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2, 3'-DISUBSTITUTED-2-(2'-CARBOXYCYCLOPROPYL)GLYCINES AS POTENT AND SELECTIVE ANTAGONISTS OF METABOTROPIC GLUTAMATE RECEPTORS

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Abstract: 2-(9-Xanthylmethyl)-2-(2'-carboxycyclopropyl) glycine **6e** is a novel metabotropic glutamate receptor antagonist. A series of *alpha*, C-3' disubstituted (carboxycyclopropyl)glycines **6f-n** were prepared. Antagonist activity was observed for all these compounds at group 2 and group 3 mGluRs. Although they were slightly less active on group 2 mGluRs than non C-3' substituted **6e**, the compounds **6f-n** were more selective with lesser or no activity on group 1 mGluR subtypes (IC_{50} values greater than 100μm).

Introduction

Excitatory aminoacids (EAA), represented by L-glutamate (L-Glu), are major excitatory neurotransmitters in the mammalian central nervous system (CNS).[1] Pharmacological and molecular studies have demonstrated two classes of glutamate receptors. Ionotropic glutamate receptors are ligand-gated ion channels that exist as heteromeric protein complexes composed of heterogeneous subunit proteins.[2] In contrast, metabotropic glutamate receptors (mGluRs) are coupled to cellular effectors via GTP-binding proteins. There currently exist eight distinct mGluR proteins which have been classified into three groups.[3] 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) and include mGluR2 and 3. Finally, group 3 mGluRs (mGluR4, mGluR6, mGluR7 and mGluR8) also negatively coupled to cAMP but show different ligand selectivity.

(Carboxycyclopropyl)glycines (CCG), conformationally constrained analogs of L-glutamate, have been shown to be a valuable source of potent and selective ligands. (2S, 1'S, 2'S)-2-(2'-Carboxycyclopropyl)glycine **1** (L-CCG-I),[4] (2S, 2'R, 3'R)-2-(2',3'-dicarboxycyclopropyl)glycine **2** (DCG-IV)[5] and (2S, 1'S, 2'S, 3'R)-2-(2'-carboxy-3'-methoxymethylcyclopropyl)glycine **3** (*cis*-MCG-I),[6] are among the most widely used agonists for group 2 mGluRs. Incorporation of a lipophilic side chain both at the C-3' of cyclopropane (**4**)[7] or in the *alpha* position (C-2) to the aminoacid (**5**) leads to potent and relatively selective mGluR antagonists with no activity on iGluRs.[8] In this study, we have decided to investigate the effects of substitution on the cyclopropane C-3' position whilst retaining the *alpha* substituent and the synthesis and pharmacological characterization of a series of disubstituted CCGs **6** (see Figure 1) is described.

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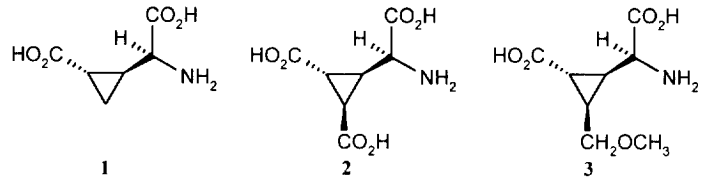
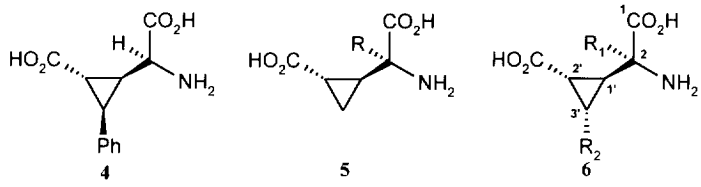
Agonists



