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Synthesis of Phenylglycine Derivatives as Potent and Selective Antagonists of Group III Metabotropic Glutamate Receptors

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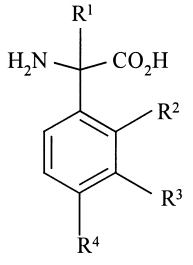
Abstract—The syntheses of a range of ring and α-substituted 4-phosphonophenylglycines are described. A brief discussion of the antagonist activities of compounds **4**–**10** on group I, II and III metabotropic glutamate (mGlu) receptors expressed in the neonatal rat spinal cord is included. © 2001 Elsevier Science Ltd. All rights reserved.

It is now accepted that (*S*)-glutamate ((*S*)-Glu) is the principle excitatory neurotransmitter in the vertebrate central nervous system (CNS).[1] Due to its inherent structural flexibility, (*S*)-Glu acts at a wide variety of glutamate receptor subtypes.[1–3] These glutamate or excitatory amino acid receptors can be divided into two general classes; ionotropic glutamate (iGlu) receptors and metabotropic glutamate (mGlu) receptors, on the basis of molecular cloning, pharmacology and electrophysiology.[1,3] The iGlu receptors include the *N*-methyl-D-aspartate (NMDA), (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainic acid receptor subtypes. These mediate fast synaptic responses via ligand gated ion channels.[3] The mGlu receptors are coupled to intracellular second messenger systems via G-proteins and play a modulatory role in synaptic transmission.[1,2] To date, eight mGlu receptor subtypes have been cloned and these have been divided into three groups on the basis of amino acid sequence homology, pharmacology and coupling to second messenger systems. Group I ($mGlu_1$ and $mGlu_5$) mGlu receptors are selectively activated by (*S*)-3,5-dihydroxyphenylglycine (DHPG). Group II mGlu receptors ($mGlu_2$ and $mGlu_3$) are selectively activated by (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate (APDC) whilst those in group III ($mGlu_{4,6,7,8}$) are selectively activated by (*S*)-2-amino-4-phosphonobutanoic acid ((*S*)-AP4).[2]

Whilst in-roads have been made into the development of group selective antagonists very few show useful selectivity for group III mGlu receptors.[2]

One of the first antagonists to show selectivity for mGlu over iGlu receptors, (*S*)-α-methyl-4-carboxyphenylglycine (MCPG **1**, Fig. 1), displayed activity at all three groups of mGlu receptors.[1,2,4,5] Bioisosteric replacement of the terminal carboxyl group of MCPG with a phosphono group[6,7] to give α-methyl-4-phosphonophenylglycine (MPPG, **2**) led to improved selectivity for group III over group II mGlu receptors, and abolished activity on group I mGlu receptors (see Table 1). A further improvement in potency and selectivity for group III over group II mGlu receptors was obtained by substitution of the α-methyl group for an α-cyclopropyl group to give (*RS*)-α-cyclopropyl-4-phosphonophenylglycine (CPPG, **3**) (see Table 1).[8,9]

In recent years there has been much interest in the possible therapeutic applications of mGlu receptor



1. R^1=Me, R^2=R^3=H, R^4=CO_2H
2. R^1=Me, R^2=R^2=H, R^4= PO_3H_2
3. R^1=Cycpyl, R^2=R^3=H, R^4= PO_3H_2
4. R^1=Me, R^2=R^4=H, R^3=$CH_2PO_3H_2$
5. R^1=Me, R^2=H, R^3=Cl, R^4=PO_3H_2
6. R^1=Me, R^2=H, R^3=OMe, R^4=PO_3H_2
7. R^1=Me, R^2=H, R^3=OMe, R^4=PO_3EtH
8. R^1=R^3= Me, R^2=H, R^4=PO_3H_2
9. R^1=R^2= Me, R^3=H, R^4=PO_3H_2
10. R^1= c-Pr, R^2=H, R^3=OMe, R^4=PO_3H_2

Figure 1. Phenylglycine based group III mGlu receptor antagonists.

