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(2S,4S)-2-AMINO-4-(2,2-DIPHENYLETHYL)PENTANEDIOIC ACID SELECTIVE GROUP 2 METABOTROPIC GLUTAMATE RECEPTOR ANTAGONIST

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Abstract: (2*S*,4*S*)-2-Amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-dioic acid **1m**, is a novel metabotropic glutamate receptor (mGluR) antagonist with insignificant ionotropic affinity. It is a selective antagonist of negatively-coupled cAMP-linked mGluRs with no effect on phosphoinositide coupled mGluRs. A series of 4-substituted glutamic acid analogues were prepared and it was found that compound **1k** is tenfold more potent than **1m**. Compound **1k** has neither significant affinity for ionotropic glutamate receptors nor group 1 and 3 metabotropic receptors.

Introduction

L-Glutamate (Glu) is the major excitatory aminoacid (EAA) neurotransmitter in the mammalian central nervous system (CNS). Glutamate receptors are subdivided into ionotropic (iGluRs),[1,2] and metabotropic glutamate receptors (mGluRs).[3,4] Ionotropic glutamate receptors mediate fast synaptic transmission through ligand-gated ion channels. Metabotropic receptors are coupled to G-proteins and modulate second messanger levels in neurons. Subtypes of mGluRs have been cloned and fall into three groups.[5,6] Group 1 mGluRs are positively coupled to phosphoinositide hydrolysis and include mGluR1 and 5. Group 2 mGluRs are negatively coupled to the formation of cyclic adenosine 5′-monophosphate (cAMP) formation and include mGluR2 and 3. Finally, group 3 mGluRs (mGluR4, mGluR6, mGluR7 and mGluR8) also negatively couples to cAMP but shows different ligand selectivity.

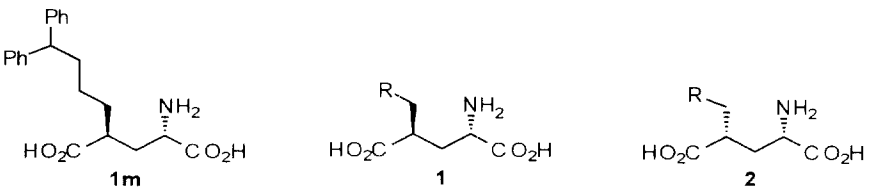

Figure 1

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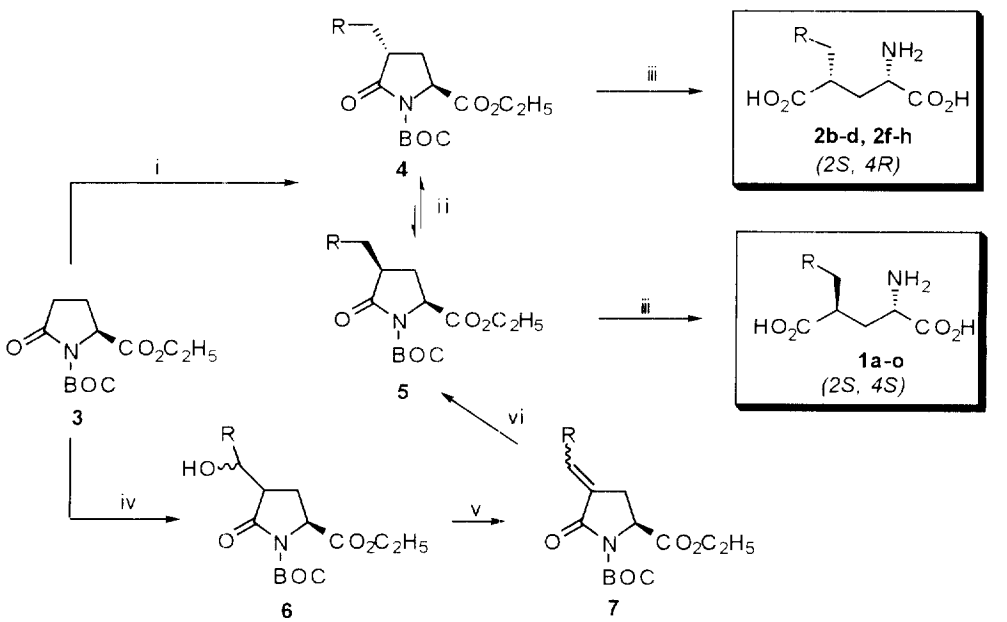
As part of an ongoing effort to discover subtype selective mGluR agonists and antagonists, we reported that (2*S*, 4*S*)-2-amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-dioic acid (**1m**) is a potent and selective antagonist for metabotropic group 2 mGluR (human mGluR2), with no effects on group 1.[7] In this paper we present the structure activity relationship for a series of 4-substituted glutamic acids (general formula **1** and **2**) which are group 2 antagonists. We now report the effect of chain length, the presence of one or two phenyl rings at the terminus and conformational restricted substitutents.