1 2 3

Antagonists



4 5 6

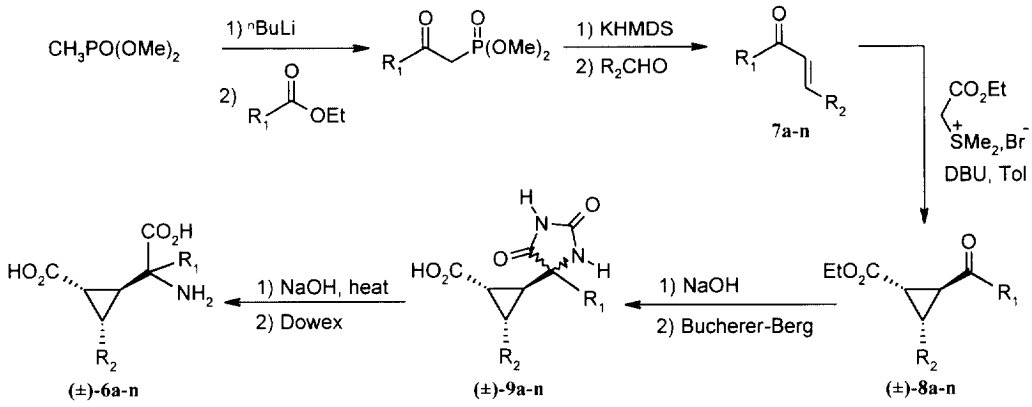
Figure 1

Results and Discussions

We envisioned 2'-ethoxycarbonyl-3'-substituted cyclopropyl alkyl ketones **8** as precursors for the disubstituted CCGs **6**, since classical amino acid synthesis techniques would convert the ketone to the *alpha* amino acid (Scheme 1). To this end, we have studied the cyclopropanation of acyclic enones **7** with a stabilized sulfur ylide with the ethoxycarbonyl moiety already in place.[11] It should be noted that in the reaction *three contiguous stereogenic centres* are created. Noteworthy, we found that cyclopropanation of α, β-unsaturated ketones with ethyl (dimethyl sulfuranylidene) acetate (EDSA),[9] generated *in situ* from the corresponding sulfonium bromide salt and DBU in toluene, leads exclusively to racemic products **8** with high degree of stereocontrol.[10] The E enone **7**, in turn, was prepared by a Horner-Emmons reaction from the desired stabilized phosphonate with the corresponding aldehyde.[11] In this way, R_1 and R_2 can be chosen at will.

The conversion of ketones **8** to the amino acid was accomplished by Bucherer-Berg reaction. We showed it was more convenient to first hydrolyze the ester to the corresponding acid with 1N sodium hydroxide because of solubility problems. Thus, the corresponding ketoacids were reacted with 5 equiv of potasium cyanide and 9 equiv of ammonium carbonate in aqueous ethanol in a sealed tube at 100°C for 6 hours. As predicted, there was no selectivity in the formation of stereoisomers at the C-5 centre of hydantoins **9**, and all were obtained as a 1:1 inseparable mixture of two racemic diastereomers.

The hydrolysis of hydantoins **9** turned to be difficult. The best results were obtained using 1N sodium hydroxide in a hydrogenation bomb at 150°C during 24 hours. Final amino acids **6a-n** (as mixtures of four stereoisomers) were purified by cation exchange chromatography or by precipitation of the zwitterion in water.[11]



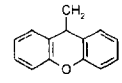
Scheme 1

All compounds shown in Table 1 were initially evaluated as metabotropic ligands in an ACPD sensitive [^{3}H]-glutamate binding assay using rat forebrain membranes. This binding assay identifies affinity for group 2 mGluR receptors.[12] The series of *alpha* 9-xanthylmethyl substituted **6f-n** were also evaluated for functional activity in nonneuronal cell lines (RGT) expressing either cloned human mGluR2 or mGluR3.[13] None of the compounds produced agonist effects. Instead, reversal of 1*S*,3*R*-ACPD-induced inhibition of forskolin-stimulated cyclic-AMP formation was observed (IC_{50} values in Table 1) indicating that all these compounds are antagonists like the parent compound **6e**.