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ligands.[1,2,10] Before the therapeutic potential and physiological significance of the mGlu receptor subtypes can be fully explored, it is necessary to develop potent and selective ligands for these receptors. Here, we report the synthesis and initial pharmacological evaluation of seven new phenylglycine derivatives and discuss structural features required for selective antagonist activity at group III mGlu receptors.

Chemistry

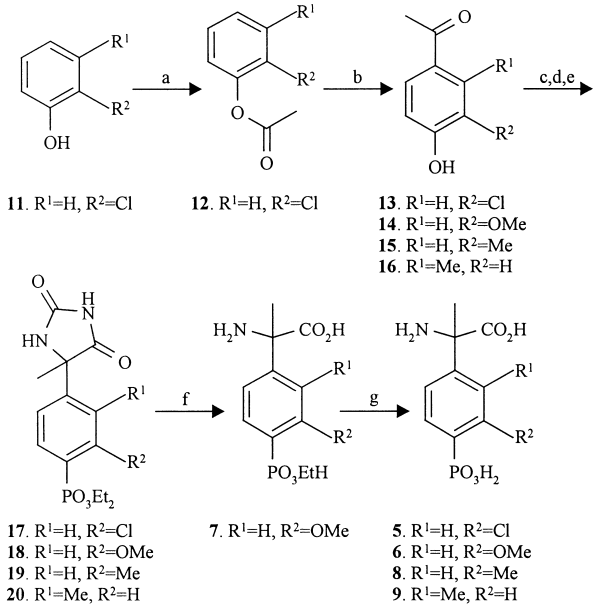
A convenient synthesis of the phenylglycine derivatives **5**–**9** was devised starting from the appropriately substituted acetophenones (**13**–**16**).

As one such acetophenone (**13**) was not commercially available it was synthesized from 2-chlorophenol (Scheme 1). The phenolic alcohol (**11**) was acylated under Schotten–Baumen conditions[11] to give **12** in 79% yield, which was subsequently subjected to a Fries rearrangement[12] to give **13** in 59% yield.

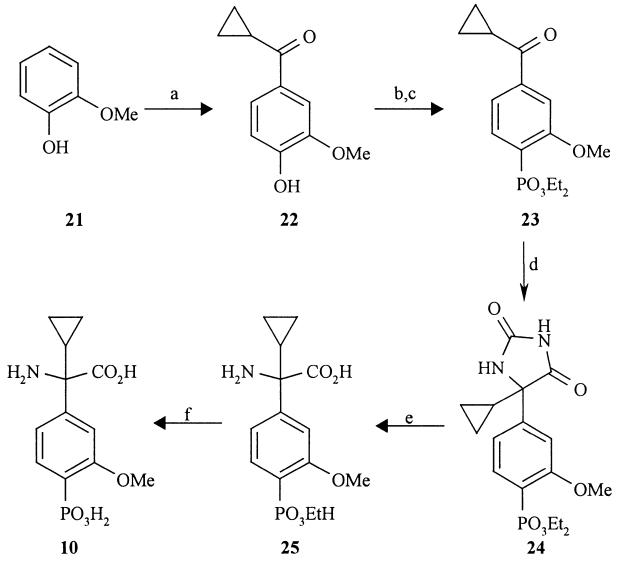
Triflation of the phenols (**13**–**16**)[13] and subsequent treatment with diethyl phosphite and a palladium catalyst[14] yielded the corresponding 4-phosphono-substituted acetophenones. These were converted to the 5′-methylhydantoins (**17**–**20**) via the Bucherer–Bergs reaction.[15] In the case of hydantoin (**18**), base hydrolysis gave the monoethyl ester of the phosphonate (**7**) in 34% yield. The monoethyl ester is exclusively formed due to the formation of the monosodium salt of the monoethoxyphosphono group, which prevented further hydroxyl attack. For other hydantoins the monoesters were not isolated as the diacids (**5**, **6**, **8** and **9**) were obtained directly by hydrolysis in 6 N HCl (5–50% yield). Steps d, e, f and g in Scheme 1 were carried out in an acid digestion bomb (Parr Instruments, Illinois), as optimal yields were obtained under these conditions.