Results and Discussions

The (2*S*,4*R*) and (2*S*,4*S*) 4-substituted glutamic acids (**1** and **2**) were stereoselectively prepared from ethyl *N*-Boc pyroglutamate **3** (Scheme 1). Selective generation of the pyroglutamate lactame enolate with LiHMDS in THF at -78°C, and subsequent reaction with activated electrophiles,[8] gave rise exclusively to (2*S*,4*R*) ethyl 4-substituted pyroglutamates **4** with benzyl halides or a thermodynamic equilibrium mixture (3:1 ratio) with allyl halides and cinnamyl halides. Where necessary the major diastereomer **4** was easily separated from the minor one **5** by flash chromatography.



(i) LiHMDS, THF/-78°C, then RCH_2-X; (ii) KCN/DMF; (iii) 6N HCl, reflux or LiOH/THF/H_2O, HCl_g/EtOAc; (iv) LiHMDS, THF/-78°C, then RCHO/$Et_2O.BF_3$; (v) MsCl/Et_3N; (vi) H_2/PtO_2.

Scheme 1

Subsequent hydrolysis of these substituted pyroglutamates either under acidic (6N HCl/reflux) or base followed by acid (LiOH, THF/H_2O, then HCl/EtOAc) at room temperature, yielded the corresponding free aminoacids **2** (isolated as zwitterions after ion exchange chromatography). While this method turned out to be synthetically useful for glutamic acids **2**, another approach was developed for the C-4 epimers **1**. Thus, BF_3·OEt mediated aldol condensation[9] of the lactam enolate of **3** with aldehydes gave rise to a mixture of aldols **6** in good to excellent yields. No attempt was made to separate these diastereomeric mixtures as in the

following step both chiral centres were going to be lost. These mixtures were treated with mesyl chloride in the presence of excess triethylamine to yield the 4-alkylidene pyroglutamates **7** which were stereoselectively hydrogenated on the least hindered face to give the *cis* 4-substituted pyroglutamates **5** in quantitative yield. Finally, hydrolysis of these compounds yielded the (2*S*, 4*S*) 4-substituted glutamic acids **1**. It should be pointed that this is a much shorter synthesis of **1m** than that previously reported.[7] The optical purity of the final products **1** and **2** was >95% (detection limit determined by doping experiments).

Table 1

Compd	R	IC_{50} μM ACPD Sens. [^{3}H]glut	Compd	R	IC_{50} μM ACPD Sens. [^{3}H]glut
1a	Phenyl	8.99			
1b	2-Naphthyl	3.33	**2b**	2-Naphthyl	8.82
1c	p-CH_3-Phenyl	4.60	**2c**	p-CH_3-Phenyl	7.85
1d	p-CF_3-Phenyl	3.16	**2d**	p-CF_3-Phenyl	>100
1e	m-OCH_3-Phenyl	9.28			
1f	Benzyl	4.65	**2f**	Benzyl	14.50
1g	2-Phenylethyl	1.84	**2g**	2-Phenylethyl	21.10
1h	3-Phenylpropyl	4.69	**2h**	3-Phenylpropyl	>100
1i	4-Phenylbutyl	2.49			
1j[10]	-$(CH_2)_2$-CHPh-$(CH_2)_2$-	3.20			
1k	Diphenylmethyl	0.38			
1l	2, 2-Diphenylethyl	1.16			
1m	3, 3-Diphenylpropyl	3.38			
1n	4, 4-Diphenylbutyl	3.40			
1o[10]	-$(CH_2)_2$-CPh_2-$(CH_2)_2$-	1.40			

