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Metabotropic glutamate receptor orthosteric ligands and their binding sites

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Key words : orthosteric agonists; antagonists; selectivity; Venus Flytrap; binding site; 3D-structure

Abstract

Metabotropic glutamate receptors (mGluRs) have been discovered almost four decades ago. Since then, their pharmacology has been largely developed as well as their structural organization. Indeed mGluRs are attractive therapeutic targets for numerous psychiatric and neurological disorders because of their modulating role of synaptic transmission. The more recent drug discovery programs have mostly concentrated on allosteric modulators. However, orthosteric agonists and antagonists have remained unavoidable pharmacological tools as, although not expected, many of them can reach the brain, or can be modified to reach the brain. This review focuses on the most common orthosteric ligands as well as on the few allosteric modulators interacting with the glutamate binding domain. The 3D-structures of these ligands at their binding sites are reported. For most of them, X-Ray structures or docked homology models are available. Because of the high conservation of the binding site, subtype selective agonists were not easy to find. Yet, some were discovered when extending their chemical structures in order to reach selective sites of the receptors.

1. Introduction

The glutamate saga counts an important milestone with the pioneering work of J.C. Watkins and his memorable article with R.H. Evans in 1981 putting together all data to confirm its neurotransmitter role (Watkins and Evans, 1981). Evidence for acidic amino acid-mediated synaptic excitation was soundly demonstrated. At that time only NMDA and non-NMDA, quisqualate (now named AMPA) and kainate, receptors were considered. The characterization of these excitatory amino acid receptors was strongly based on the specific pharmacological tools that are selective agonists and antagonists. A few years later besides these ionotropic glutamate receptors which are ligand-gated ion channels, a new type of receptors that were able to directly stimulate inositol phosphate formation were discovered (Nicoletti et al., 1986; Sladeczek et al., 1985). These receptors activate phosphoinositide hydrolysis and were named metabotropic glutamate receptors (mGluRs) (Sugiyama et al., 1987; Sugiyama et al., 1989). The first subtype was cloned in 1991 followed by the seven other subtypes (Conn and Pin, 1997; Masu et al., 1991). Their signaling transduction was well established through coupling with G-proteins (Conn and Pin, 1997). mGluRs were indeed the first members of class C G-Protein Coupled Receptor (GPCR) family (Pin et al., 2003). Once the eight subtypes were identified, they were classified in three groups according to their sequence identity, transduction mechanism and pharmacological profile (Fig. 1) (Conn and Pin, 1997; Pin and Acher, 2002; Pin et al., 1999; Pin and Duvoisin, 1995).

Group I includes mGlu1 and mGlu5 receptors, group II, mGlu2 and mGlu3; and group III, mGlu4, 6, 7 and 8. Group I receptors are mostly postsynaptic and activate phospholipase C (PLC). They are selectively activated by 3,5-DHPG. Group II receptors are mostly presynaptic and inhibit adenylyl cyclase (AC). LY354740 is one of their most potent and specific agonist. Group III receptors are also presynaptic, inhibit AC and are selectively activated by L-AP4, a phosphonate analogue of glutamate. Activation of presynaptic receptors reduces the release of glutamate, which may be beneficial in excitotoxicity related disorders. Recently, different mGluR subunits (either 1 and 5, or any group II and III subunits) were found to assemble into heterodimeric entities (Doumazane et al., 2011; Habrian et al., 2019; Scholler et al., 2017a) increasing the potential number of mGluR dimers by 16. Evidence for

the existence of such heterodimers in the brain has been provided for mGlu2-4 (Lee et al., 2020; Moreno Delgado et al., 2017; Yin et al., 2014).

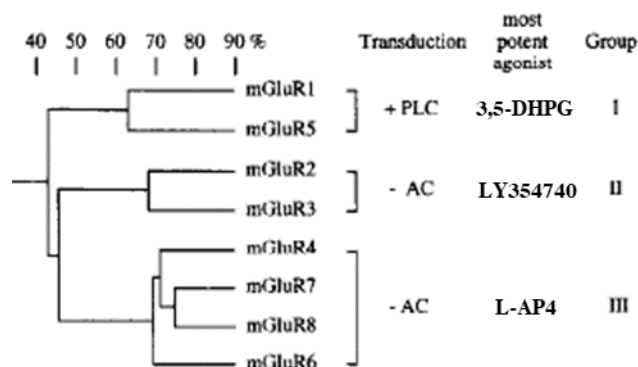


Figure 1. Dendrogram and pharmacological classification of the members of the mGluR family (updated figure from reference (Pin and Duvoisin, 1995)).

Whereas ionotropic glutamate receptors are essential for synaptic excitatory neurotransmission, mGlu receptors modulate the synaptic event in the central nervous system (CNS). Because such a role allows a better fine tuning with pharmacological agents, mGluRs were soon perceived as promising therapeutic targets for numerous psychiatric and neurological disorders (Crupi et al., 2019). Such a situation boosted their structural and pharmacological investigations. Here we review these two aspects from the first discoveries to recent ones.

2. Structural investigations into mGluRs

2.1 From sequence to 3D-structures

Among the GPCRs, class C receptors hold 7 transmembrane helices (7TM) as members of the other classes. Yet their hallmark is a large extracellular amino-terminal domain (ATD) composed of a binding domain linked to the 7TM by a cysteine-rich domain (CRD) for most of them but the GABAB receptor where the CRD is replaced by a stalk domain (Conn and Pin, 1997; Niswender and Conn, 2010; Pin and Duvoisin, 1995; Shaye et al., 2020).

Initially this ATD was represented as a linear thread (Fig. 2A) (Pin and Duvoisin, 1995) until P. O'Hara and colleagues reported a similarity between the ATD of mGlu1 and Leucine-Isoleucine-Valine Periplasmic Binding Protein (LIVBP), a bacterial protein for which an X-ray structure was available (O'Hara et al., 1993; Sack et al., 1989). Their discovery was based on sensitive sequence analysis techniques revealing 22.3% identity over the first 496 N-terminal residues. LIVBP is a member of the large family of periplasmic binding proteins (PBP) involved in the transport of sugars, amino acids and other nutrients. These proteins, including LIVBP, consist of two distinct globular domains connected by a flexible hinge made of three short peptide segments (Sack et al., 1989). Their substrate binds close to the hinge, first to an open conformation of the bilobate protein then is trapped upon closing and delivered to a transmembrane carrier bringing the substrate into the bacteria. It was thus proposed that the ligand binding domain of mGluRs folds similarly to PBPs with two lobes linked by a flexible hinge (O'Hara et al., 1993). Glutamate, the endogenous agonist of mGluRs would bind to an open conformation and then activation of the receptor would occur upon closing of the lobes. This binding domain of mGluRs was then named the Venus FlyTrap domain (VFT) by analogy with the carnivorous plant. Because of the low sequence identity and the LIVBP template available as an open conformation only, the generation of an accurate closed homology model of an mGluR VFT was not straightforward (Fig. 2B) (Bessis et al., 2000), although, the binding motif involved in the interaction with the amino-acid moiety was well conserved between mGlu1 and LIVBP. The first crystal structure of mGlu1 VFT confirmed the computing hypothesis (Kunishima et al., 2000) and provided a template for homology models of the various mGluR VFTs (Fig.3A) (Bertrand et al., 2002). It was then only in 2019 that the structure of the full length mGlu5 receptor was solved by cryo-EM (Fig. 3B) (Koehl et al., 2019). In 1996, Romano et al identified that mGluRs are disulfide-linked homodimers (Romano et al., 1996). The X-Ray and cryo-EM structures of the VFT domains and the full-length receptor confirmed the obligatory dimeric structure that explains the activation mechanism (Koehl et al., 2019; Kunishima et al., 2000). Indeed, the VFT closing causes the CRD's to move closer and induces a rotation of the 7TM one relative to the other, which can then initiate signaling (Fig. 3B and 4). The binding of agonists to the dimer

inactive resting state with both VFTs open (Roo) promotes the shift to the active state, with both VFTs closed (Acc) through an intermediate state (Fig. 4) (Kniazeff et al., 2004; Liauw et al., 2021). Detailed steps of the activation mechanism are still under investigation. Such a mechanism leads to the surprising activation of a single G protein per dimer, and this was clearly demonstrated with an mGlu2 cryo-EM structure and with the heterodimers such as mGlu2-4 in which the mGlu4 subunit is responsible for G protein activation (Liu et al., 2017; Seven et al., 2021).

