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Design and synthesis of APTCs (aminopyrrolidinetricarboxylic acids): Identification of a new group III metabotropic glutamate receptor selective agonist

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Abstract—A new family of mGlu receptor orthosteric ligands called APTCs was designed and synthesized using a parallel chemistry approach. Amongst 65 molecules tested on mGlu4, mGlu6 and mGlu8 subtypes, (2*S*,4*S*)-4-amino-1-[(*E*)-3-carboxyacryloyl]pyrrolidine-2,4-dicarboxylic acid (**8a06**—FP0429) has been shown to be a full mGlu4 agonist and a partial mGlu8 agonist. In addition, **8a06** was shown to be selective versus group I and II mGlu subtypes. A possible explanation for this efficacy difference is proposed by docking experiment performed with molecular model of the receptor.

Glutamate, the most commonly occurring neurotransmitter in the CNS, plays a critical role in a large number of physiological processes. It operates through two families of receptors: the ionotropic (iGlu) and the metabotropic (mGlu) receptors. mGlu receptors have been divided in three groups according to their sequence homologies, pharmacological properties and signal transduction pathways.[1] Group III mGlu receptors include mGlu4, mGlu6, mGlu7 and mGlu8 receptors, which are negatively coupled to cAMP formation. They are mainly localised in the central nervous system with the exception of mGlu6 which is found mostly in the retina. Their potential roles for the treatments of anxiety, Parkinson's disease and epilepsy have been suggested.[2]

mGlu receptor ligands can be classified in two families according to their site of interaction: orthosteric ligands competing with glutamate in the venus fly trap (VFT) domain and allosteric modulators interacting with the transmembrane domain.[3] To date, only few orthosteric agonists have been described for group III mGlu receptors. The most representative ones are L-AP4, R, S-PPG,[4] S-DCPG[5] and ACPT-I[6] (Fig. 1). They have low-micromolar to sub-micromolar potencies for mGlu4, mGlu6 and mGlu8 and high-micromolar potency for mGlu7 receptor.

Because of their simple amino acid structures, mGlu orthosteric ligands are not subject to parallel chemistry. To our knowledge, there is no report of chemical approach offering rapid access to a large number of such

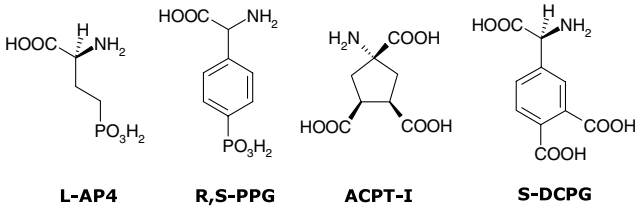

Figure 1. Structures of standard group III mGlu receptor agonists.

Keywords: Metabotropic glutamate receptor; mGlu4; Excitatory amino acids.
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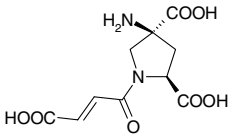
polar derivatives. In this communication, we report the design and the synthesis of a new orthosteric ligand family named APTC (for aminopyrrolidine-tricarboxylic acid), and the pharmacological characterization of **8a06** (FP0429), a hit showing micromolar potencies on group III subtypes.

APTCs design is based on structural modifications of tricarboxylic acid ACPTs, family of molecules in which selective group III agonists were found.[6] The aim was to obtain a closed scaffold, that is, an α-amino acid on a five-membered ring with highly polar side chains attached to positions 3 and 4. These side chains could interact with the highly basic distal binding pocket of the receptors. In addition, our objective was also to obtain a new scaffold amenable to parallel synthesis. Just like ACPTs are ACPD's analogues with an additional COOH group, we anticipated that functionalizing APDCs on the pyrrolidine nitrogen with polar groups would afford the expected family of ligands (Fig. 2).