Compound **10** was synthesized from 2-methoxyphenol (guaiacol, **21**, Scheme 2). Treatment of **21** with aluminium chloride and 4-bromobutyryl chloride in nitrobenzene initially esterified the phenolic alcohol which subsequently underwent an in situ Fries rearrangement to yield the corresponding butyrophenone. This was extracted into two equivalents of 2 M NaOH to simultaneously isolate it from the nitrobenzene and effect cyclisation, yielding the cyclopropyl phenyl ketone (**22**) in 32% overall yield. Triflation of the phenol[13] and subsequent treatment with diethyl phosphite and a palladium catalyst[14] yielded the 4-phosphono substituted analogue (**23**). The ketone (**23**) was converted to a 5′-cyclopropyl-hydantoin (**24**) via the Bucherer–Bergs reaction.[15] Base hydrolysis of **24** gave the monoethyl phosphonate ester of amino acid **25**. This prior base hydrolysis allowed a shorter treatment with acid for the subsequent hydrolysis than would otherwise have been required thus ensuring that opening of the cyclopropyl ring did not occur. The diacid (**10**) was obtained by hydrolysis of the monoester (**25**) in 6 N HCl for 3 h at reflux (35% yield).

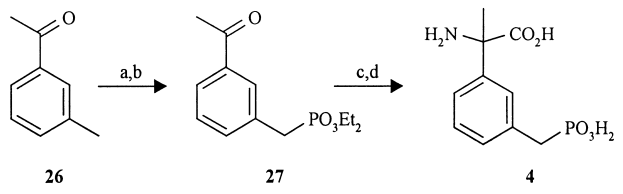
Compound **4** was synthesized from 3-methyl-acetophenone (**26**) (Scheme 3). Bromination of **26** and a subsequent Arbuzov reaction[16] furnished **27** in 73% overall yield. The ketone (**27**) was converted to the hydantoin[15] in excellent yield (97%) and acid hydrolysis gave the amino acid **4** in 21% yield.[17]



Scheme 1. (a) NaOH soln. (10% w/v), crushed ice, acetic anhydride 79%; (b) Fries rearrangement, $AlCl_3$, 120 °C, 59%; (c) 4-DMAP, 2,6-lutidine, trifluoromethanesulfonic anhydride, CH_2Cl_2, N_2 atmosphere, −78 °C, 62%–93%; (d) diethyl phosphite, tetrakistriphenylphosphine palladium(0), anhydrous Et_3N, bomb, 80 °C, 70–83%; (e) Bucherer–Bergs reaction: $(NH_4)_2CO_3$, KCN, 50% EtOH, bomb, 80 °C, 37–82%; (f) 4 N NaOH, bomb, 120 °C, 34%; (g) 6 N HCl, bomb, 120 °C, 5–50%.



Scheme 2. (a) (i) $AlCl_3$, 4-bromobutyrylchloride, nitrobenzene; (ii) 2 N NaOH, 32% overall yield; (b) 4-DMAP, 2,6-lutidine, trifluoromethanesulfonic anhydride, CH_2Cl_2, N_2 atmosphere, −78 °C, 51%; (c) diethyl phosphite, tetrakistriphenylphosphine palladium(0), anhydrous Et_3N, bomb, 80 °C, 27%; (d) $(NH_4)_2CO_3$, KCN, 50% EtOH, bomb, 80 °C, 34%; (e) 2 N NaOH, bomb, 120 °C, 34%; (f) 6 N HCl, reflux, 3 h, 35%.



Scheme 3. (a) NBS, CCl_4, hv; (b) $P(OEt)_3$; (c) $(NH_4)_2CO_3$, KCN, 50% EtOH, 80 °C, 24 h, 96.5% yield; (d) hydrolysis, 6 N HCl, bomb, 120 °C, 24 h, 21% yield. Stages a and b overall yield 73%.