Table 1[a]

Compd	R_1	R_2	IC_{50} μM ACPD Sens. [^{3}H]glut bind	IC_{50} μM mGluR2	IC_{50} μM mGluR3
6a	CH_3	CH_3	>10	NT	NT
6b	$Ph(CH_2)_2$	CH_3	5.07	NT	NT
6c	$Ph(CH_2)_2$	$Ph(CH_2)_2$	2.54	NT	NT
6d	$Ph(CH_2)_2$	$CH_3(CH_2)_4$	0.53	NT	NT
6e[b]	9-Xanthylmethyl[c]	H	0.010	0.20	0.016
6f	9-Xanthylmethyl[c]	CH_3	0.071	NT	0.1-1
6g	9-Xanthylmethyl[c]	CH_3CH_2	NT	2.85	0.076
6h	9-Xanthylmethyl[c]	$CH_3(CH_2)_2$	NT	>10	0.196
6i	9-Xanthylmethyl[c]	$CH_3(CH_2)_3$	NT	1.32	0.029
6j	9-Xanthylmethyl[c]	$(CH_3)_2CHCH_2$	NT	1.37	0.040
6k	9-Xanthylmethyl[c]	$CH_3(CH_2)_4$	0.050	NT	0.01-0.1
6l	9-Xanthylmethyl[c]	$CH_3(CH_2)_5$	NT	1.87	0.122
6m	9-Xanthylmethyl[c]	$CH_3(CH_2)_8$	NT	2.16	>1
6n	9-Xanthylmethyl[c]	$Ph(CH_2)_2$	NT	1.67	0.125

a All compounds were evaluated as a mixture of four stereoisomers.

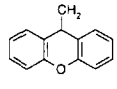
b Compound **6e** was included for comparison purposes, ref 8. NT: not tested. c 

It has been shown that incorporation of a substituent on the amino acid carbon converted agonist **1** into an antagonist **5** (figure 1). Affinity for group 2 mGluRs was optimal when the *alpha* substituent changed from alkyl to phenylethyl and to xanthylmethyl.[8] We observed exactly the same trend for the 2, 3'-disubstituted compounds **6**. Thus, for a given substituent at the C-3' position (methyl) the affinity increases with the nature of the substituent at the *alpha* position in the same way as previously reported (**6f** 72-times more active than **6b** which is more active than **6a**).

On the other hand, for a given R_1 (phenylethyl) the activity increases with the chain length (**6d** 10-fold more potent than **6b**) while only a 2-fold improvement was obtained when R_2 is phenylethyl. However, for the 9-xanthylmethyl (**6f-n**), the substitution at the C-3' centre slightly changed the affinity for group 2 mGluR receptors. All compounds were found to be functional antagonists at mGluR2 and 3. The presence of the extra substituent does not apparently affect the activity of parent **6e**, indicating that a large steric bulk is allowed in this position for these stereoisomers. In comparing the activities of the compounds on mGluR2 and mGluR3, it was evident that in most cases, the compounds were 10-50 times more potent at inhibiting mGluR3 than mGluR2.

Table 2[a]

Compd	R_1	R_2	IC_{50} μM mGluR1	IC_{50} μM mGluR5	IC_{50} μM mGluR4	IC_{50} μM mGluR7	IC_{50} μM mGluR8
6e	9-Xanthylmethyl[b,c]	H	7.8	8.2	22.0	0.99	0.17
6f	9-Xanthylmethyl[c]	CH_3	>100	>100	62.85	23.07	1.23
6g	9-Xanthylmethyl[c]	CH_3CH_2	>100	>100	60.35	19.66	2.52
6h	9-Xanthylmethyl[c]	$CH_3(CH_2)_2$	>100	>100	NT	67.82	2.25
6i	9-Xanthylmethyl[c]	$CH_3(CH_2)_3$	57.0	>100	10.08	9.42	0.49
6j	9-Xanthylmethyl[c]	$(CH_3)_2CHCH_2$	>100	>100	8.68	2.60	0.38
6k	9-Xanthylmethyl[c]	$CH_3(CH_2)_4$	18.0	>100	9.45	7.61	0.34
6l	9-Xanthylmethyl[c]	$CH_3(CH_2)_5$	54.9	56.1	12.53	6.25	0.75
6m	9-Xanthylmethyl[c]	$CH_3(CH_2)_8$	5.7	5.4	20.12	7.82	5.18
6n	9-Xanthylmethyl[c]	$Ph(CH_2)_2$	>100	>100	11.41	15.35	0.99

a All compounds were evaluated as a mixture of four stereoisomers.
b Data for the enantiomer (2S, 1'S, 2'S)-2-(9-Xanthylmethyl)-2-(2'-carboxycyclopropyl)glycine. NT: not tested. c 