Whereas orthosteric ligands binding to the VFT concentrated the initial efforts in drug discovery, positive and negative allosteric modulators (PAM and NAM, respectively) binding to the 7TM were later discovered (Lindsley et al., 2016). Recently PAMs and NAMs interacting with the VFT have also been identified (Gee et al., 2014; Scholler et al., 2017b). In the present review, we will focus on all types of ligand binding to the VFT of mGlu receptors, essentially the orthosteric agonists and competitive antagonists, as well as the PAMs and NAMs interacting with the VFT.

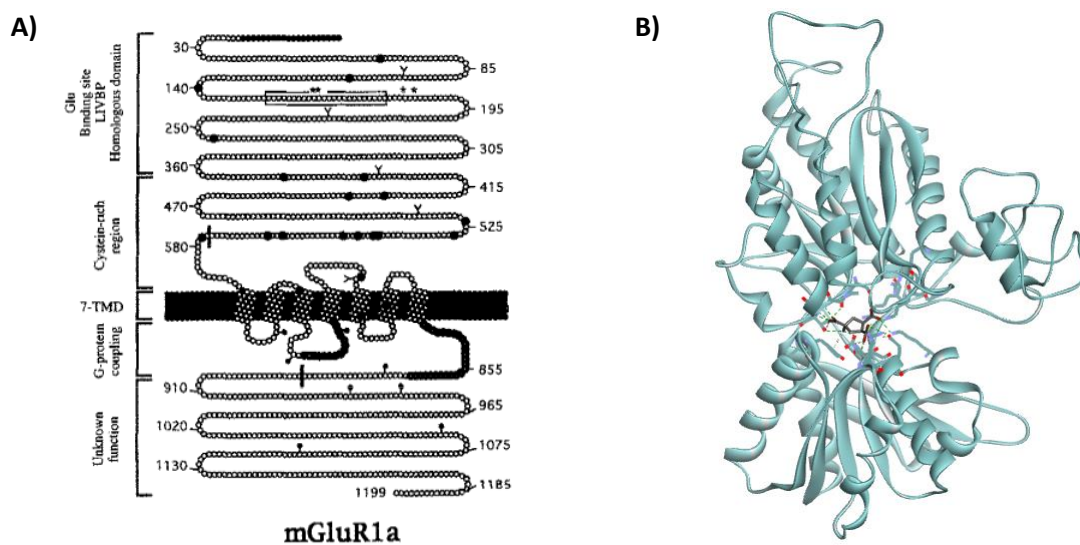


Figure 2. A) Initial schematic representation of mGlu1a receptor (Pin and Duvoisin, 1995). B) First homology model of mGlu4 VFT domain bound to the agonist ACPT-I in the closed conformation (Bessis et al., 2000). Discovery Studio 2019 display as for all figures of 3D-structures.

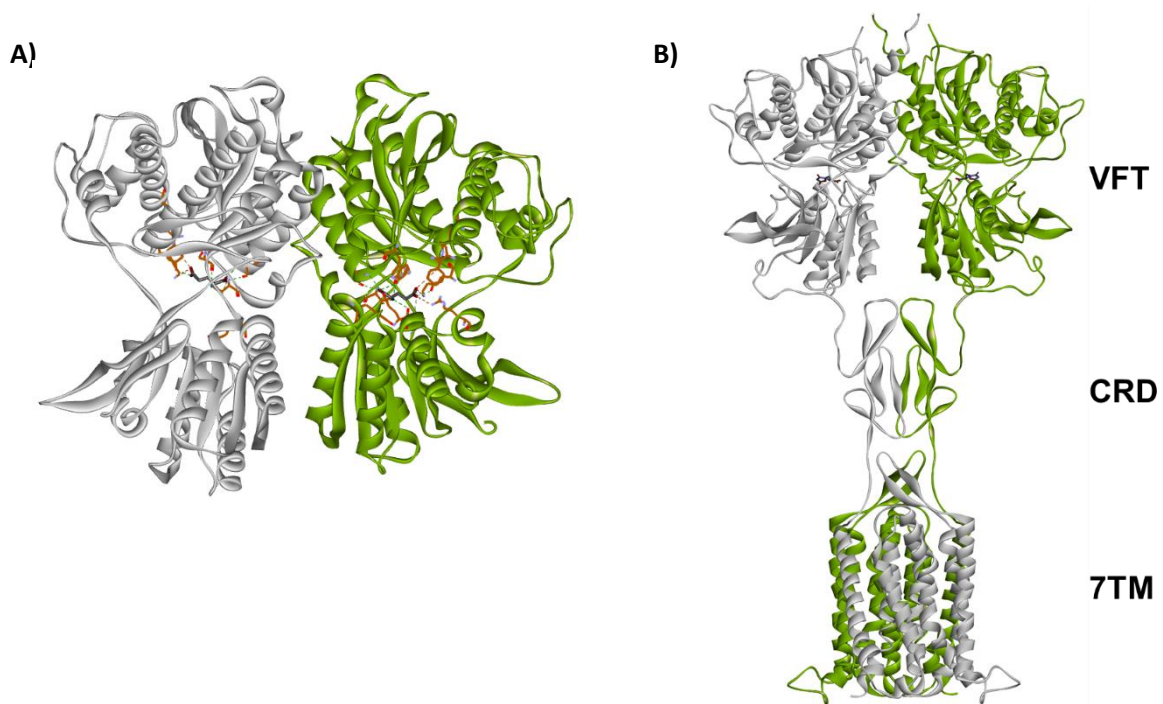


Figure 3. A) Co-crystal structure of the VFT domain of mGlu1 receptor dimer bound with glutamate (PDB ID 1EWK) (Kunishima et al., 2000). Protomer A (green) is closed whereas protomer B (gray) is open. Side chains of binding residues are displayed. Interactions are shown as dashed lines. B) Cryo-EM structure of full-length mGlu5 dimer in the active conformation (PDB ID 6N51) (Koehl et al., 2019). Protomer A (green) and B (gray) include a closed VFT bound to quisqualate.

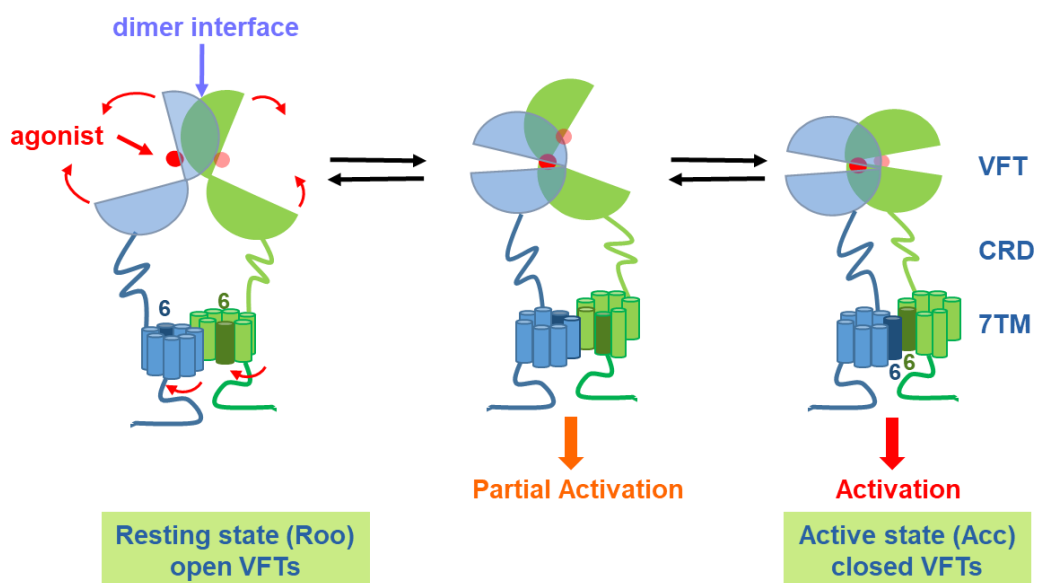


Figure 4. Schematic representation of mGlu receptor activation mechanism. Upon binding of agonists, the equilibrium is shifted from a resting to an intermediate then to an active state of the receptor

(Kniazeff et al., 2004; Liauw et al., 2021). The VFTs are both open in the Roo state and closed in the Acc state. An intermediate state with one VFT open and the other one closed has been proposed (Liauw et al., 2021).

