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Synthesis, Molecular Modeling and Preliminary Biological Evaluation of 1-Amino-3-phosphono-3-cyclopentene-1-carboxylic Acid and 1-Amino-3-phosphono-2-cyclopentene-1-carboxylic Acid, Two Novel Agonists of Metabotropic Glutamate Receptors of Group III

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Abstract—On the basis of a pharmacophore definition of $mGlu_4$ agonists, the two novel semi-rigid derivatives **12** and **13** were designed and synthesized. The preliminary biological evaluation demonstrated that both compounds interact with $hmGlu_{4a}$, while ineffective at group II receptor subtypes. In particular, derivative **13** is a full $hmGlu_{4a}$ agonist with an $EC_{50} = 17$ μM.

Introduction

Most neurons and glia in the central nervous system (CNS) express multiple receptor subtypes responding to L-Glutamic acid, the major excitatory neurotransmitter in the central nervous system (CNS) of vertebrates. Among these, metabotropic glutamate (mGlu) receptors constitute an heterogeneous family of G-protein coupled receptors. So far, at least eight mGlu subtypes have been cloned and classified into three groups, according to sequence homology, transduction mechanisms and agonist pharmacology. Group I includes $mGlu_1$ and $mGlu_5$, which are positively coupled to PI hydrolysis whereas group II ($mGlu_2$, $mGlu_3$) and group III ($mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$) are both negatively coupled to the activity of adenylyl cyclase but are endowed with different pharmacology and neuronal localization.[1]

Over the past decade, the therapeutic potentialities associated with the modulation of individual mGlu subtypes have continuously increased as potent and subtype-selective ligands have become available. So far, most of the interest has been devoted to group I and group II mGlu receptor subtypes. In particular, the prevalently postsynaptic group I receptor subtypes are involved in the amplification of the excitotoxic insult, whereas group II receptor subtypes may contribute to the attenuation of the neuronal damage, also by releasing neurotrophic factors.

Recent evidences, however, point out an important role for group III mGlu receptor subtypes, and $mGlu_4$ in particular, in a variety of CNS diseases.[2] Indeed, it has been observed that the presynaptically localized $mGlu_4$ receptor subtypes play an important role in the homeostasis of extracellular glutamic acid under ischemic/hypoxic conditions and that genetically modified $mGlu_4$-lacking mice are much more vulnerable to excitotoxic insults than wild type animals.[3] These observations bring support to the idea that III mGlu receptor agonists may be of potential therapeutic relevance in attenuating neuronal damage following excitotoxic insults.

L-1-Amino-4-phosphonobutyric acid (L-AP4, **1**) is the prototype of a group III agonist, and is characterized by the substitution of the ω-carboxylate of L-Glu by a phosphonate moiety. L-AP4 (**1**) is an agonist at all the group III subtypes. Conformationally constrained analogues

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of L-AP4 (**1**) are endowed with agonist activity at group III receptor subtypes in the low micromolar range of potencies [4] (Chart 1). They include cyclopentyl derivatives **3**, **4** and the cyclopropyl derivatives **5**, **6**. Of remarkable interest is the activity of (*RS*)-PPG (**7**)[5] and that of the 1-amino-3-(phosphonomethylene)cyclobutanecarboxylic acid derivative **8**, which are not L-AP4 derivatives but still retain a high potency and selectivity towards group III receptor subtypes. Interestingly, also conformationally constrained L-Glu derivatives display agonist activity at $mGlu_4$. Among these, the broad mGlu agonist (1*S*,3*R*)-ACPD (**11**) and the tri-carboxy derivatives[6] ACPT-I (**9**) and (+)-ACPT-III (**10**) which have agonist potency similar to that of ω-phosphono derivatives with marked selectivity toward $mGlu_4$ subtype.

With the aim of understanding the conformational and topological requirements common to all the above group III agonists, we have undertaken a molecular modeling study.