All compounds shown in Table 1 were initially evaluated as metabotropic ligands in rat forebrain membranes by measuring the displacement of ACPD-sensitive [^{3}H]glutamate.[11] This assay was performed under conditions where [^{3}H]glutamate binding is ACPD-sensitive, but insensitive to the mGluR1 and 5 (group 1) agonist quisqualate or the mGluR4, 6, 7 and 8 (group 3) agonist L-AP4. The most active compounds were also tested as iGluR ligands using [^{3}H]-CGS-19755, [^{3}H]-AMPA and [^{3}H]-Kainate displacement from rat forebrain preparations (Table 2).

The general conclusions that can be drawn from data shown in Table 1 are the following. The metabotropic glutamate receptor affinity of 4-substituted glutamic acids resides in the 2*S*, 4*S* isomers (**1**), its 2*S*, 4*R* counterparts being less potent (**2**). Thus, **1b-d** and **1f-h** have lower IC_{50} values than their **2** counterpart isomers. When only one phenyl ring is present at the terminus the 3 and 5 carbon chain compounds (**1g** IC_{50} = 1.84 μm and **1i** IC_{50} = 2.49 μm) are more potent than their 2 and 4 chain counterparts (**1f** IC_{50} = 4.65 μm and **1h** IC_{50} = 4.69 μm). However, with two phenyl rings the activity diminishes with the increasing length (**1k-n**). For the same chain the two phenyl rings series is more active than the one phenyl series. Thus, compounds **1k-m** are more potent than **1f-h**, an exception to this rule are **1i** and **1n**. Finally, the conformational constraint of the alkyl substituent does not affect significantly the affinity thus, the cyclohexyl derivatives **1j** and **1o** were as potent as their n-alkyl analogues **1i** and **1n**.

Table 2

Compd	R	$IC_{50}\mu M$ [^3H] CGS19755	$IC_{50}\mu M$ [^3H] AMPA	$IC_{50}\mu M$ [^3H] Kainate	$IC_{50}\mu M$ ACPD[11] Sens[^3H]glutamate
1g	2-Phenylethyl	5.40	34.03	9.20	1.84
1k	Diphenylmethyl	>100	>100	>100	0.38
1l	2, 2-Diphenylethyl	13.20	14.49	39.27	1.16
1m	3, 3-Diphenylpropyl	147.00	>100	>100	3.38

Compounds **1k** and **1m** were further examined in functional assays for agonist and antagonist activities in non-neuronal cells (RGT) expressing human mGluR subtypes using approximate $EC_{80\text{-}90}$ agonist challenges at each receptor[12] (table 3). 1*S*,3*R*-ACPD-stimulated phosphoinositide hydrolysis was measured for mGluR1 and mGluR5. For group 2 mGluRs (mGlu2 and mGlu3), we examined ACPD-induced inhibition of forskolin (15 μM)-stimulated cAMP formation. For group 3 mGluRs (mGlu4, 7, and 8), L-AP4-induced inhibition of forskolin-stimulated cAMP formation was used. Compounds **1k** and **1m** had no agonist or antagonist (agonist reversal) activities at up to 300 μM concentrations in any of the group 1 or group 3 mGluRs. In contrast, compound **1m** reversed the effect of ACPD with similar activity at mGlu2 and mGlu3. Compound **1k** was a more potent antagonist at either group 2 mGluR with a low μM IC_{50} at the mGlu3 receptor. Thus, both **1k** and **1m** are highly selective group 2 mGluR antagonists.