2.2 Binding motif of agonists in mGluRs

The glutamate binding network is composed of a proximal motif interacting with the amino-acid moiety of glutamate and a distal motif binding the side-chain carboxylate. The proximal binding motif is conserved in all mGlu receptors (Acher and Bertrand, 2005). It builds up a signature motif present in all proteins that belong to the LIVBP-like superfamily and bind to amino acids (Acher and Bertrand, 2005). In contrast, variable residues are found among the distal binding residues, in addition to two conserved basic residues (Fig. 5). These variable residues at the glutamate binding site (Table 1) allowed to discover group selective orthosteric agonists (see below). These group selective orthosteric agonists were early identified, however sub-type selectivity was more challenging to obtain. It required more extended ligands that interact with residues further away from the glutamate binding site (see LY2812223, LY2794193 and LSP4-2022) (Goudet et al., 2012; Monn et al., 2018; Monn et al., 2015a).

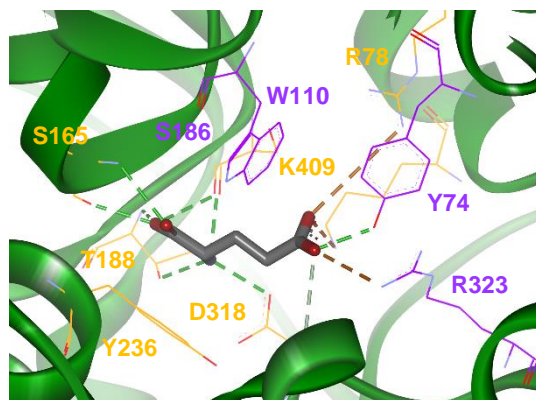


Figure 5. Glutamate (sticks) bound to the VFT closed conformation of mGlu1 receptor (green ribbon) (PDB ID 1EWK:A). Conserved residue side chains are colored in orange (S165, T188, Y236, D318, R78, K409) and group-selective residue side chains in violet (Y74, W110, R323) (Kunishima et al., 2000). Non-bond interactions are displayed as dashed lines (green for H-bonds, brown for salt bridges, van der Waals contacts are not shown).

Table 1. Alignment of group selective residues at the glutamate binding site of the 8 rat mGlu receptor subtypes.

Receptor subtypes	Aligned subtype selective residues					
	helix- α 1	loop β 5- α 2	loop β 6- α 3	loop β 10- α 7	loop β 11- α 8	loop β 12- α 9
mGlu1R	Y₇₄	W₁₁₀	G₁₆₃S₁₆₄	S₂₆₃N₂₆₄A₂₆₅G₂₆₆ - -	E₂₉₂G₂₉₃M₂₉₄	G₃₁₉ R₃₂₃
mGlu5R	Y₆₄	W₁₀₀	G₁₄₉S₁₅₀	S₂₄₉N₂₅₀A₂₅₁G₂₅₂ - -	E₂₇₈G₂₇₉M₂₈₀	G₃₀₅ R₃₀₉
mGlu2R	R₅₇	S₉₃	S₁₄₃Y₁₄₄	R₂₄₃A₂₄₄M₂₄₅S₂₄₆ - -	R₂₇₁S₂₇₂E₂₇₃	G₂₉₆ L₃₀₀
mGlu3R	R₆₄	S₁₀₀	S₁₄₉Y₁₅₀	R₂₄₉S₂₅₀N₂₅₁I₂₅₂ - -	R₂₇₇S₂₇₈D₂₇₉	G₃₀₂ Q₃₀₆
mGlu4R	K₇₄	S₁₁₀	S₁₅₇G₁₅₈	R₂₅₈E₂₅₉P₂₆₀K₂₆₁ - -	N₂₈₆E₂₈₇D₂₈₈	S₃₁₃ K₃₁₇
mGlu6R	Q₅₈	S₉₄	S₁₄₆A₁₄₇	R₂₄₇E₂₄₈P₂₄₉K₂₅₀ - -	N₂₇₅E₂₇₆D₂₇₇	S₃₀₂ K₃₀₆
mGlu7R	N₇₄	S₁₁₀	S₁₅₇G₁₅₈	Q₂₅₈E₂₅₉R₂₆₀K₂₆₁D₂₆₂R₂₆₃	N₂₈₈D₂₈₈E₂₈₉	S₃₁₅ K₃₁₉
mGlu8R	K₇₁	S₁₀₇	A₁₅₄A₁₅₅	R₂₅₅E₂₅₆P₂₅₇R₂₅₈ - -	N₂₈₃E₂₈₄D₂₈₅	S₃₁₀ K₃₁₄

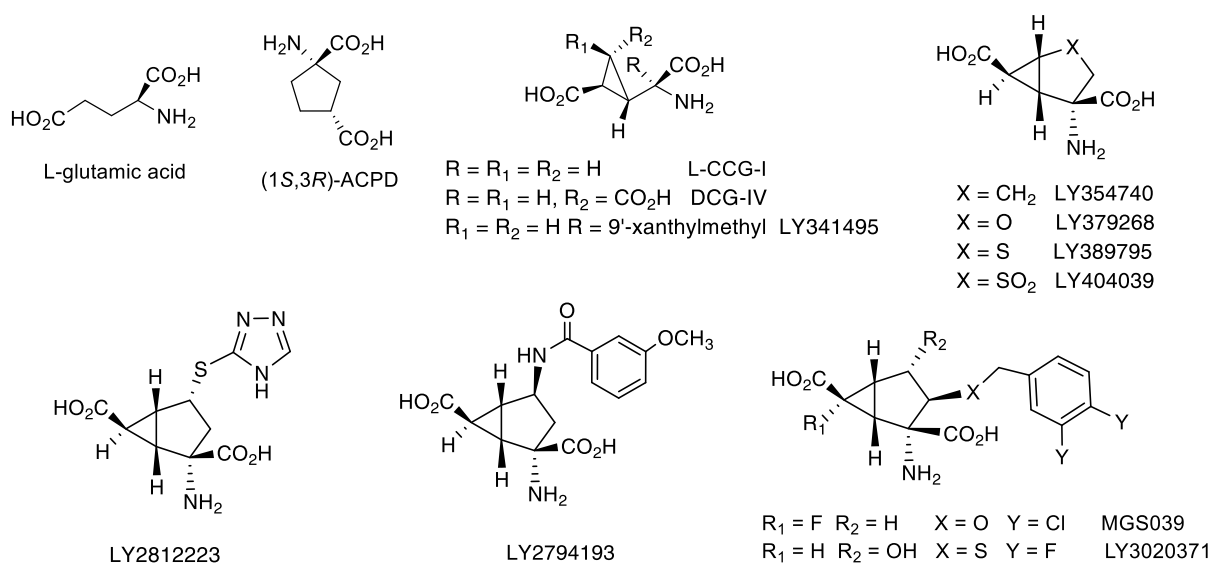
3. Molecular pharmacology of mGluRs

3.1 Agonists and their binding sites

As mentioned above, mGlu receptors participate in the modulation of synaptic transmission and neuronal excitability. As such, they provide a major opportunity for the development of novel treatments for psychiatric and neurological disorders. Indeed numerous drug discovery programs have been carried out since the cellular expression of the 8 mGlu receptors. Tremendous efforts led to the development of agonists, antagonists and allosteric modulators (Niswender and Conn, 2010). Since the discovery of the first mGlu1 negative allosteric modulator CPCCOEt in the late nineties, (Litschig et al., 1999) research was mostly focused on allosteric modulators (Lindsley et al., 2016). However, agonists and antagonists binding at the glutamate binding site remain extensively used as pharmacological tools. Here we review the most popular ones and describe their binding mode at the VFT domain of mGlu receptors (Fig. 6).