Group III agonist pharmacophore

Also in analogy with other glutamate receptor families, a five points pharmacophoric model was chosen. These five points include a hydrogen bonding donor atom (the positively charged amino acidic nitrogen), two acceptor atoms (the two oxygen atoms of the carboxylic moiety of the aminoacidic group) and two donor sites (ELPs) belonging to the distal phosphonate or carboxylate moiety. Thus, three ELPs were added to each of the phosphonate oxygens and two ELPs were added to each of the carboxylate oxygens.

Derivatives **1**–**11** were built from standard dictionary within the SYBYL program, energy minimized by the Tripos force field (electrostatic term included, dielectric constant of 80) and then submitted to a conformational search performed by using the multisearch routine, as implemented in SYBYL, based on the Montecarlo algorithm. One hundred conformers were generated for each derivative. Those conformers with an energy higher than 6 *K*cal/mol from the global minumum were discarded. After completation of the conformational sampling, the DISCO algorithm was run.[7] The pharmacophore search yielded 128 solutions that were reduced to 10 after elimination of those solution endowed with a maximum tolerance and a RMS fit higher than 1. These 10 final solutions were graphically examined and found to be very similar each to the others only differing by slight different superimposition of individual ligands. The solution endowed with the lowest mean energy of the contributing conformers was chosen as pharmacophore model. The 3-D representation of the resulting pharmacophore is shown in Figure 1. More in detail, all the derivatives contribute to the pharmacophore with conformers less than 2 *K*cal/mol from the global minimum with the exception of compound 2 and 9, whose proposed bioactive conformers are 4.1 and 3.7 *K*cal/mol above the global minimum, respectively.

It should be mentioned that while this paper was in preparation, a study by Bessis et al. has appeared describing a pharmacophore model for $mGlu_4$ agonists.[8] A major difference between that study and our own is that we have included in the search (*RS*)-PPG (**7**), which is the commonly used pharmacological tool for studying group III receptors and has a potency comparable with L-AP4 derivatives. Because of its unique structure among the other group III agonists, (*RS*)-PPG (**7**) strongly influences the topology of the pharmacophore. The results of the pharmacophore search point out that L-AP4 (**1**) interacts