In APTCs, two asymmetric centres are present. We decided to control both by choosing the stereochemical synthesis depicted in Scheme 1. Starting material was either 2*S*,4*R* or 2*R*,4*R* hydroxyproline which were converted into hydantoins **3a** and **3b** following similar procedures used by Monn et al. for the synthesis of APDCs.[7] Diastereoisomers **3a** and **3b** were then hydrolysed, esterified and separated using flash chromatography to afford the four isomers **4a**–**d**. These aminoesters were boc-protected (**5a**–**d**) and hydrogenolysed to the corresponding pyrrolidines **6a**–**d**. The four protected platforms were capped in parallel with electrophilic species to give **7(a–d)XX** using solid supported reagents and scavengers. Final deprotections yielded the desired APTCs **8(a–d)XX**.[8]

Using 25 different R groups (Scheme 1), chosen in order to introduce an additional polar group on the N-1 position, a virtual library of one hundred APTCs was designed. Half of these groups is made of a carboxylic acid, where as the other half contains non-acidic polar functions. Synthetic efforts led to the synthesis of 65 final compounds giving a success rate of 65% for the library synthesis.

Synthesized APTCs were screened on three group III mGlu receptors (mGlu4, mGlu6 and mGlu8) in functional assays at 450 and 300 μM, for their agonist and antagonist activities, respectively.[9] **8a06** (Fig. 3) was



FP0429 (8a06)

Figure 3. 8a06 structure.

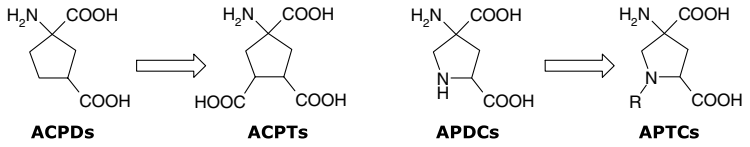
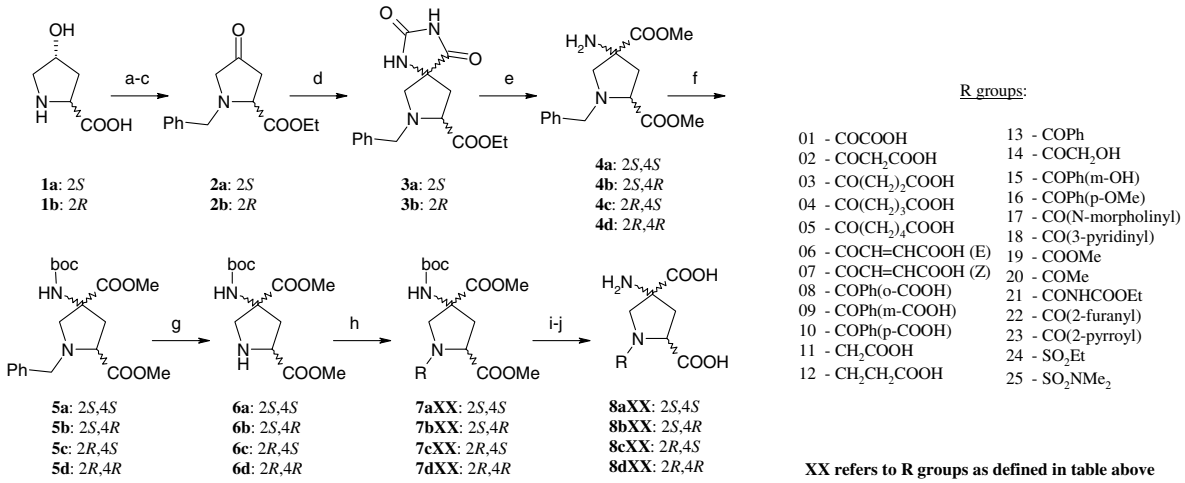


Figure 2. Design of APTC family.