Pharmacology

All experiments were performed on isolated hemisected spinal cords of 1–5 day Wistar rats of either sex.[18] Recordings were taken from the ventral root following stimulation of the corresponding dorsal root (30 V, 2 pulses min^{-1}). To isolate the fast non-NMDA component of the response (i.e., the fDR-VRP), DR-VRPs were recorded in the presence of 2 mM $MgSO_4$ and 50 μM (*R*)-2-amino-5-phophono-pentanoate (AP5).[19] The novel phenylglycines were tested for their ability to antagonize either group II ((1*S*,3*S*)-ACPD) or ((2*R*,4*R*)-APDC) used as the agonist challenge) or group III ((*S*)-AP4 used as the agonist challenge) mGlu receptor agonist-induced depressions of the fDR-VRP (see Table 1).[20]

Table 1. Pharmacological data given as apparent K_Ds (μM)±SEM (values are as a result of three separate determinations) unless otherwise stated

Compound	Antagonist activity at mGlu receptors in rat spinal cord:		
	Group I	Group II	Group III
1 (MCPG)	144±12[21]	479±37[5]	227±12[5]
2 (MPPG)	n.e. at 1 mM[6]	113±13[6]	9.2±0.3[6]
3 (CPPG)	70±12% at 1 mM[a, 8]	53[8]	1.7[8]
4	62±1% antag.[a]	20±6 % pot.[b]	n.e. at 200 μM
5	ne at 1 mM	726±125	6.8±1.5[e]
6	—	51±1%[c]	4.8±0.8
7	—	—	13±2%[d]
8	—	—	5.5±0.6
9	—	—	180±49
10	—	—	n.e. at 50 μM

[a]% Antagonism of (1*S*,3*R*)-ACPD response at 200 μM, $n=3$.
[b]% Potentiation of (1*S*,3*S*)-ACPD response at 200 μM, $n=4$.
[c]% Antagonism of (2*R*,4*R*)-APDC response at 1 mM.
[d]% Antagonism of (*S*)-AP4 at 100 μM.

For potent group III mGlu receptor antagonists, concentration–response curves to (*S*)-AP4 and either (1*S*,3*S*)-ACPD or (2*R*,4*R*)-APDC (to assess selectivity) were constructed in the absence and presence of the novel antagonist and an apparent K_D value obtained. In cases where antagonist potency was low, a one point assay (performed in triplicate) was carried out in which a single concentration of antagonist was applied and observed for its ability to antagonize a single agonist induced response (results being expressed as a percentage antagonism).

Antagonist action on group II mGlu receptors was not determined when little or no antagonist action of a compound was observed when tested at 100 μM against group III mGlu receptor agonist-induced responses.

To examine the effect of **4** and **5** (1 mM) on direct depolarisation of spinal motoneurones induced by the group I mGlu receptor ligand (*S*)-3,5-DHPG experiments were conducted in standard medium (excluding Mg^{2+}/AP5) that contained tetrodotoxin (TTX; 10^{-5} M for 2 min, then 10^{-7} M continuously). The degree of depolarisation of the motoneuron was measured by peak amplitude. The agonist was applied in the absence and presence of the antagonist (1 mM) and the ability of the antagonist to reduce the peak amplitude of the response was measured.

Structure–activity conclusions

From the pharmacological data presented in Table 1 we propose a number of structural features required for optimal antagonist activity at group III mGlu receptors present on primary afferent terminals.

A terminal phosphono group confers selective activity for group III versus group I mGlu receptors as compounds **2**–**5** showed no appreciable antagonist activity at group I mGlu receptors.

It would appear that a co-linear arrangement of the bonds attaching the phosphonate and glycine unit to the phenyl ring is necessary for optimal activity at group III mGlu receptors, as the 3-phosphonomethyl analogue (**4**) which has the same inter-acidic group chain length as MPPG (**2**) was almost completely inactive.