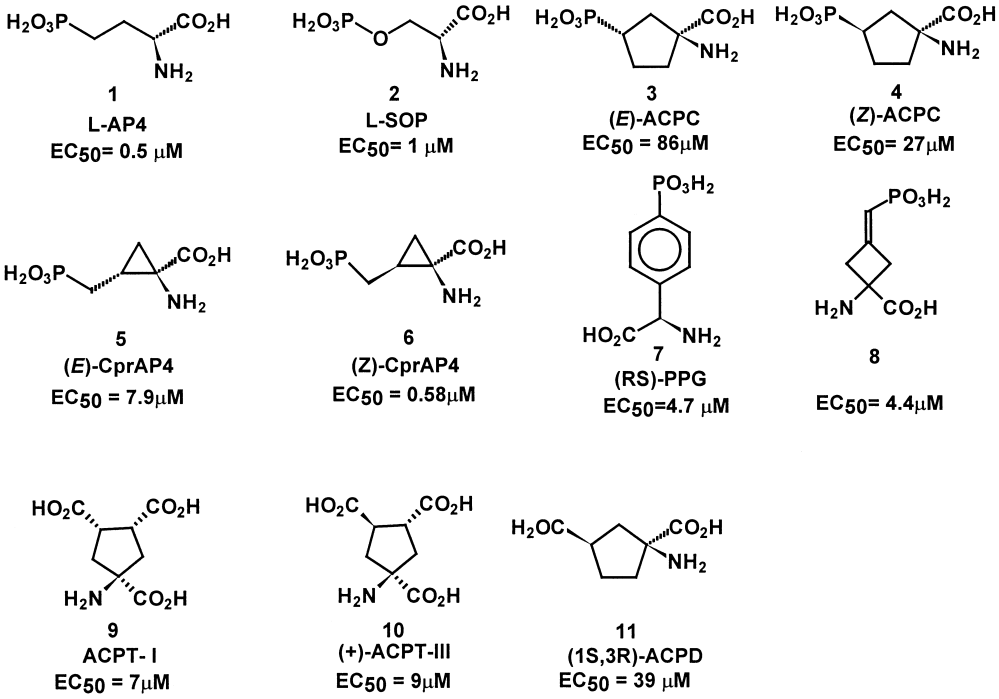


Chart 1.

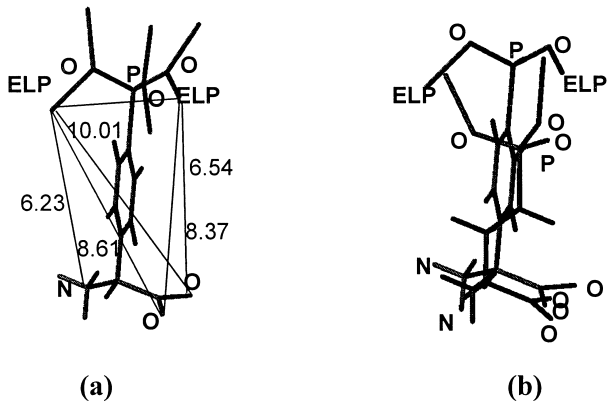


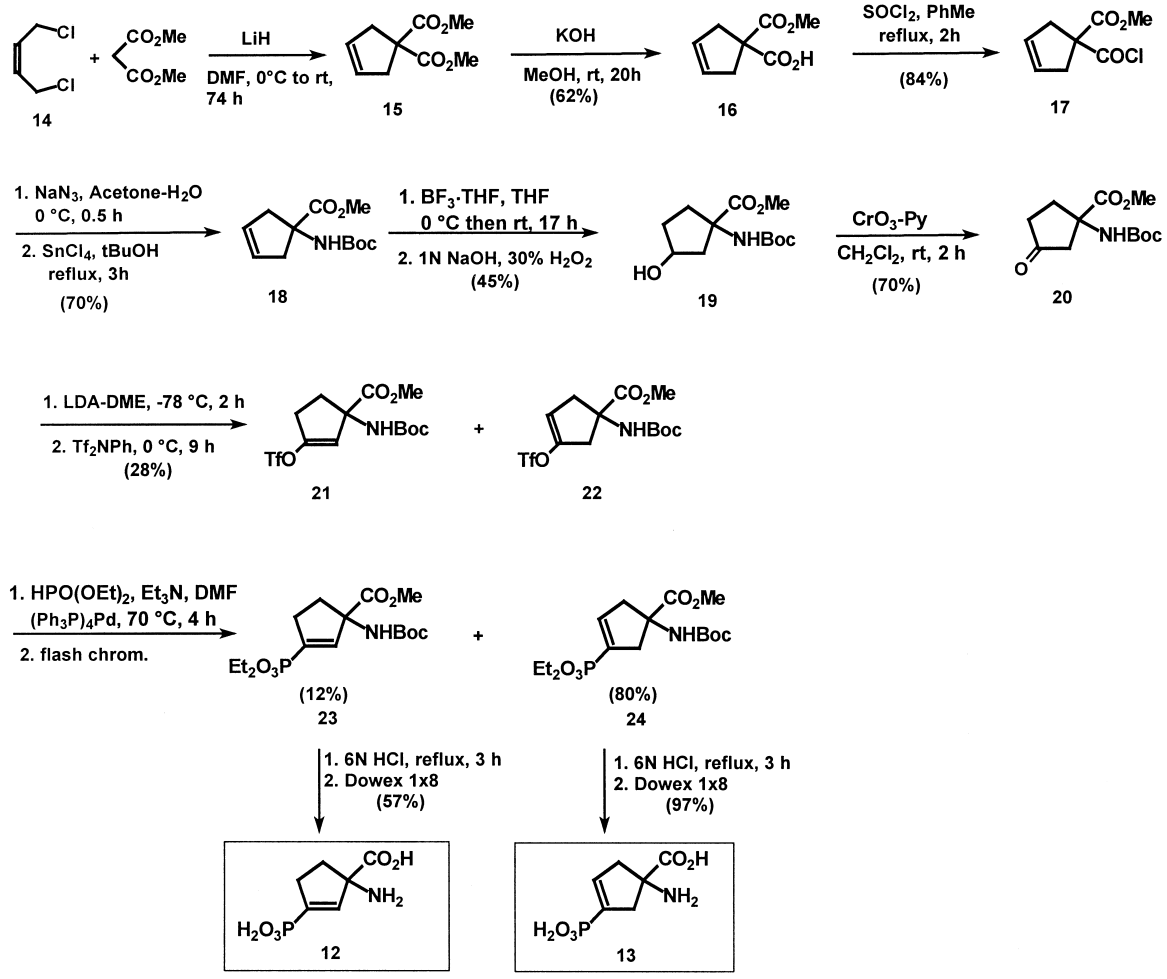
Figure 1. (a) Depiction of group III pharmacophore model. PPG (**8**) (S-configuration at the α-carbon atom) is included for clarity; (b). Superimposition of L-AP4 (**1**) to PPG (**8**).