Scheme 1. Synthesis of APTCs. Reagents and conditions: (a) $SOCl_2$, EtOH, reflux, 95–99%; (b) $PhCH_2Br$, Et_3N, DCM, reflux, 79–90%; (c) 1—Oxalyl chloride, DCM, DMSO, −78 °C, 2—Et_3N, −78 °C–rt, 92–96%; (d) $(NH_4)_2CO_3$, KCN, EtOH, H_2O, 50–55 °C, 68–78%; (e) 1—NaOH 2 M, reflux, 2—$SOCl_2$, MeOH, reflux, 3—diastereoisomer separation by standard chromatography, 7–15% for *cis* diastereoisomers, 30–60% for *trans* diastereoisomers; (f) Boc_2O, DCM, rt, 67–95%; (g) NH_4HCO_2, Pd-C 10%, MeOH, reflux, 57–95%; (h) 1—electrophilic agent, PS-DEA, DCM, rt, 2—AMPS, DCM, rt; (i) LiOH 2 M, THF, rt; (j) HCl 2 M in ether, AcOH, rt, 5–50% for the three last steps.

detected with strong agonist responses on mGlu4 and lower one on mGlu6 and mGlu8 at 450 μM.[10] It is interesting to notice that amongst inactive APTCs on mGlu4, some are structurally very close to **8a06**. It is the case of **8a03** or **8d06** which differ only by saturation of the double bond or stereochemistry of the pyrrolidine position 2, respectively. This result further illustrates the particularly restricting feature of group III mGlu active sites.

We then determined EC_{50} values for mGlu4, 6 and 8 and selectivity against other mGlu subtypes (Table 1). **8a06** activates both mGlu4 and mGlu8 with micromolar potency (EC_{50} = 48.3 μM and EC_{50} = 56.2 μM, respectively) and mGlu6 with high micromolar potency (EC_{50} = 378 μM). It shows also selectivity for group III mGlu receptors as no agonist nor antagonist activity was detected on subtypes mGlu1, mGlu5 and mGlu2 up to 5 mM. Interestingly, although **8a06** shares a similar potency on mGlu4 and mGlu8, its efficacy for the two subtypes differs significantly. **8a06** is a full agonist on mGlu4 with a maximal response almost comparable to that of glutamate (E_{max} = 75%) and a partial agonist on mGlu8 with a E_{max} value of 36% of glutamate response (see Fig. 4).

In order to further understand the interaction of **8a06** with mGlu4, a docking experiment was performed using the receptor molecular model and docking protocol recently described by Bertrand et al.[11] This protocol consists in the flexible fitting of the ligand within a rigid receptor using the shape-based docking algorithm LigandFit. The obtained poses were subsequently scored using the LigScore scoring function. The best pose was then energy minimized with CHARMm allowing full flexibility for the ligand and only side-chain flexibility for the receptor. All calculations were carried out in the Discovery Studio 1.5 environment (Accelrys, San Diego, California). Results show that **8a06** fits nicely in a dense H-bond network, interacting with a dozen amino acids of mGlu4 active site. The glycine entity of **8a06** is bound to the set of residues that build up the signature motif common to all proteins of the fold family (S159, A180, T182, D202, Y230, D312).[11,12] The distal functions of **8a06**, one amide and two acidic groups, are bound to the same residues as ACPT-I one's (K71, K74, R78, S110, S157, R258, N286, S313, K317, K405)[11] (Fig. 5). Yet, because of conjugation, the fumaric acid amide substituent is rigid, thus when all polar bindings are fitted, one of the ethylenic protons is found in close contact with those of G158 (2.6 Å)

Table 1. Activities of **8a06** and standard group III agonists on rat mGlu receptors (EC_{50} values in μM)[9]

	Group I		Group II		Group III			
	mGlu1	mGlu5	mGlu2	mGlu3	mGlu4	mGlu6	mGlu7	mGlu8
8a06[a]	>5000	>5000	>5000	NT	**48.3 (5.2)**	378 (112)	NT	**56.2 (14.6)**
L-AP4[b]	>1000	>1000	>1000	>1000	0.5–1	0.6–0.9	160–800	0.6
RS-PPG[c]	>500	>500	>300	>200	5.2	4.7	185	0.21
S-DCPG[d]	ant @ 32	>100	>100	>100	8.8	3.6	>100	0.031
ACPT-I	ant @ 1000[e]	>1000[a]	>1000[e]	NT	7.2[e]	18.4[f]	1200[f]	10.1[f]

