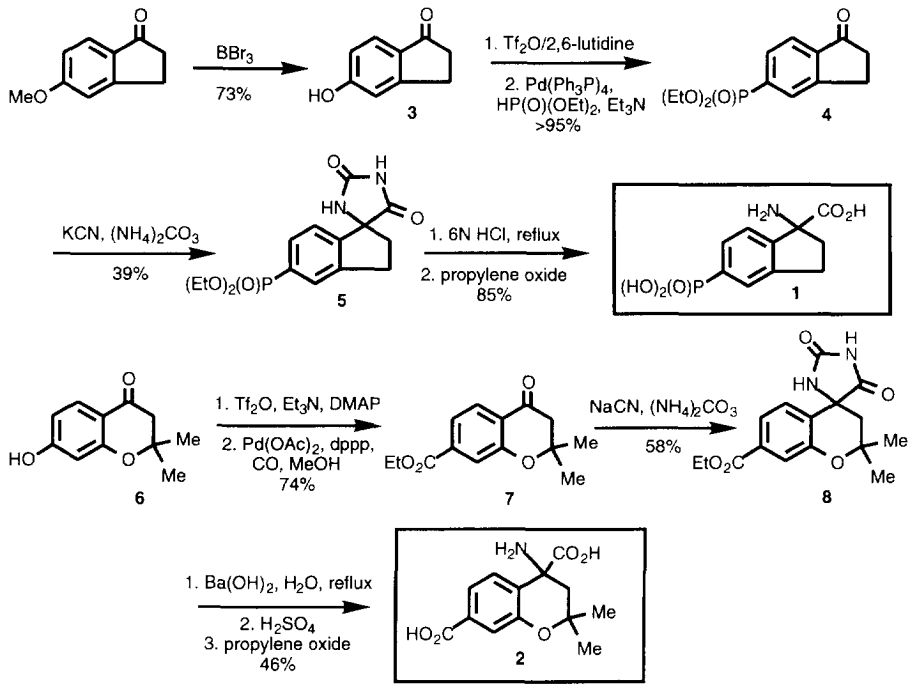
In order to better characterize the roles of GluRs in physiological processes, there is an important need to identify novel, high affinity ligands which are family and subtype specific. Due to Watkins' pioneering work, a number of phenylglycine derivatives have been identified that exhibit agonist, antagonist, or partial agonist actions depending on the system in which they are tested; however, some phenylglycines have more selective actions.[3] Among these compounds, 4-carboxy-3-hydroxyphenylglycine (4C3HPG) was described as a potent antagonist of group I mGluRs, but also as a relatively potent agonist of group II mGluRs. In contrast, α-methyl-4-carboxyphenylglycine (MCPG) inhibits a variety of ACPD-induced effects and has been shown to be a low potency antagonist of group I and II mGluRs.[4] Another derivative, 4-carboxyphenylglycine is an antagonist of group I mGluRs, showing a certain selectivity for mGluR1 over mGluR5.[5] Extensive studies are being carried out with α-alkyl substituted isosteres of these phenylglycines, including some phosphono and tetrazolyl derivatives.[6] These studies have provided some knowledge of the steric and electronic requirements for the particular mGluR subtypes, and identified α-methyl-4-phosphonophenylglycine (MPPG) as a potent

antagonist for group III receptors.[6] Recently, α-methyl-3-carboxyphenylalanine was reported as a potent antagonist of these receptors.[7] A different derivative of phenylglycine, 3,5-dihydroxyphenylglycine (DHPG) was reported to act as a selective agonist of group I mGluRs.[8] Lastly, Pellicciari has described the rigidified MCPG analogue, 1-aminoindan-1,5-dicarboxylic acid, as a selective, albeit low potency antagonist of transfected mGluR1 receptors.[9]

Herein we describe the chemistry and biology of two new additional members of this phenylglycine family of mGluR ligands, the cyclic MPPG analogue **1**, (*R,S*)-1-amino-5-phosphonoindan-1-carboxylic acid, and the novel chroman analogue of MCPG, compound **2**, (*R,S*)-4-amino-2,2-dimethylchroman-4,7-dicarboxylic acid.