Most orthosteric ligands derive from glutamate. In addition, glutamate binds to all subtypes, in a common extended conformation (Bessis et al., 1999; Jullian et al., 1999). Accordingly, the group selectivity of agonists is due to specific chemical features that allow selective interactions at each binding site as described in the following paragraphs. These features derive from classical medicinal chemistry approach including addition of constrained rings or chemical groups. Thus the first selective mGluR agonist to be discovered was the five membered ring **(1S,2R)-ACPD** (Manzoni et al., 1990). It was of great utility for the initial investigations of the physiological roles of the mGluRs in mammalian brain, although it activates both group I and group II receptors and is moderately potent. **L-CCG-I** is another constrained glutamate derivative where a 3-membered -ring has been introduced. This compound activates all subtypes of mGluRs since it adopts preferentially the common extended bioactive conformation. Consequently, it is more active than glutamate. Other constrained glutamate analogs as **LY354740**, provide potency and selectivity (see below). On the other hand, **L-AP4** illustrates the group selectivity brought by an additional acidic function provided by the phosphonate group (Fig. 6).

It is to be mentioned here, that agonists of group II and III receptors have been more developed because of their presynaptic location and thus beneficial physiological effects resulting from their activation. The opposite situation for postsynaptic group I receptors, led to intensive search for inhibitors and more specifically negative allosteric modulators that are not reviewed here.



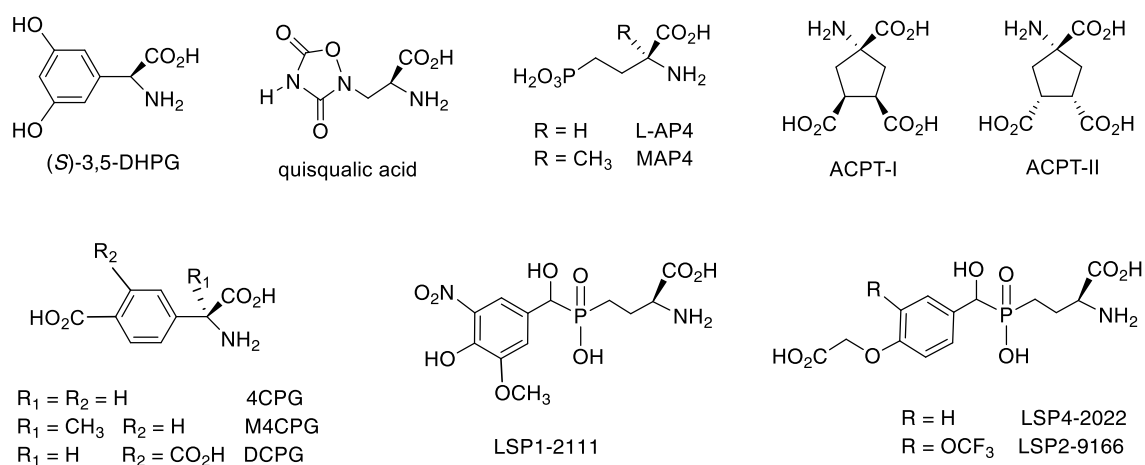


Figure 6. Chemical structures of most common and selective orthosteric agonists and antagonists of mGlu receptors

Table 2. Potencies of cited mGlu receptor agonists in functional assays (EC₅₀, μ M)

Receptor	mGlu1	mGlu5	mGlu2	mGlu3	mGlu4	mGlu6	mGlu7	mGlu8
Glu (Pin and Acher, 2002) ^a	0.32	0.70	5.9	0.34	1.6	12.3	869	9.5
(1S,3R)-ACPD (Pin and Acher, 2002) ^a	3.2	2.1	24	18	74	83.7	832	137
3,5-DHPG (Pin and Acher, 2002) ^a	0.9	3.9	>1000	106	NE	>1000	>1000	>1000
Quis (Pin and Acher, 2002) ^a	0.01	0.03	108	121	112	103	>1000	378
LCCG-I (Pin and Acher, 2002) ^a	2.3	1.5	0.2	0.5	1.4	2.4	46	1.9
LY354740 (Monn et al., 2007) ^b	NE ^c	NE ^a	0.0111	0.0380	NE ^c	-	NE ^c	11.5
LY379268 (Monn et al., 2007) ^b	NE ^c	NE ^c	0.0027	0.0046	NE ^c	-	NE ^c	1.69
LY389795 (Monn et al., 2007) ^b	NE ^c	NE ^c	0.0039	0.0076	NE ^c	-	NE ^c	7.34
LY404039 (Monn et al., 2007) ^b	NE ^c	NE ^c	0.0234	0.048	NE ^c	-	NE ^c	47.8
LY2812223 (Monn et al., 2015a) ^a	> 25	> 25	0.0211	> 25	> 25	> 25	> 25	> 25
LY2794193 (Monn et al., 2018) ^a	> 25	> 25	0.277	0.0116	11.8	19.5	> 25	> 25
L-AP4 (Selvam et al., 2018) ^a	NE ^d	NE ^d	NE ^b	NE ^d	0.13	1.03	>100	0.29
ACPT-I (Selvam et al., 2010) ^a	NE ^d	NE ^d	NE ^d	NE ^d	1.74	10.6	280	5.1

LSP1-2111 (Selvam et al., 2018) ^a	NE ^d	NE ^d	NE ^d	NE ^d	0.9	3.2)	101	19.4
LSP1-2022 (Selvam et al., 2018) ^a	NE ^d	NE ^d	NE ^d	NE ^d	0.11	4.2	11.6	29.2
LSP2-9166 (Belhocine et al., 2018) ^a	NE ^d	NE ^d	NE ^d	NE ^d	0.07	13.8	2.2	48
(S)-DCPG (Thomas et al., 2001) ^{a,b}	32	> 100	> 100	> 100	8.8	3.6	> 100	0.031

^aWhole cell IP₃ production or Ca²⁺ release and fluorescence assay; ^b functional cAMP responses in mGlu receptor-expressing cells. NE no effect at ^c100 μM and ^d300μM. “-“ not tested

3.1.1 Group I (mGlu1 and mGlu5) agonists

Two agonists that activate mGlu1 and mGlu5 receptors, have been early identified: (S)-3,5-DHPG and quisqualate (Schoepp et al., 1999). They are described hereafter.

(S)-3,5-DHPG

(S)-3,5-DHPG selectively activates Group I mGluRs (Fig. 6, Table 2). It is devoid of activity on both group II and group III mGluRs, as well as glutamate transporters (Schoepp et al., 1994; Schoepp et al., 1999). (S)-3,5-DHPG has been docked at the mGlu1 binding site (Bertrand et al., 2002). It shows how this ligand takes advantage of Y74, W110, E292 that are group I selective residues (Fig. 7, Table 1). A similar binding occurs at mGlu5 receptor.

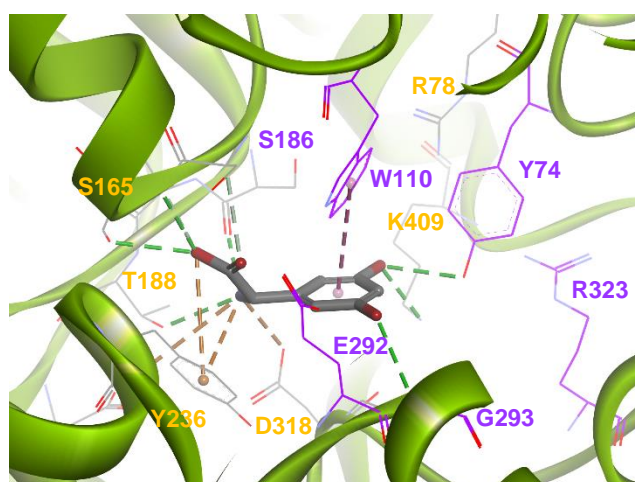


Figure 7. (S)-3,5-DHPG docked at mGlu1 binding site using the PDB 1EWK:A structure as template.

The mGlu1 backbone ribbon is colored in green, the group selective residue side chains in violet (Y74,

W110, E292, G293, R323), other labels as in Fig. 5. New docking performed with Discovery Studio (Biovia Dassault Systèmes) and Gold (Cambridge Crystallographic Data Centre) softwares.

Quisqualate

Although quisqualate is not as selective as 3,5-DHPG because it also activates AMPA receptors, it activates much more potently mGlu1 and mGlu5 (Fig.6, Table 2). It is frequently used when selectivity is not an issue (e.g. quisqualate is present in the mGlu5 full-length Cryo-EM structure (Fig.3B)). Similarly to 3,5-DHPG, the crystal structure of the mGlu VFT with bound quisqualate (Koehl et al., 2019) revealed interactions with the conserved and group selective (Y74, W110, R323) residues at the glutamate binding site (Fig. 8, Table 1)).