NT: not tested.
[a] Values are means of three independent experiments, standard deviation is given in parentheses.
[b] Taken from Refs. 3 and 13.
[c] On human mGlu, taken from Ref. 4.
[d] On human mGlu, taken from Ref. 5.
[e] Taken from Ref. 6.
[f] Personal communication from Dr. Jean-Philippe Pin.

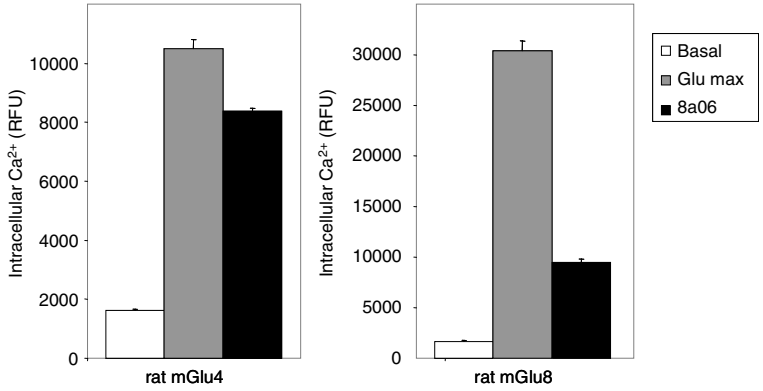


Figure 4. 8a06 efficacies on mGlu4 and mGlu8. Intracellular calcium mobilization in HEK 293 expressing the indicated receptor, under basal conditions (white bars), 500 μM glutamate (grey bars) and 1 mM **8a06** (black bars) activations. No activity was detected with either Glutamate or **8a06** on mock-transfected cells.

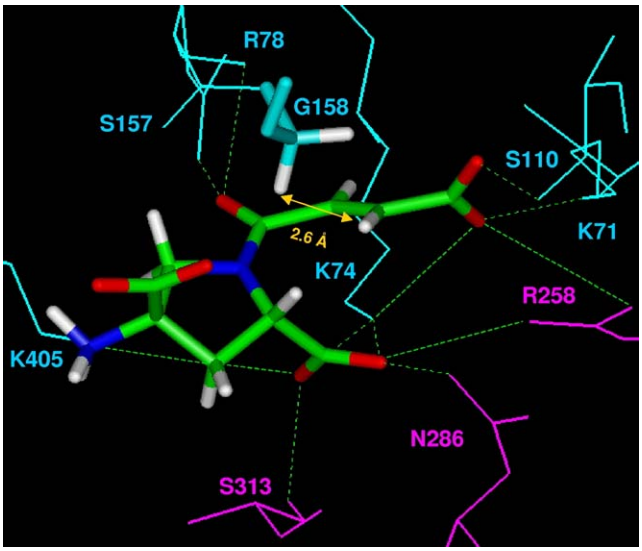


Figure 5. Agonist **8a06** docked at mGlu4 active site.

(Fig. 5). Consequently, any bulkier residue as an alanine would induce some distortion of the binding network. Indeed, the corresponding amino acid of G158 in mGlu6 and mGlu8 is no longer a glycine but an alanine. In addition, S157 to which **8a06** is also bound (Fig. 5) is replaced by an alanine in mGlu8. These differences may be responsible for the different behaviour observed with **8a06** on these subtypes. The latter assumption should be validated by using mGlu4, 6 and 8 mutants where the crucial amino acids would be replaced.

In conclusion, a chemical library of new ligands was designed and synthesized using parallel chemistry approach. An agonist for group III was identified with different efficacies for subtypes mGlu4 and mGlu8. This ligand will be of great interest to further understand pharmacological role and therapeutic potential of mGlu4 subtype.

