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Design, Synthesis and Preliminary Evaluation of Novel 3′-Substituted Carboxycyclopropylglycines as Antagonists at Group 2 Metabotropic Glutamate Receptors

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Abstract—Two novel 3′-substituted carboxycylopropylglycines, (2*S*,1′*S*,2′*S*,3′*R*)-2-(3′-xanthenylmethyl-2′-carboxycyclopropyl)-glycine (**8a**) and (2*S*,1′*S*,2′*S*,3′*R*)-2-(3′-xanthenylethyl-2′-carboxycyclopropyl)glycine (**8b**), were synthesized and evaluated as mGluR ligands. Compound **8b** showed to be a potent group II antagonist with submicromolar activity.

Metabotropic glutamate receptors constitute a heterogeneous family of G-protein coupled receptors responding to synaptically released glutamic acid (L-Glu, **1**). At least eight genes encode for molecularly diverse mGluR subtypes, named mGluR1–mGluR8.[1] The urgent need of clarifying the multiplicity of physiological and pathological effects in which these individual subtypes of metabotropic glutamate receptors (mGluRs) are involved is at the basis of the continuous quest for subtype-selective mGluR ligands. The class of 2-(carboxycyclopropyl)glycines, conformationally constrained analogues of L-Glu (**1**),[2] has been a valuable source of potent and selective ligands for the family of glutamate receptors. In particular, (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I, **2**), which mimics an extended conformation of L-Glu (**1**), is a potent and rather selective agonist for group II metabotropic glutamate (mGlu) receptors although also active as group I agonist.[3]

It has been shown that the nucleus of L-CCG-I (**2**) is amenable to a large number of chemical manipulations which have allowed the synthesis of a variety of ligands endowed with a considerable diversity in functional profiles and affinity values for individual mGlu receptor subtypes. In past years, two families of substituted CCGs, in particular, have been designed, synthesized and biologically evaluated as mGluR ligands, the family of 3′-substituted CCGs and the family of 2-substituted CCGs (Chart 1).

In 1996, for the first time we reported that the stereoselective introduction of a bulky, hydrophobic group such as the phenyl ring in the 3′-position confers antagonism, as demonstrated by the activity of (2*S*,1′*S*,2′*S*,3′*R*)-2-(2′-carboxy-3′-phenyl-cyclopropyl)-glycine (PCCG-4, **3**),[4] at that time the most potent group II antagonist reported. PCCG-4 (**3**) was identified as the only mGluR2 antagonist out of a library of all the 16 PCCG stereoisomers, thus pointing out the relevance of the stereosubstitution on the cyclopropane ring. The possibility of achieving functional antagonism by 3′-substitution was also exploited by Collado et al. who reported the synthesis and the evaluation of a series of 2,3′-disubstituted CCGs (see, e.g., **4** and **5**),[5] in which linear and branched alkyl substituents are inserted into the 3′-position while retaining the α-substitution (methyl, phenylethyl, or 9-xanthenylmethyl).

Interestingly, the 2,3′ disubstitution never results in an increase of potency with respect to the parent, 2-substituted, derivative and only in the best cases the

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potency is maintained. These results clearly indicate that the effect of the 2,3′ disubstitution is not additive but, on the contrary, hydrophobic substituents on 2- or 3′-position are likely to compete for the same receptor binding pocket.

As far as the 2-substitution is concerned, 2-substitution of L-CCG-I (**2**) converts the activity of the parent derivative **2** into group II mGluR antagonism (Chart 2). Thus, the 2-methyl derivative of L-CCG-I (**2**), MCCG-I (**6**),[6] is a modestly potent though selective group II antagonist. In a fundamental work, Ornstein et al. have demonstrated that the 2-position of L-CCG-I (**2**) can be further functionalized and alkyl-, aryl-, alkyl-aryl-, cycloalkyl-2-substituted CCGs invariably retain the antagonist character of MCCG-I (**6**) with considerable increase in potency. Indeed, the nature of the 2-substituent greatly influences the affinity for group II receptor subtypes, the xanthenylmethyl group (LY341495, **7**) being the optimal one.[7,8] Compound **7** displays nanomolar potency as a group II antagonist and is currently used as radiolabeled ligand in its tritiated form.[9] These structure–activity relationships prompted us to further explore the possibility of achieving significant antagonism towards group II mGluRs by functionalizing in a stereospecific way the 3′-position of CCGs with substituents of different lengths and steric demand. In particular, we envisaged the synthesis and the preliminary evaluation of carboxycyclopropylglycines 3′-substituted with 9-xanthenylmethyl (XM-CCG-I, **8a**) and 9-xanthenylethyl (XE-CCG-I, **8b**).