Chemistry. As outlined in the accompanying scheme, we synthesized the rigidified MPPG analogue **1** starting from 5-methoxy-1-indanone which was deprotected by treatment with boron tribromide to yield **3** (Scheme 1). Compound **3** was converted to the corresponding triflate,[10] and this intermediate was coupled with diethyl phosphite catalyzed by tetrakis(triphenylphosphine)palladium[11] to afford the diethyl arylphosphonate **4**. Application of the Bucherer-Bergs reaction[12] to **4** gave the corresponding hydantoin **5**, which was refluxed in 6N HCl and then treated with propylene oxide to deliver **1** in 85% yield.[13] Related chemistry was used to construct the chroman analogue **2**. 2,2-Dimethyl-7-hydroxy-4-chromanone **6** was synthesized from resorcinol according to a known procedure.[14] Compound **6** was converted to its triflate intermediate, and then a palladium-catalyzed carbonylation reaction[15] was carried out to provide ester **7**. This ester was submitted to the Bucherer-Bergs reaction to generate **8**. The hydrolysis of **8** with barium hydroxide at reflux followed by ion exchange resin chromatography (Dowex) afforded the MCPG analogue **2** in 46% yield.[16]

Scheme 1. Synthesis of New Phenylgylcine Analogues.



Biology. Methods. Chinese hamster ovary (CHO) cells stably expressing mGluR1a, mGluR5a, mGluR2, or mGluR6 were cultured as described previously[17] and used for measurements of phosphoinositide (PI) hydrolysis or cAMP formation. For measurements of PI hydrolysis, cells expressing mGluR1a or mGluR5a were cultured in 24-well plates and then labelled overnight with 1 μCi/mL of [^{3}H]*myo*-inositol (specific activity 17 Ci/mmol, Amersham). CHO cells expressing mGluR2 or mGluR6 were cultured in 96-well plates. Measurements of PI hydrolysis and of forskolin-induced cAMP formation were performed as described previously.[18,19] Results from these experiments are provided in the accompanying Table 1.

Results. As shown in Table 1, the cyclic chroman analogue **2** of MCPG is an antagonist of mGluR1a, but also shows activity at mGluR2. The compound is much less active at mGluR5a, and inactive at mGluR6. In contrast, the other known cyclic analogue of MCPG, 1-aminoindan-1,5-dicarboxylic acid, has been reported to be group I selective, and to possess an IC_{50} of about 200 μM.[9]

Table 1. Selectivity of compounds **1** and **2** for the mGluR subtypes. (values are IC_{50}s, μM).[a]

Group	Receptor	Analogue 1	Analogue 2	MPPG
I	mGluR1a	NE	93 ± 14	NE
	mGluR5a	NE	355 ± 59	NE
II	mGluR2	30 ± 5	43 ± 12	11 ± 3
III	mGluR6	NE	NE	480 ± 81

[a]NE = no significant effect observed at 1 mM; values are means ± SEM from 3-4 experiments.