with group III receptor in a $g^{-}g^{-}$ conformation (*N*-C2-C3-C4 = −56.9°; C2-C3-C4-C5 = −60°). The *syn* ELPs of the phosphonate group are superimposed to the corresponding *anti* ELPs of the phosphonate of PPG (**7**). On the basis of the pharmacophore model, we reasoned that the two cyclopentene derivatives **12** and **13**, which are endowed with the required relative disposition between pharmacophoric groups can potentially display group III agonist activity.[9] The synthesis and the preliminary biological evaluation of two new conformationally constrained analogues of L-AP4, namely 1-amino-3-phosphono-2-cyclopentene- and 1-amino-3-phosphono-3-cyclopentene-1-carboxylic acids **12** and **13**, respectively, are reported herein.

Chemistry

The synthesis of the two phosphonocyclopentene amino acids (**12** and **13**) is depicted in Scheme 1.

The diester **15**, readily obtained from dimethyl malonate and *Z*-1,4-dichloro-2-butene,[10] was submitted to monohydrolysis to give the corresponding monoester **16** which was then transformed into the protected amino acid **18** via the Curtius rearrangement of the corresponding acyl azide. Hydroboration–oxidation reaction of **18** gave the alcohol **19**[11] which was in turn oxidized (**20**) and reacted with *N*-phenyl bis(trifluoromethanesulfonimide)[12] thus affording an inseparable mixture of the two corresponding enol triflates **21** and **22**. Pd(0)-catalyzed coupling of the mixture of **21** and **22** with diethyl phosphite[13] yielded the two protected phosphonocyclopentene amino acids (**23** and **24**) which were then separated (flash chromatography)



Scheme 1.