Our general approach to the desired trisubstituted cyclopropylaminoacids **8a–b** (Scheme 2) with definite stereochemistry at all four chiral centers involved four key steps: (a) a Still–Gennari[10] modified Horner–Emmons olefination affording the (Z)-α,β-unsaturated esters **14a–b**; (b) a Cu(II)-catalyzed intramolecular cyclopropanation of the allylic diazoesters **16a–b** affording the racemic *endo*-lactones **17a–b**; (c) a selective inversion of the configuration at C-1 of the cyclopropylalcohols **18a–b** leading to the epimeric compounds **19a–b**; (d) a diastereoselective Strecker synthesis employing *R*-(−)-α-phenylglycinol as chiral auxiliary[11] affording predominantly the (*S*)-α-amino nitriles and **22a–b**.

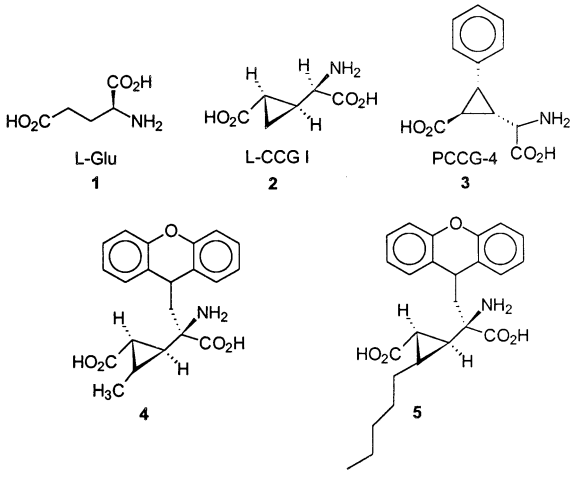
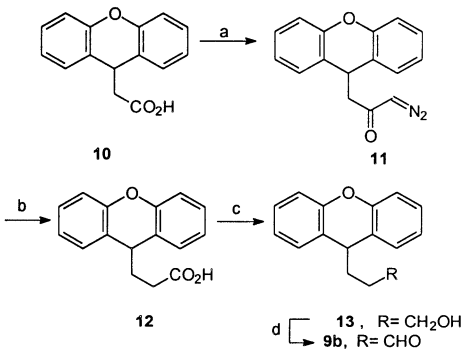


Chart 1.



Scheme 1. (a) (i) $SOCl_2$, C_6H_6, reflux; (ii) CH_2N_2, Et_2O, rt; (b) (i) PhCOOAg, Et_3N, THF–H_2O, rt; (c) $LiAlH_4$, THF, reflux; (d) PCC, CH_2Cl_2, rt.

The starting aldehyde **9a**, needed for the Wittig olefination, was prepared by known procedures[12–15] whereas 3-(9*H*-9-xanthenyl)propanal (**9b**) was synthesized via a route developed in our laboratories and based on an Arndt–Eistert homologation[16] (Scheme 1).

Reaction of the aldehydes **9a–b** with bis(trifluoroethyl)phosphonoacetate ethyl ester[17] in the presence of strongly dissociated base system ($KN(TMS)_2$/18-Crown-6) according to Still and Gennari[10] afforded the corresponding (*Z*)-α,β-unsaturated esters **14a–b** which were reduced by DIBAL-H (CH_2Cl_2, −78 °C) to the corresponding allylic alcohols **15a–b** and then converted in satisfactory yields into the corresponding allylic diazoesters **16a–b**.[18] Bis(*N*-*tert*-butylsalicylaldimine) copper(II)-catalyzed decomposition of **16a–b** gave the racemic *endo*-6-substituted-3-oxabicyclo[3.1.0]-hexan-2-ones **17a–b** (57–77%), which were quantitatively converted into the corresponding hydroxymethyl morpholine amides **18a–b**.[19] Selective inversion of the configuration at C-1 of alcohols **18a–b** (LiHMDS, THF) afforded, as previously described,[4] the isomeric alcohols **19a–b** which were submitted to a Swern oxidation[20] giving the corresponding aldehydes **19a–b** in good yields.