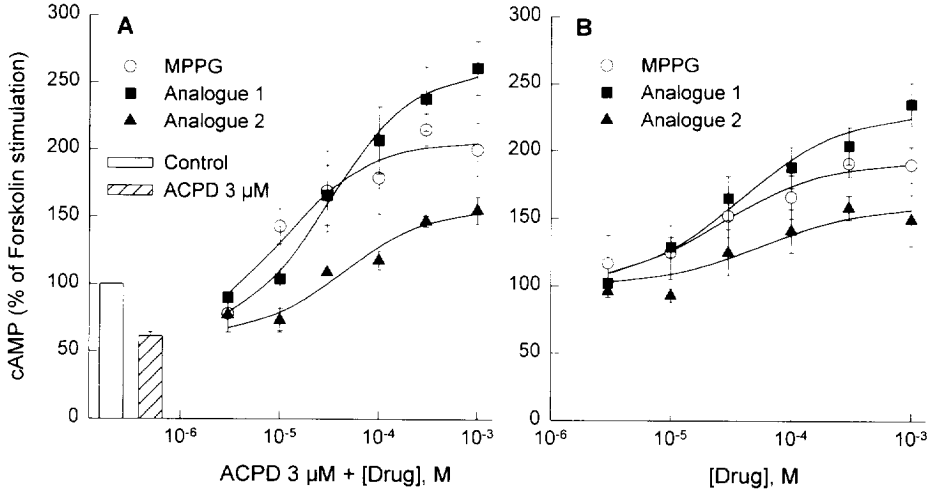


Figure 1. Dose response curves for the effects of compounds **1** and **2** and MPPG at mGluR2 receptors. **A**. Antagonism of the inhibitory effect of 3 μM ACPD, and **B**. effect on basal (agonist independent) receptor activity. The points represent means ± SEM from four experiments. The forskolin stimulated cAMP production in control conditions was 199 ± 16 pmol cAMP/mg protein/5 min.

Of greater interest, however, is the activity shown by the cyclic analogue **1** of MPPG. The compound appears to be selective for group II receptors, with an IC_{50} of 30 μM at mGluR2 when applied in presence of 3 μM ACPD. Its inhibitory potency is only slightly less than that of MPPG, thus defining the preferred conformation of MPPG at the group II receptors. The transfection of CHO cells with mGluR2 receptors results in the expression of receptors endowed with agonist-independent intrinsic activity. When tested in presence of 3 μM ACPD (Figure 1A), all three compounds were able not only to reverse the effect of ACPD, but were able to induce an elevation in the level of cAMP formation above that caused by forskolin alone. When tested in absence of agonists (Figure 1B) all three compounds increased the level of forskolin-stimulated cAMP formation but with a different efficacy. Similar inverse agonist effects were observed with other known group II antagonists, such as MCCG, MCPG, and α-ethylglutamate (data not shown). Inverse agonism was not observed with mGluR6 receptors expressed in the same CHO cells and assayed under identical conditions.

Summary. The syntheses of two new structurally rigid phenylglycine analogues, one of MPPG and and the other of MCPG, are disclosed herein. The cyclic chroman analogue **2** of MCPG is an antagonist of mGluR1a, but also shows activity at mGluR2. The compound is much less active at mGluR5a, and inactive at mGluR6. Analogue **1**, on the other hand, appears to be a particularly promising pharmacological tool in that it was found to be a selective group II antagonist with an IC_{50} of 30 μM. Analogues **1** and **2** and MPPG when tested in the absence of agonists were found to increase the level of forskolin-stimulated cAMP formation. Further experiments to explore the intrinsic activity of mGluR2 and other mGluRs are planned.

Acknowledgment. We are indebted to the National Natural Science Foundation of China, the Chinese Academy of Sciences, the Alexis Corporation, and NIH grant NS01720 (JTW) for support of these studies.

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13. Selected data for **1**: ^{1}H NMR (300 MHz, D_2O) δ 7.58 (d, J = 12.3 Hz, 1H), 7.52 (dd, J = 11.8, 8.7 Hz, 1H), 7.27 (dd, J = 8.7, 2.4 Hz, 1H), 3.10 (t, J = 6.9 Hz, 2H), 2.68 (dt, J = 14.8, 6.9 Hz, 1H), 2.25 (dt, J = 14.8, 6.9 Hz, 1H); MS, *m/z* 257 (M^+), 241, 219, 195, 188, 161.
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19. Tückmantel, W.; Kozikowski, A. P.; Wang, S.; Pshenichkin, S.; Wroblewski, J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 601. Analogue **1** is available from the Alexis Biochemicals Corporation.

(*Received in USA* 18 *November* 1996; *accepted* 4 *April* 1997)