Acknowledgments

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8. Typical experimental procedure for capping and deprotections of intermediates **6a–d**: Electrophilic species (0.33 mmol) was added to a solution of **6a–d** (50 mg, 0.165 mmol) and PS-DEA (200 mg, 0.33 mmol) in DCM (2 mL). The resulting suspension was shaken for 20 h. Then AMPS (200 mg, 0.33 mmol) was added to the solution, and this latter was shaken for a further 20 h. Resins were filtered and the solvent was evaporated. Lithium hydroxide (2 M solution, 0.3 mL) was added to a solution of crude ester **7(a–d)XX** in THF (1 mL). The resulting mixture was stirred at RT for 12 h, then acidified to pH 1 with 1 M hydrochloric acid, extracted with AcOEt, washed with brine, dried over $MgSO_4$ and evaporated. Hydrochloric acid (2 M solution in ether, 0.2 mL) was added to a solution of crude boc-amine **8(a–d)XX** in acetic acid (0.5 mL). The resulting solution was stirred for 6 h at RT. Solvents were evaporated and the resulting solid was suspended in ether, filtered and dried to give expected product as a white solid. All compounds were identified by ^1H NMR and LC/ES–MS and data were consistent with the proposed structures.
9. Intracellular calcium measurements: HEK 293 cells were transiently transfected with plasmid DNA encoding rat mGlu receptors, and a chimeric G protein to couple the naturally Gi/o coupled receptors (subtypes 2, 4, 6 and 8) to the Phospholipase C/Ca^{2+} pathway (Frauli, M.; Neuville, P.; Vol, C.; Pin, J.-P.; Prezeau, L. *Neuropharmacology* **2006**, *50*, 245). In summary, cells were seeded in poly-ornithine-coated, black-walled, clear bottomed, 96-well plates and cultured for 24 h after transfection. Cells were then washed with freshly prepared buffer (1× HBSS supplemented with 20 mM Hepes, 1 mM $MgSO_4$, 3.3 mM Na_2CO_3, 1.3 mM $CaCl_2$, 0.5% BSA and 2.5 mM Probenecid) and loaded with 1 μM Ca^{2+}-sensitive fluorescent dye Fluo4AM (Molecular Probes). After loading, cells were washed twice with buffer and then incubated in 50 μL of buffer. Addition of compounds (50 μl of 2×-drug solution) and intracellular Ca^{2+} measurements were performed by the fluorescence microplate reader FlexStation (Molecular Devices) at sampling intervals of 1.5 s for 60 s (excitation 485 nm, emission 525 nm). Agonist or antagonist activities were evaluated in comparison to basal

signal or signal evoked by Glutamate EC80 alone, respectively. Dose–response curves were fitted using the GraphPad Prism program (San Diego). All values are means ± SEM of three independent experiments performed in triplicate.

10. Compound **8a06** was resynthesized using non-parallel procedure. Characteristic data for **8a06**: ^{1}H NMR (D_2O, 400 MHz) δ mixture of rotamers, 7.21 ($1H^{major}$, d, J = 15.5 Hz), 7.10 ($1H^{minor}$, d, J = 15.5 Hz), 6.68 ($1H^{major}$, d, J = 15.2 Hz), 6.65 ($1H^{minor}$, d, J = 15.5 Hz), 4.58 (1H, m), 4.27 (1H, d, J = 12.0 Hz), 4.13 (1H, m), 3.94 ($1H^{minor}$, d, J = 14.0 Hz), 3.02 ($1H^{minor}$, m), 2.93 ($1H^{major}$, dd, J = 9.9 and 14.6 Hz), 2.47 ($1H^{minor}$, m), 2.93 ($1H^{major}$, dd, J = 4.4 and 14.0 Hz). ES–MS (m/z): 273 $(M+H)^{+}$.
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