A diastereoselective Strecker synthesis[11] involving the nucleophilic addition of a cyanide ion to the Schiff bases

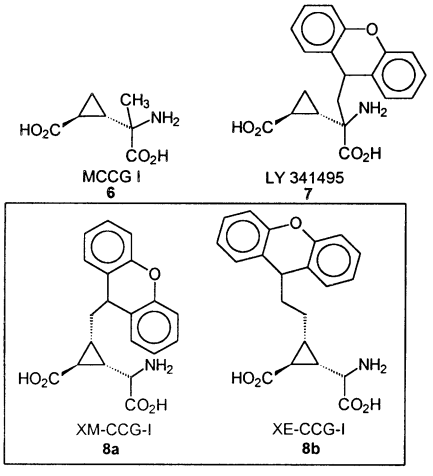
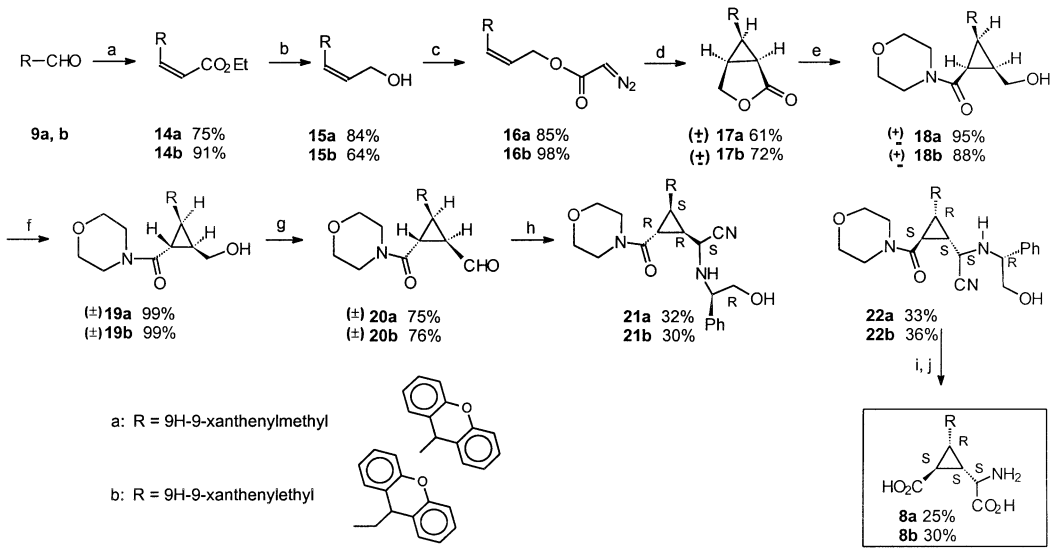


Chart 2.



Scheme 2. (a) $(CF_3CH_2)_2POCH_2COOEt$, $KN(TMS)_2$, 18-Crown-6, THF, −78 °C; (b) DIBAL-H, CH_2Cl_2, −78 °C; (c) pTsNHN=CHCOCl, *N*,*N*-dimethylanilin Et_3N, CH_2Cl_2, 0 °C; (d) $Cu(TBS)_2$, PhMe, reflux; (e) morpholine, $AlMe_3$, CH_2Cl_2, reflux; (f) Li-HMDS, THF, rt; (g) $(COCl)_2$, DMSO, Et3N, CH_2Cl_2, −60 °C; (h) (i) *R*-(−) α-phenylglycinol, TMSCN, MeOH, rt; (ii) mpc; (i) (i) HCl(g) MeOH, rt; (ii) $Pb(OAc)_4$, CH_2Cl_2–MeOH, 0 °C; (iii) 3 N HCl, Et_2O, rt; (j) (i) 0.5 N NaOH, 100 °C; (ii) Dowex 50WX2-200; (iii) RP-8 mpc.

formed by condensing each racemic aldehyde (**20a**–**b**) with optically active *R*-(−)-α-phenylglycinol allowed us to obtain a mixture of the expected four α-amino nitriles, as two major and two minor components in ca. 8:2 ratio. Because *R*-(−)-α-phenylglycinol preferentially induces opposite chirality in the newly formed asymmetric center the two more abundant constituents derived from each aldehyde were identified as (2*S*)-[(*R*)-(phenylglycinyl)amino nitriles (**21a**–**b** and **22a**–**b**) and separated by medium pressure chromatography. On the basis of their elution sequence during silica gel chromatography and according to our previous report,[4] to the first eluting **21a**–**b** were assigned the (1′*R*,2′*R*,3′*S*) absolute configuration and to **22a**–**b** the opposite (1′*S*,2′*S*,3′*R*) configuration. The α-aminonitriles **22a**–**b**, characterized by the same absolute configuration as PCCG-4 (**3**), were then converted into the corresponding methyl esters by treatment with gaseous hydrogen chloride in methanol. Oxidative cleavage with lead tetracetate of the α-aminoesters gave the corresponding imines which were hydrolyzed using mild acidic conditions (3 N HCl in Et_2O, rt) to afford the corresponding free amines. Final hydrolysis was performed under basic conditions (0.5 N NaOH, reflux) affording the title 3′-substituted 2-(2′-carboxy)cyclopropylglycines **8a**–**b** after purification by ion exchange resin chromatography followed by reversed phase chromatography (RP-8).