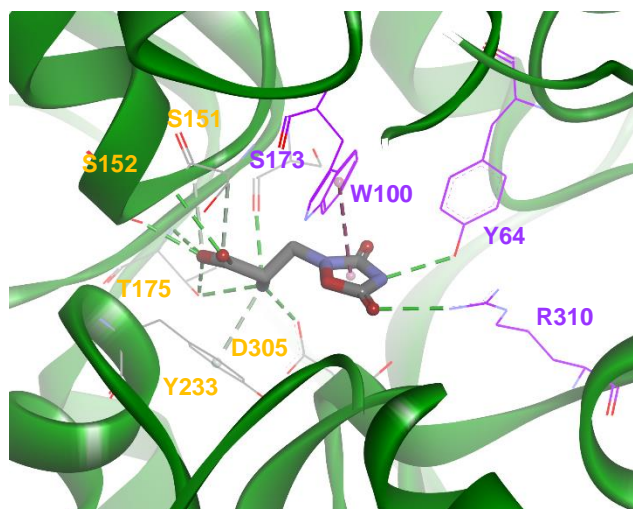


Figure 8. Crystal structure of quisqualate bound to mGlu5 glutamate binding site (PDB ID 6N50) (Koehl et al., 2019). The mGlu5 backbone ribbon is colored in green, the group selective residue side chains in violet (Y74, W110, R323), other labels as in previous figures.

3.1.2 Group II (mGlu2 and mGlu3) agonists

(1S,3R)-ACPD

(1S,3R)-ACPD was the first mGluR agonist that was able to discriminate between ionotropic and metabotropic glutamate receptors. It shows moderate potency at group I and II receptors (Fig. 6, Table

2) (Pin and Acher, 2002). The X-Ray structure of (1*S*,3*R*)-ACPD bound to mGlu3 VFT shows that the five-membered ring is well accommodated at the glutamate binding site (Fig. 9).

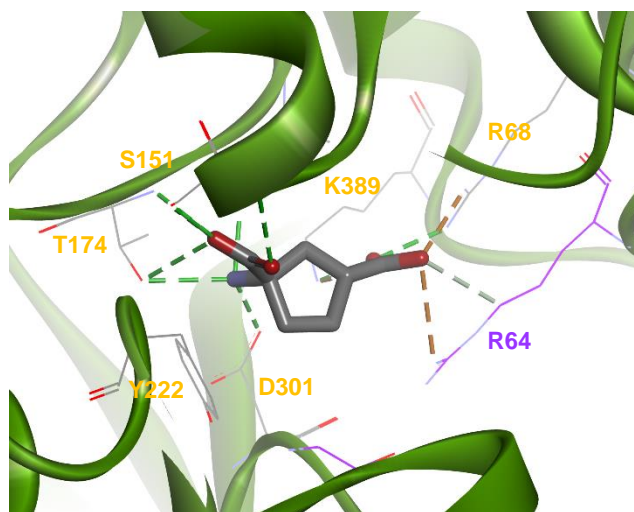


Figure 9. Crystal structure of (1*S*,3*R*)-ACPD bound to mGlu3 glutamate binding site (PDB ID 2E4X) (Muto et al., 2007). The mGlu3 backbone ribbon is colored in green, the group selective residue side chains in violet, other labels as in previous figures.

LY354740 and derivatives

In the mid-nineties Darryl Schoepp at Eli Lilly initiated a large drug discovery program on orthosteric agonists of mGlu2/3 receptors. This program was led by Jim Monn and his colleagues. When adding a second ring to LCCG-I, they discovered **LY354740** (Monn et al., 1997) and a series of group II nanomolar agonists (Monn et al., 2007) (Fig. 6, Table 2). These compounds showed antipsychotic potential in animal studies. Among them **LY404039** was clinically tested as its oral prodrug Pomaglumetad Methionil (**LY2140023**). It was safe, well tolerated and resulted in statistically significant improvements in the first phase 2 clinical trial for schizophrenia (Patil et al., 2007). However, further trials failed to demonstrate the efficacy of LY2140023 in the treatment of positive and negative symptoms of schizophrenia for patients that had been previously treated with serotonergic atypical antipsychotics (e.g., clozapine and olanzapine). It was later rationalized by an

epigenetic downregulation of the transcription of mGlu2 by the chronic atypical antipsychotic treatment (de la Fuente Revenga et al., 2019).

The clinical development was stopped but these agonists remain excellent pharmacological tools.

Several X-Ray structures of these compounds bound to mGlu2 and mGlu3 have been disclosed. They show the interactions of the amino acid moiety and the distal carboxylate with the conserved residues as well as interactions with group II selective residues (R57, Y144, G296, R271 in mGlu2, Fig. 10 and Table 1).

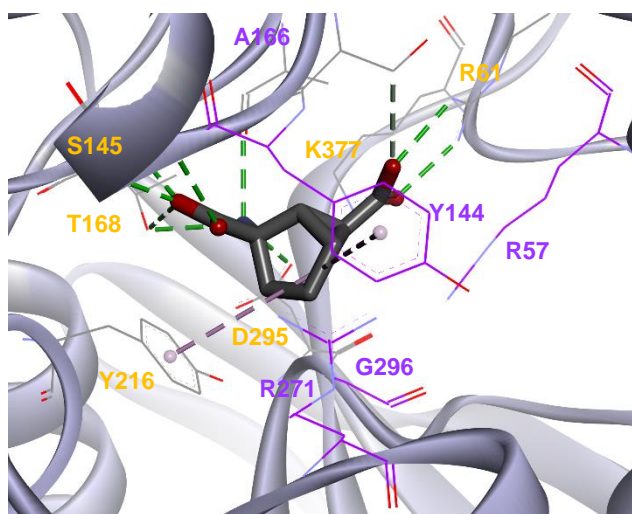


Figure 10. Crystal structure of LY354740 bound to mGlu2 glutamate binding site (PDB ID 4XAQ (Monn et al., 2015b)). The mGlu2 backbone ribbon is colored in mauve, the group selective residue side chains in violet (R57, Y144, G296, R271), other labels as in previous figures.

LY2812223 mGlu2 selective agonist

Subtype selectivity between mGlu2 and mGlu3 was found when adding a thiotriazole substituent in the C4 position of LY354740 providing LY2812223 (Fig. 6) (Monn et al., 2015a). This compound is a potent mGlu2 agonist and a weak partial agonist or antagonist at mGlu3 depending on the functional assay (Table 2). The X-Ray structures of LY2812223 bound to mGlu2 and mGlu3 VFTs reveal that its amino diacidic functions bind to the conserved motif as in all mGlu receptors (Fig. 11A and 11B). In the mGlu2 structure, the thiotriazole chemical group interacts with R271 and the first residues of helix $\alpha 8$ (Fig. 11A and 11C), which are not observed at mGlu3 (Fig. 11B). This-structure also shows

that Y144 has flipped from its usual conformation (Fig. 10, 11A and B). Thus, the pharmacological difference between mGlu2 and mGlu3 may be interpreted with the subtle difference in the molecular network involving these two residues and the selective D146/E273 which are S152/D279 in mGlu3 (Fig.11A-C, Table 1)). A detailed study of this network has been previously investigated and revealed the difference in chloride sensitivity between mGlu2 and mGlu3 subtypes (Tora et al., 2018).