Substitution on the phenyl ring of MPPG at the 3 position leads to similar antagonist activity at group III mGlu receptors and this activity is independent of the electronic effects of the substituent i.e., no real differences between K_D values for the 3-chloro (**5**) (electron withdrawing) and the 3-methyl (**8**) (electron donating) substituted phenylglycines. Thus, the group III mGlu receptor binding site can accommodate substituents at least as large as methoxy at the 3-position of the phenyl ring of MPPG.

However, the most significant effect of 3-substitution, is that of the reduction of activity at group II mGlu receptors. Addition of a chloro group at the 3-position of the phenyl ring of MPPG conferred ~100-fold selectivity for group III over group II mGlu receptors. A 3-methoxy substituent conferred selectivity in excess of 200-fold for group III over group II mGlu receptors. This is probably due to the steric bulk of the substituent at the 3-position of MPPG being well tolerated by group III but not group II mGlu receptors.

Substitution at the 2-position of the phenyl ring as exemplified by **9** reduces antagonist potency at group III mGlu receptors. This may be due to the steric bulk at the 2-position being unacceptable to group III mGlu receptors, or more likely due to the 2-methyl group altering the preferred orientation of the glycine unit relative to the phenyl ring forcing the 2-methyl group into excluded space in the receptor. This is supported by the overlaid minimum energy conformations of **6** and **9** showing the two phenyl rings perpendicular to each other (Fig. 2).

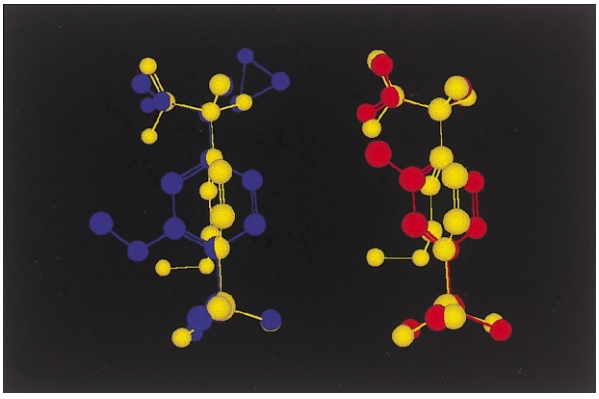


Figure 2. Left: Overlay of **6** (yellow) and **10** (blue). Right: Overlay of **6** (yellow) and **9** (red). Optimised in Chem-X and displayed in Chem3D.

Both OH groups on the phosphonate moiety may be needed for optimal binding to the group III mGlu receptor, as the mono ethyl ester (**7**) was much less active than the corresponding diacid (**6**). Alternatively, steric hindrance due to the ethyl group may be responsible for the reduction in activity.

Compound **10**, an α-cyclopropyl-substituted analogue of **6**, was surprisingly inactive at concentrations up to 50 μM at group III mGlu receptors. One explanation for this could be that CPPG and MPPG have different preferred orientations of the glycine unit relative to the plane of the phenyl ring and therefore the 3-methoxy substituent on the phenyl ring of **10** may probe excluded space in the receptor binding site. When energy minimised conformations of compounds **6** and **10** are overlaid it can be seen that the two phenyl rings are perpendicular to each other supporting this theory (Fig. 2).

Concluding Remarks

As the 3-chloro- and 3-methoxy-MPPG analogues (**5** and **6**, respectively) are at least 100-fold selective for group III over group II mGlu receptors they are the most potent selective group III mGlu receptor antagonists yet reported. As such they are likely to be useful tools to elucidate the physiological roles of group III mGlu receptors in the CNS. Recent work has suggested that the depressant action of (*S*)-AP4 on synaptic transmission in the spinal cord is mediated by mGlu8 receptors[2,22] and therefore the compounds described in this report are likely to be mGlu8 receptor antagonists. We are currently characterising **5**, **6** and **8** on all eight mGlu receptor subtypes in order to determine their subtype selectivity.

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