The new derivatives **8a** and **8b** were tested as potential mGluR ligands in: (i) hmGluR1b (CHO cells) or hmGluR5a (Ltk- cells) assay by measuring inositol triphosphate formation,[21,22]; (ii) GTP(γ)^{35}S stimulation assay for mGluR2 (CHO cells) receptors,[23] (iii) [^{3}H]glutamate binding assay for mGluR3 (HEK cells) receptors.[24] The results are summarized in Tables 1–3.

As summarized in Tables 1–3, the new derivatives **8a**–**b** show activity as group II mGluR antagonists albeit with significant differences in potency and subtype selectivity. On the overall, these data support the notion that the stereospecific 3′-substitution on CCGs is sterically well tolerated and that the insertion of hydrophobic groups results in group II antagonism. Furthermore, our present data demonstrate that the 3′-substitution alone is sufficient to achieve submicromolar potency as mGluR2/R3 antagonists regardless of the presence of a 2-position substituent. When considered together with the data of Collado et al.,[5] these results clearly indicate

Table 1. Antagonism of the stimulation of GTP(γ)^{35}S binding by glutamate in membranes from CHO cells expressing human mGluR2 receptors

Compd	IC_{50} (μM)	K_i (μM)	Hill-coeff.	*N*
8a	6.4±1.3	2.7	0.93±0.14	4
8b	0.20±0.02	0.085	0.92±0.09	4
7[a]	0.20			

GTP(γ)^{35}S binding was measured as described in the methods section. The IC_{50} values obtained from inhibition curves as shown in Charts 1 and 2 were converted into inhibition constants K_i with the Cheng–Prusoff equation (EC_{50} for glutamate = 7.4 μM). None of the Hill-coefficients were significantly different from unity.
[a]Compound **7** is included for reference purposes. Data taken from ref 7.

Table 2. Binding characteristics at rat recombinant mGluR3 receptors expressed in HEK293 cells

Compd	IC_{50} (μM)	Hill-coeff.	*N*
8a	1.3±0.3	0.78±0.06	3
8b	0.075±0.012	1.09±0.14	3
7[a]	0.016		

[^{3}H]glutamate binding was measured as described in the methods section. The data shown are means ± SEM from (*n*) independent experiments. The Hill-coefficients were not significantly different from unity. Because the concentration of radioligand used was well below its K_D value, IC_{50} values given are essentially identical to K_iS.
[a]Compound **7** is included for reference purposes. Data taken from ref 7.

Table 3. Effects on group I mGluR — mediated phosphoinositol metabolism

Compd	mGluR1b	mGluR5a
8a	10 μM: 131 ± 30 (2) 50 μM: 95 ± 5 (2)	10 μM: 101 ± 7 (3) 50 μM: 94 ± 8 (3)
8b	10 μM: 110 ± 37 (2) 50 μM: 111 ± 15 (2)	10 μM: 103 ± 10 (3) 50 μM: 91 ± 7 (3)

Phosphoinositol metabolism was measured as described in the methods section. The numbers shown represent activities in percent of the respective controls with quisqualate (40 μM for mGluR1b and 0.3 μM for mGluR5) and are expressed as means ± SEM from (*n*) independent experiments.

that the 3′- and the 2-substituents compete for the same additional binding pocket in the receptor. An ethylene spacer (**8b**) between the cyclopropane ring and the aromatic moiety is better than the methylene one (**8a**). Despite the clear indications that 2- or 3′-substituted CCGs bind to the same additional pocket in the receptors, there are some differences between the two classes of derivatives that are worth pointing out. The most relevant one is the observation that, despite a small decrease in potency, our XE-CCGI (**8b**) shows a complete selectivity for group II over group I subtypes (Tables 1–3), whereas LY341495 (**7**) is a significantly potent antagonist also at group I or group III receptor subtypes. A certain degree of group-selectivity can thus be achieved by 3′-substitution, a finding that can certainly be exploited in the design of new, more potent and selective, antagonists.

In conclusion, the new derivatives **8a** and **8b** represent novel group II antagonists endowed with interesting potencies and selectivity and can be considered suitable tools for either the pharmacological characterization or for the structural analysis of the group II subtypes.

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