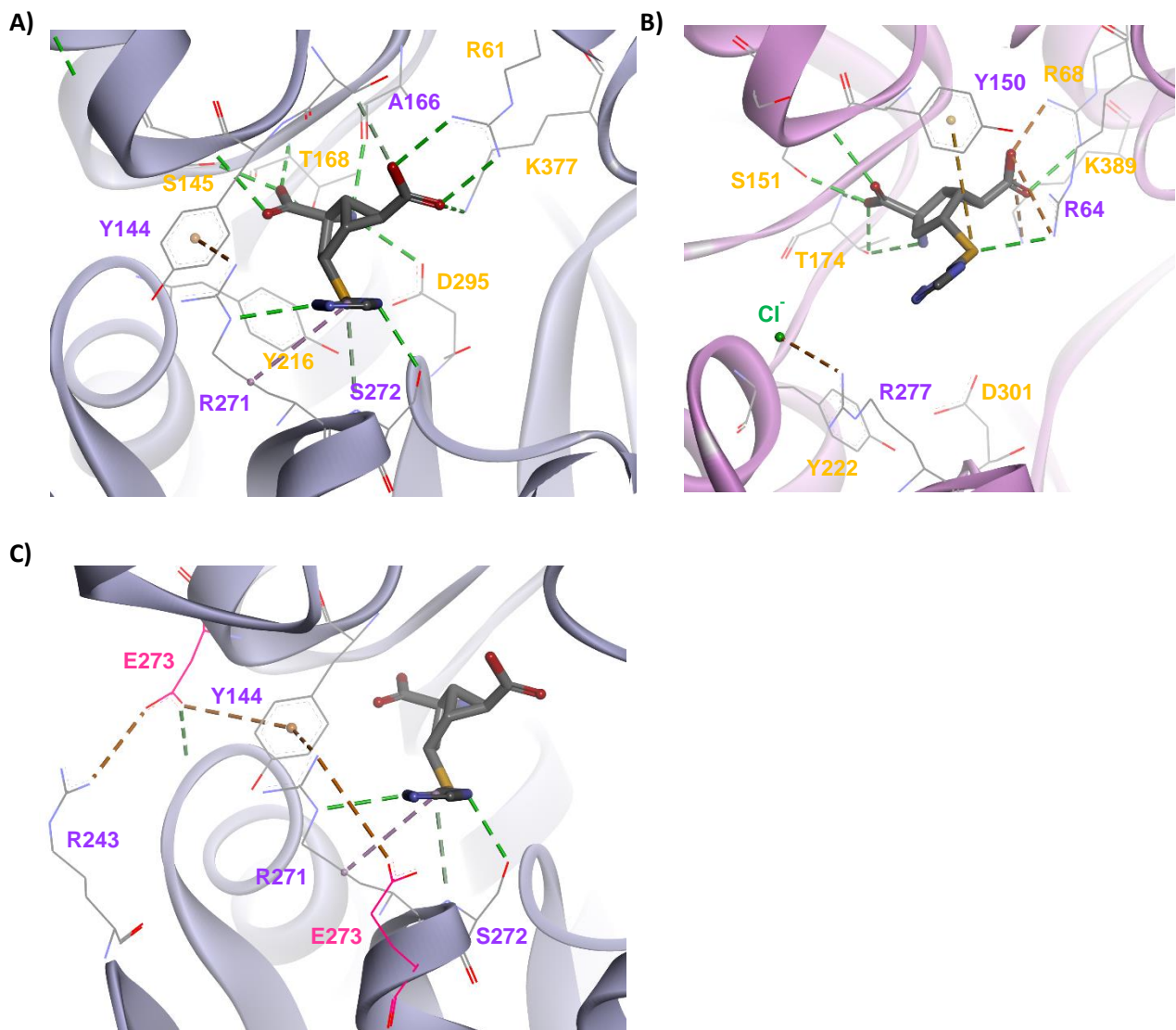


Figure 11. Crystal structure of LY2812223 bound to mGlu2 glutamate binding site (A, PDB ID 5CNJ, mauve ribbon) and mGlu3 (B, PDB ID 5CNM pink ribbon) (Monn et al., 2015a). LY2812223 is bound to an mGlu2 closed conformation (A) and an mGlu3 open conformation (B). Comparison between panels A and B exhibit the flip of Y144. C) Same as panel A, where the network around Y144 and

R271 is displayed. The side chains of subtype selective D146 and E273 are colored in pink. mGlu2 ribbon as in Fig. 10 and mGlu3 ribbon in light pink, other labels as in previous figures.

LY2794193 mGlu3 selective agonist

Adding a 3-methoxy-benzamide substituent in the 4 position of LY354740 provided this time the mGlu3 selectivity over mGlu2 (LY2794193, Fig. 6, Table 2) (Monn et al., 2018). The X-Ray structure of this compound bound to mGlu3 shows the conserved interactions as for LY2812223 and all mGlu agonists. The benzamide ring is intercalated between the side chains of Y150 and R277 (Fig. 12). R277 is positioned by a salt bridge with D279. This residue is mGlu3 selective and shorter than its homologous E273 in mGlu2 (Table 1). This difference may affect the network around R271 in mGlu2 and in particular the salt bridge with D146 (Tora et al., 2018) and explain the selectivity of LY2794193 versus mGlu2 (Monn et al., 2018).

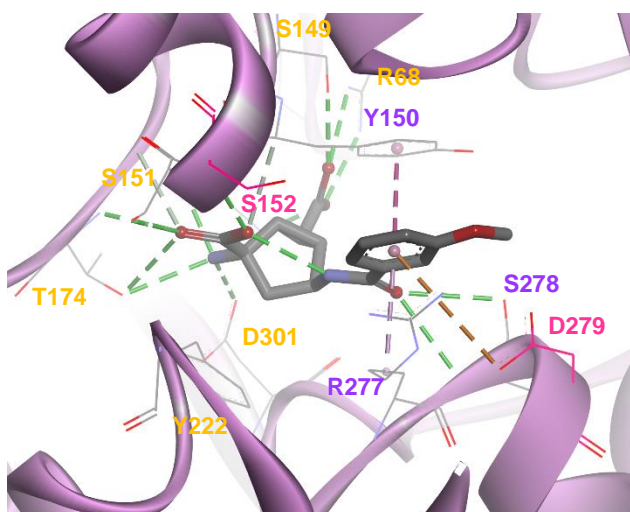


Figure 12. Crystal structure of LY2794193 bound to mGlu3 glutamate binding site in a closed conformation (PDB ID 6B7H) (Monn et al., 2018). The benzamide moiety is anchored by H-bonds between its carbonyl group and S278, by a π - π stacking with Y150 and a π -alkyl interaction with R277. Subtype selective residue side chains are colored in pink, other labels as in previous figures.

3.1.3 Group III (mGlu4, mGlu6, mGlu7 and mGlu8) agonists

L-AP4

L-AP4 was known for its specific properties in regulating brain synapses much before the discovery of group III mGlu receptors (Fig. 6). It had been shown that L-AP4 potently blocks excitation of the hippocampal dentate gyrus granule cells via the lateral perforant path (Koerner and Cotman, 1981). It was only a few years later that C. Thomsen and colleagues demonstrated that L-AP4 stimulates the mGlu4 receptor which is negatively coupled to the cAMP cascade and results in a decrease in presynaptic mediated glutamate release (Thomsen et al., 1992). L-AP4 possesses no activity at group I or group II mGlu receptor subtypes (Table 2). It activates all group III receptors (mGlu4, 6, 7, 8) and is more potent than glutamate. Indeed the additional acidic group from the distal phosphonate of L-AP4, can establish ionic interactions with the highly basic environment at the distal binding site of mGlu4 and 8 (Fig. 13). Of notice, mGlu6 is only found in the retina as a postsynaptic receptor and is responsible for the night vision. mGlu7 is activated by millimolar concentration of glutamate and hundreds of μM concentration of L-AP4. D. Hampson has shown that this weak activity is a consequence of a longer loop $\beta 10\text{-}\alpha 7$ that hampers the VFT closing (Table 1) (Rosemond et al., 2004).

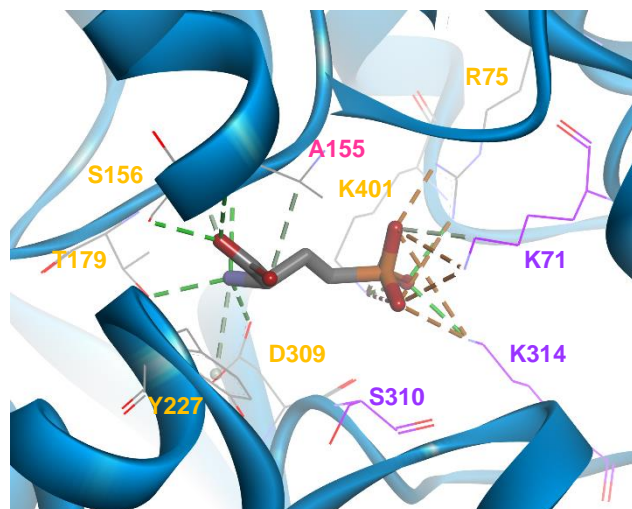


Figure 13. Crystal structure of L-AP4 bound to mGlu8 glutamate binding site (PDB ID 6BT5) (Schkeryantz et al., 2018). The mGlu8 backbone ribbon is colored in blue, the group selective residue side chains in violet (K71, S310, K314), other labels as in previous figures.