At this point, we decided to investigate the effects of the compounds on functional responses for cloned human group 1 and 3 metabotropic glutamate receptors. Quisqualate-stimulated phosphoinositide hydrolysis was measured for mGluR1 and 5 as described previously.[14] For group 3 mGluRs (mGluR4, 7 and

8), L-AP4 induced inhibition of forskolin-stimulated c-AMP formation was used.[15] To our surprise, except for **6k** and **6m**, no significant antagonist activity was observed for either mGluR1 or 5. In contrast, a number of compounds showed antagonist effects on group 3 mGluRs with submicromolar IC_{50} values observed for mGluR8. Although, **6f-n** are less active than **6e**.

In summary, we prepared a series of (carboxycyclopropyl)glycines, compounds **6**, in which two substituents were incorporated (*alpha* and C-3'). The affinity for group 2 mGluRs was optimal when the *alpha* substituent was 9-xanthylmethyl. For this given *alpha* substituent, the C-3' substitution does slightly affect the activity. Noteworthy, no potent group 1 activity was shown although group 3 activity was retained. We can conclude that these disubstituted CCGs although slightly less potent than the previous reported **6e** are more selective since they do not exhibit group 1 activity.

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11 General experimental procedure: **Phosphonates Synthesis**: To a solution of dimethyl methylphosphonate (7.80g, 63 mmol) in THF (63 mL) at -78°C. n-Butyllithium (69 mmol) was dropwise added. After 15 minutes at this temperature the corresponding ester was added (31.5 mmol) in THF (30 mL) and the resulting solution stirred for 30 minutes at -78°C. The dry ice bath was removed and stirring continued for an additional hour. After quenching with saturated aqueous solution of NH_4Cl, the combined organic layer were dried over Na_2SO_4, evaporated and chromatographied.

(E)-1-(9-Xanthyl)-6-phenyl-3-hexen-2-one (7n): using Hexane/Ethyl acetate 5:1 as eluent. IR (KBr) 2930, 1647, 1681, 1458, 1258 cm^{-1}. H NMR ($CDCl_3$) 7.30-6.98 (m, 13H), 6.62 (dt, J=6.8 and 15.9 Hz, 1H), 5.94 (dt, J=1.4 and 15.9 Hz, 1H), 4.64 (t, J=6.7 Hz, 1H), 2.87 (d, J=6.7 Hz, 2H), 2.60 (m, 2H), 2.35 (m, 2H). C NMR ($CDCl_3$) 198.0, 152.1, 147.1, 140.5, 131.1, 128.7, 128.4, 128.2, 127.7, 126.1, 125.2, 123.3, 116.4, 50.6, 34.7, 34.1, 34.0.

Cyclopropanation: The carbethoxymethyl dimethylsulfonium bromide (3.44 mmol) together with DBU (2.87 mmol) was stirred in chloroform (1.17 mL) for 30 minutes. Then the enone (2.87 mmol) in 1.7 mL of $CHCl_3$ was

added and the resulting solution stirred overnight. The following day 0.5 equiv. of preformed ylide was added and stirred for 2 days. The crude mixture was diluted with dichloromethane (10 mL) and washed twice with 0.5N HCl (2x4 mL). The organic layer was dried over Na_2SO_4 and evaporated to yield a crude which was purified by column chromatography using ethyl acetate-hexane mixtures as eluent.