Table 3

Human mGluR	Agonist	$IC_{50}\mu M$ 1k	$IC_{50}\mu M$ 1m
mGlu1a[a]	ACPD (100 μM)	>300	>300
mGlu5a[a]	ACPD (100 μM)	>300	>300
mGlu2[b]	ACPD (3 μM)	18±3	50±19
mGlu3[b]	ACPD (3 μM)	6.1±1.3	30±10
mGlu4a[b]	L-AP4 (3 μM)	>300	>300
mGlu7a[b]	L-AP4 (1000 μM)	>300	>300
mGlu8[b]	L-AP4 (0.3 μM)	>300	>300

[a] Phosphoinositide hydrolysis. [b] Cyclic Adenosine 5'-Monophosphate (cAMP) formation.

In summary, we have explored the structure activity relationship surrounding the previously report lead metabotropic antagonist compound **1m** and in so doing have found several compounds which are more potent. Notably, compound **1k** is nearly ten fold more potent than the parent having a shorter linker between the diphenyl group and the glutamate back bone. This compound is also devoid of ionotropic activity and metabotropic group 1 and 3 and as such should be a useful pharmacological tool in the exploration of the role group 2 metabotropic glutamate receptors in the CNS.

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Experimental Section

(2S,4S)-2-amino-4-(2,2-diphenylethyl)pentanedioic acid, hydrochloride (1k)

To a solution of ethyl N-Boc pyroglutamate **3** (15.6 mmol) in THF (50 mL) stirred at -78°C was added a 1M solution of lithium hexamethyldisilazide in THF (18.7 mL, 18.7 mmol, 1.1 equiv). The reaction mixture was stirred for 1 hour at -78°C prior to the addition of a solution of diphenylacetaldehyde (17.2 mmol) and $Et_2O{\cdot}BF_3$ (17.2 mmol) in THF (50 mL). The reaction mixture was stirred for 1 hour at -78°C, quenched with saturated ammonium chloride solution (100 mL) and extracted with ethyl ether (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated to dryness. Purification of the crude by flash chromatography (Hexane/ ethyl acetate 2:1) gave a mixture of aldols **6**, which were dissolved in CH_2Cl_2 (30 mL) and treated with methanesulfonyl chloride (1.34 mL, 17.2 mmol) and triethylamine (18 mL, 172 mmol). After stirring this solution for two days at room temperature it was quenched with water and extracted with dichloromethane (3 x 50 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure affording **7k** as a mixture of *E* and *Z* isomers. This mixture was hydrogenated without further purification. Thus, PtO_2 (0.65 mmol) was added to a solution of **7k** (6.46 mmol) in ethyl acetate (50 mL). Hydrogen (1 atm) was introduced and the heterogeneous mixture was stirred at room temperature for 4 hours. The mixture was filtered through a pad of celite and evaporated to give **5k** exclusively.

Finally, **5k** (2 mmol) and a 6N HCl solution (25 mL) was refluxed overnight. The resulting solution was evaporated to dryness. The residue was triturated with ethyl ether (3 x 20 mL).yielding **1k** as a white solid m.p. 160-2°C. $[\alpha]_D$ = +7.4° (c 1.6, MeOH). ^1H NMR (MeOH-d_4) δ 7.40-7.00 (m, 5H), 4.07 (t, J = 8.2 Hz, 1H), 3.85 (dd, J = 5.8 and 8 4 Hz, 1H), 2.80- 2.00 (m, 5H). ^{13}C NMR (MeOH-d_4) δ 178.1, 171.8, 145.4 (2C), 129.6 (2C), 129.0 (2C), 128.7 (2C), 52.7, 49.0, 41.4, 39.8, 34.0. IR (KBr pellet) 3424, 2930, 1716, 1495 cm^{-1}. HRMS (FAB) calcd for $C_{19}H_{22}NO_4$ (M^++1-HCl) 328.1549, found 328.1540.

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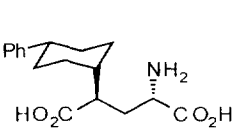
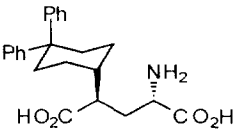
10 Structures of **1j** and **1o** are depicted:



1j



1o

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