ACPT-I

The ACPT series was discovered by introducing a carboxylate substituent in the 4-position of ACPD (Acher et al., 1997). ACPT-I was one of the first group III selective agonists. It is endowed with μM activity at mGlu4, 6, 8 and hundreds of μM at mGlu7 with no effect at other subtypes (Fig. 6, Table 2) (Acher et al., 1997; Selvam et al., 2010). The selectivity is brought by the additional carboxylate interacting with polar group selective residues (Fig. 14, Table 1)).

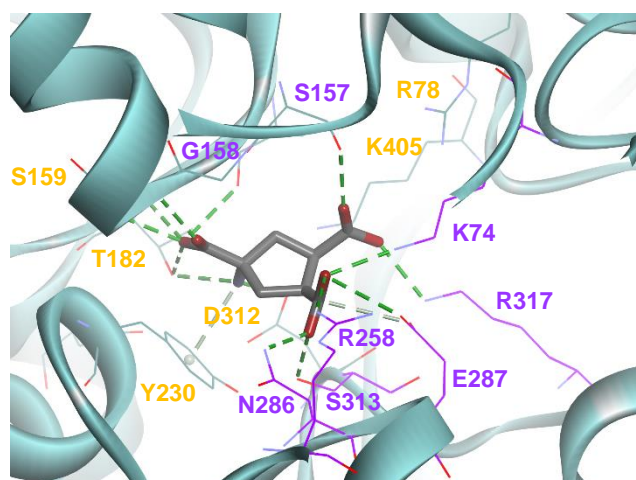


Figure 14. ACPT-1 bound to mGlu4 homology model (Bertrand et al., 2002). The mGlu4 backbone ribbon is colored in light blue, the group selective residue side chains in violet, and other labels as in previous figures.

LSP1-2111, LSP4-2022 and analogues

An initial virtual high throughput screening at mGlu4 binding site, provided a hit that was optimized in numerous mGlu4 agonists (Selvam et al., 2018; Selvam et al., 2010). The first benzylic derivatives such as LSP1-2111 were mGlu4 preferential then selectivity was reached with LSP4-2022 (Fig. 6,

Table 2) (Goudet et al., 2012; Selvam et al., 2018). These compounds hold several advantageous properties such as high aqueous solubility, brain availability, high free fraction, low P450 metabolism (Cajina et al., 2014). They are thus useful pharmacological tools and have been used in *ex-vivo* and *in vivo* studies (references cited in (Selvam et al., 2018)).

Similarly to mGlu2 and mGlu3 subtype selective agonists, the mGlu4 selectivity of LSP4-2022 originates from interaction with residues remote from the glutamate binding site (Goudet et al., 2012; Selvam et al., 2018). We have demonstrated that the distal carboxylate of LSP4-2022 mimics a chloride ion located at a structural (site 1, Fig. 15) or modulator (site 2, Fig. 16) site (Selvam et al., 2018; Tora et al., 2015). Selective residues at the extended binding site are responsible for the observed selectivity as demonstrated with mutagenesis experiments at the β_6 - α_3 loop and the β_3 - β_4 hairpin (Table 1) (Goudet et al., 2012).

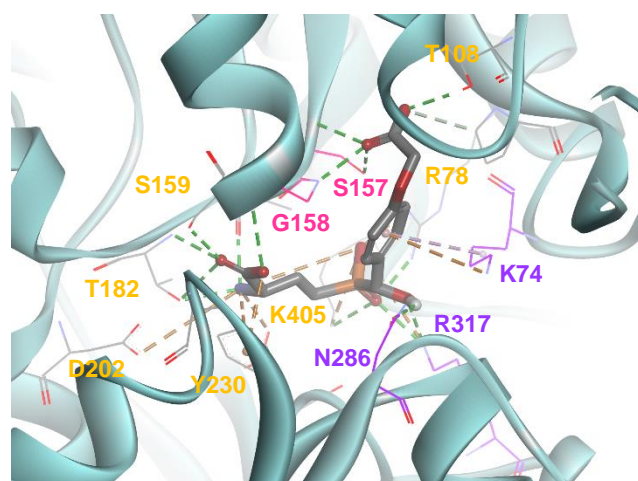


Figure 15. LSP4-2022 bound to mGlu4 homology model at chloride site 1 (Selvam et al., 2018). The mGlu4 backbone ribbon is colored in light blue, the group selective residue side chains in violet and subtype selective in pink.

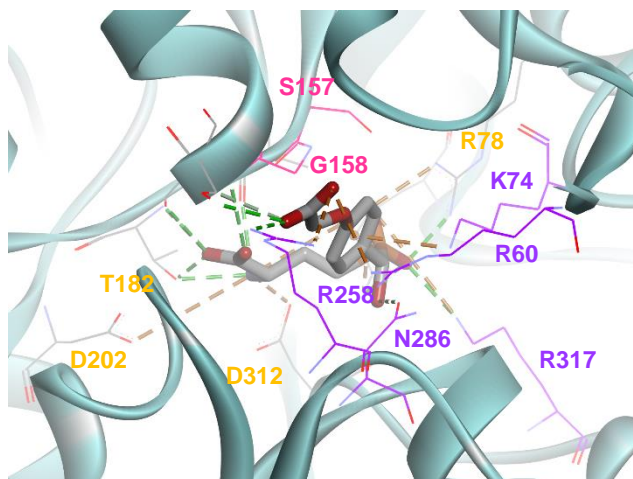


Figure 16. LSP4-2022 bound to mGlu4 homology model at chloride site 2 (Selvam et al., 2018). The mGlu4 backbone ribbon is colored in light blue, the group selective residue side chains in violet and subtype selective ones in pink.

(S)-DCPG

(S)-DCPG (Fig. 6) was shown to be a potent and mGlu8 subtype selective agonist (Table 2) (Thomas et al., 2001). This phenylglycine carries two acidic groups in its side chain similarly to ACPT-I, which provide the group III selectivity. The subtype selectivity likely originates from the hydrophobic patch (A154, A155, A177) above the phenyl ring of (S)-DCPG, as observed in the X-Ray structure (Fig. 17) (Chen et al., 2018). Interestingly, the two subtype selective alanine A154 and A155 are too bulky to allow LSP4-2022 to bind to mGlu8 and are responsible for its subtype selectivity (Goudet et al., 2012).

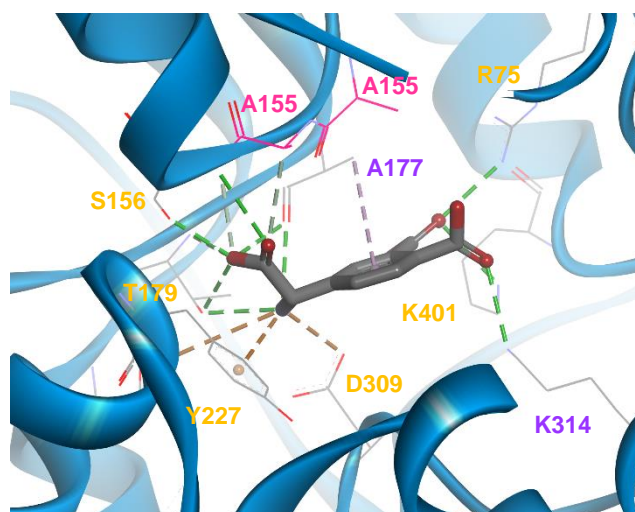


Figure 17. Crystal structure of (*S*)-DCPG bound to mGlu8 glutamate binding site in a closed conformation (PDB ID 6E5V) (Chen et al., 2018). The mGlu8 backbone ribbon is colored in blue, the subtype selective residue side chains in pink (A154, A155), other labels as in previous figures.