(1SR, 2SR, 3SR) Ethyl 2-(9-xanthylmethylcarbonyl)-3-phenethylcyclopropyl-1-carboxylate (8n): Hexane/Ethyl acetate, 9:1. IR (film) 1790, 1701, 1470, 1460,1253 cm^{-1}. H NMR ($CDCl_3$) 7.35-6.95 (m, 13H), 4.55 (t, J=6.5 Hz, 1H), 4.08 (q, J=7.0 Hz, 2H), 2.90-2.70 (m, 4H), 2.20 (m, 1H), 2.04 (t, J=5.7 Hz, 1H), 1.80-1.60 (m, 3H), 1.23 (m, 3H). C NMR ($CDCl_3$) 205.5, 169.9, 152.1, 140.9, 128.6, 128.5, 128.4, 127.9, 125.9, 124.9, 123.3, 116.5, 60.9, 54.4, 35.6, 35.3, 34.4, 29.1, 27.5, 14.2.

Bucherer Berg reaction: A solution of the ester (1 mmol) was dissolved in ethanol and then 1N NaOH (1.1 equiv) was added. The amount of ethanol used is the volume needed to run the reaction at 0.3M. The resulting solution was heated at 60°C until no starting material remains (TLC monitoring). After cooling, the reaction mixture was extracted with ether and acidified with 1N HCl (in an ice bath). The acid was extracted with ether (or ethyl acetate) twice. The organic layer was dried over Na_2SO_4 and evaporated to yield a crude which was subjected to the Bucherer-Berg reaction without any further purification.

Method A: A solution of the ketone-acid (7.34 mmol) in ethanol (10 mL) was added to a solution of KCN (5 equiv.) and $(NH_4)_2CO_3$ (7 equiv.) in water (10 mL), then this mixture was heated to 60°C for 24 hours. The mixture was cooled in an ice bath and 10% $KHSO_4$ was cautiously added till acidic pH. The mixture of hydantoines precipitate or are extracted with ethyl acetate.

Method B: The same as before but in a sealed tube in an oven at 100°C for 6 hours.

(5SR)- and (5RS)-5-((1'SR, 2'SR, 3'SR)-2'-Carboxy-3'-phenylethylcyclopropyl)-5-(9-xanthylmethyl) imidazolidine-2,4-dione (9n): Method B. Diastereomer mixture at C-5, H NMR (DMSO-d_6) 8.00 (br s, 1H), 7.80 (br s, 1H), 7.30-7.00 (m, 13H), 4.00 (m, 1H), 2.20 (m, 2H) 1.90-1.30 (m, 7H). C NMR (DMSO-d_6) 176.0, 175.6, 172.4, 172.1, 156.6, 156.5, 152.5, 141.3, 141.2, 128.3, 128.2, 127.7, 125.7, 124.7, 123.6, 116.3, 116.0, 63.0, 62.9, 40.7, 35.7, 34.8, 33.7, 27.8, 23.2, 19.7.

Basic hydrolysis: The mixture of hydantoines was treated with 1N NaOH at 150°C during 24 hours. After cooling the reaction mixture 12N HCl was added till pH=1-2 in an ice bath. Solvent evaporation yield a solid which was triturated with acetone several times in order to remove water. The resultant solid was chromatographied on Dowex resin and eluted with 10% Py or by adding water to the chloride salt the zwitterion precipitated.

(2SR)- and (2RS)-2-((1'SR, 2'SR, 3'SR)-2'-Carboxy-3'-phenylethylcyclopropyl)-2-(9-xanthylmethyl)glycine (6n): Precipitation in water. Diasteromer mixture at the aminoacid center. H NMR (D_2O/KOD) 7.40-6.80 (m, 13H), 4.00 (m, 1H), 2.50 (m, 1H), 2.20-0.90 (m, 8H). C NMR (D_2O/KOD) 182.8, 181.8, 11.6, 154.1, 154.0, 153.6, 143.6, 143.1, 129.6, 129.5, 126.7, 126.8, 124.9, 117.6, 117.3, 61.6, 60.8, 49.6, 48.1, 47.7, 38.3, 37.2, 36.0, 35.8.

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