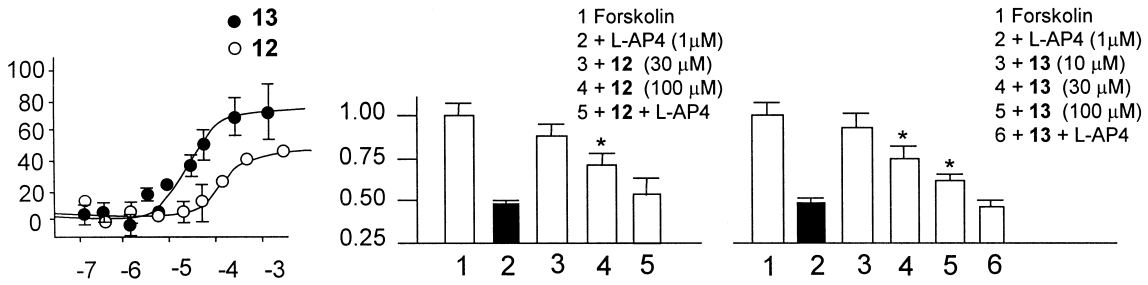


Figure 2. (a) Agonist activity of **12** and **13** determined by stimulation of [^{35}S]-GTPγS binding to membranes prepared from CHO-hmGlu$_{4a}$ cells. Data points were normalized to the stimulation obtained by 1 mM of L-Glu, and represent the mean ± SEM of three determinations from one experiment; (b) and (c) Inhibition of forskolin-stimulated cAMP accumulation in CHO cells expressing hmGlu$_{4a}$. Forskolin (10 μM) stimulated cAMP formation by about 40-fold (taken as control). All values are given as fraction of control. The effect of forskolin is inhibited by 1μM L-AP4; this submaximal agonist concentration represents approximately EC$_{80}$. To test for agonist activity, **12** and **13** were applied to forskolin-stimulated CHO cells Antagonist activity of the compounds was assessed by co-application with the sub-maximal concentration of L-AP4. Bars represent mean ± SEM of two independent experiments, $n \geq 5$. Asterisks indicate statistically significant agonist activity ($2P < 0.01$; Dunnett's *t*-test).

Table 1. Activity of **12** and **13** on two mGlu subtypes expressed in recombinant mammalian cells

Assay	Receptor subtype	**12**		**13**	
		EC$_{50}$ (μM)	IC$_{50}$ (μM)	EC$_{50}$ (μM)	IC$_{50}$ (μM)
			Group II mGlu		
GTPγS binding	Human mGlu$_2$	>300	>200	>300	>300
			Group III mGlu		
GTPγS binding					
	Human mGlu$_{4a}$	>30 (Partial agonist)	>100	17 ± 8	>100
↓ Forskolin-stimulated cAMP	Human mGlu$_{4a}$	>30	>100	≈**30** No full inhibition	>100

and hydrolyzed to give the corresponding amino acids **12** (UPF 703) and **13** (UPF 702), respectively. The structure assignment was made on the basis of the ^{1}H NMR signal of the olefinic proton in compounds **23** and **24**. Thus, in **23**, the olefinic proton appears as a doublet ($\delta = 6.5$) due to the heterocoupling with the phosphorus atom, while in **24** it appears as a multiplet due to the heterocoupling with the phosphorus atom and the allylic coupling with protons in 5-positions.

Preliminary biological evaluation

The novel derivatives **12** and **13** were evaluated for their ability to interact group II or group III mGlu in CHO cell lines expressing hmGlu$_2$ and hmGlu$_{4a}$. cAMP Accumulation[14,15] and [^{35}S]-GTPγS[16,17] assays were employed as previously described.

While ineffective at group II receptor subtypes, both **12** and **13** significantly interact with human mGlu$_{4a}$, either in the GTPγS binding assays or in the cAMP assays (Fig. 2 and Table 1). In particular, in the GTPγS assay **12** is a partial agonist, with an EC$_{50}$ >30 μM, while **13** is a full hmGlu$_{4a}$ agonist with an EC$_{50}$ = 17 μM.

In conclusion, we have reported that the cyclopentene derivatives **12** and **13** are endowed with activity at the mGlu4$_a$ receptor subtype. Compound **12** behaves as a partial agonist, while **13** is a moderately potent mGlu$_{4a}$ agonist, thus confirming our hypothesis on the group III pharmacophore requirements. It should be noted that both **12** and **13** have been tested as racemic mixture and that their affinity and functional profile (in the case of the partial agonism of **12**) may change when the pure enantiomers will be synthesized and tested.

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