3.2 Competitive antagonists

Competitive antagonists have been less developed than agonists. Yet they are unavoidable pharmacological tools. For all known competitive antagonists, the inhibition results from preventing the VFT closure and stabilization of the open conformation in the resting state (Fig. 4) (Bessis et al., 2002). They are mGluR agonist derivatives keeping their interactions with lobe 1 of the VFT but bearing an additional substituent that interacts with residues of lobe 2. Many of these orthosteric antagonists are glutamate derivatives for which the α -proton of the α -amino acid moiety has been replaced by a methyl group or a bulky substituent. This substituent interacts with the conserved tyrosine close to the hinge of the VFT and which is part of the signature motif (Fig. 5) (Acher and Bertrand, 2005). Among them, agonists such as LCCG-I, 4CPG and L-AP4 are turned into the antagonists LY341495, MCPG and MAP4 with high to moderate activity (Table 3, Fig. 18-21) (Bessis et al., 2002; Ornstein et al., 1998; Pin and Acher, 2002). The mGlu2/3 agonist LY354740 was also converted to potent competitive antagonists by addition of substituents in the 3 and 4 positions of its chemical structure affording MGS039 and LY3020371 (Fig. 6). Similarly to the previous series, their inhibition results from interaction of these substituents with the conserved tyrosine of lobe 2 and keeping the VFTs open (Fig. 22). These compounds demonstrated to be rapidly acting antidepressant (Chaki et al., 2004; Chappell et al., 2016; Witkin et al., 2017a).

Besides the steric hindrance of orthosteric antagonists with the conserved tyrosine, we identified ACPT-II for which an electrostatic repulsion is responsible for the receptor inhibition (Acher et al., 1997; Bessis et al., 2002). In ACPT-II, the carboxylate with the R configuration in the 4 position of the cyclopentyl glutamate analogue (Fig. 6) points to the conserved aspartate of the signature motif

binding the amino acid moiety (Fig. 5). The electrostatic repulsion between the two negative charges that prevents the VFT closure, was demonstrated by mutagenesis (Bessis et al., 2002).

All these competitive inhibitors behave as a wedge preventing the closure of the VFT and blocking the rigid body movement of the two lobes (Fig. 4).

Table 3. Potencies of cited mGlu receptor competitive antagonists (IC₅₀ in μM or Ki)

Receptor	mGlu1	mGlu5	mGlu2	mGlu3	mGlu4	mGlu6	mGlu7	mGlu8
LY341495 (Schoepp et al., 1999)	6.8	8.2	0.021	0.014	22	1.8	0.99	0.173
MCPG (Pin and Acher, 2002)	160	180	87	165	>1000	254	597	80
MAP4 (Pin and Acher, 2002)	-	-	114	-	88	340	167	9.1
LY3020371 (Witkin et al., 2017b)	>12.5	>12.5	0.016	0.0062	>12.5	4.01	1.26	1.77
MGS039 (Chaki et al., 2004)	>100	-	0.020	0.024	1.74	2.06	664	-
ACPT-II (Acher et al., 1997)	115	-	88	-	77	-	-	-

“-“ not tested

LY341495

LY341495 was discovered when investigating a series of α-substituted analogues of L-CCG-I at Eli Lilly (Ornstein et al., 1998). Introduction of the 9'-xanthylmethyl in that position converted the agonist into the most potent antagonist with nanomolar inhibition at mGlu2/3 (Fig. 6, Table 3). Yet, since L-CCG-I activates all mGluRs, LY341495 inhibits also the other subtypes with reasonable potency (Table 3). Crystal structures of LY341495 bound to mGlu1, mGlu3 and mGlu7 VFTs have been solved (Fig. 18-20). They all show a large opening of the VFTs.

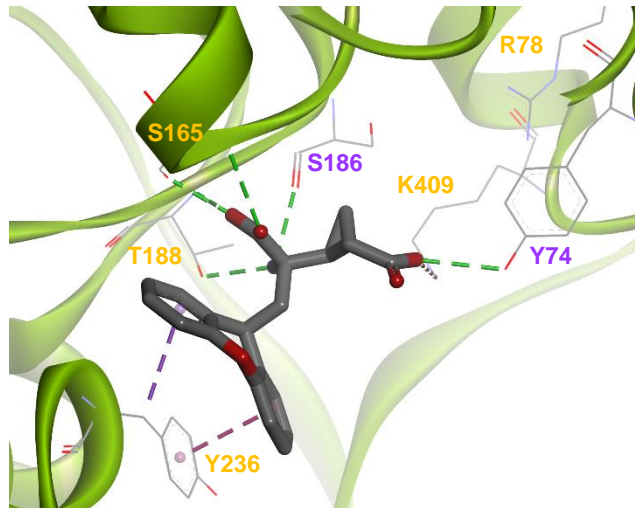


Figure 18. Crystal structure of LY341495 bound to mGlu1 glutamate binding site (PDB ID 3KS9). The mGlu1 backbone ribbon is colored in green, other labels as in previous figures. Hydrophobic interactions between the phenyl rings of the 9'-xanthylmethyl group and the side chain of Y236 prevents the closing of the VFT.

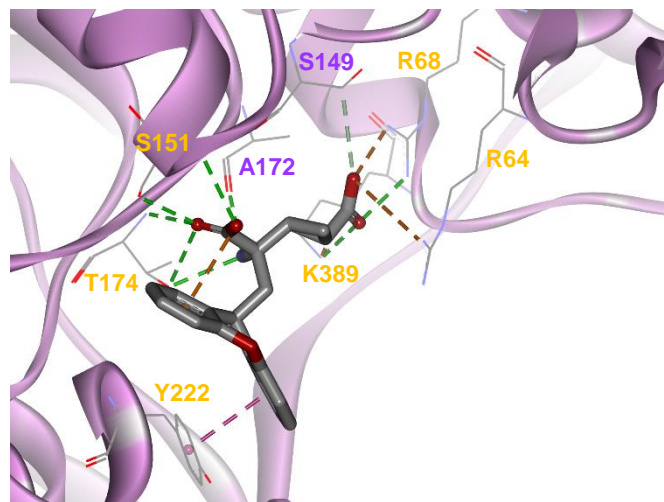


Figure 19. Crystal structure of LY341495 bound to mGlu3 glutamate binding site (PDB ID 3SM9). The mGlu3 backbone ribbon is colored in pink, other labels as in previous figures. Hydrophobic interactions between a phenyl ring of the 9'-xanthylmethyl group and the side chain of Y222 prevents the closing of the VFT as seen in mGlu1 ([Fig.18](#)).

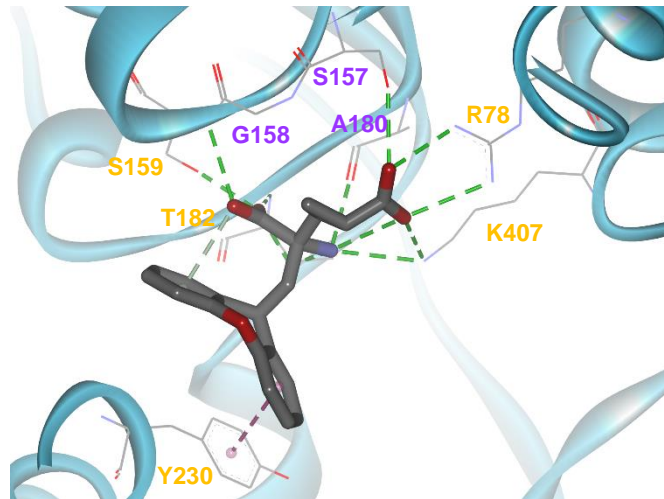


Figure20. Crystal structure of LY341495 bound to mGlu7 glutamate binding site (PDB ID 3MQ4). The mGlu7 backbone ribbon is colored in light blue, other labels as in previous figures. Hydrophobic interactions between a phenyl ring of the 9'-xanthylmethyl group and the side chain of Y230 prevents the closing of the VFT as seen in mGlu1 and mGlu3 (Fig.18, 19).

(S)-MCPG

(S)-MCPG has been the first compound reported to antagonize (1S,3R)-ACPD and L-AP4 mGlu receptor activation in the assays initially available (Coudert et al., 1996; Schoepp et al., 1999). When tested at cloned receptors, (S)-MCPG was better characterized as a weakly active and non-selective antagonist (Fig. 6, Table 3) (Pin and Acher, 2002). However, it allowed to solve the first mGlu1 VFT crystal structure kept in an open inactive conformation (Fig. 21) (Tsuchiya et al., 2002).

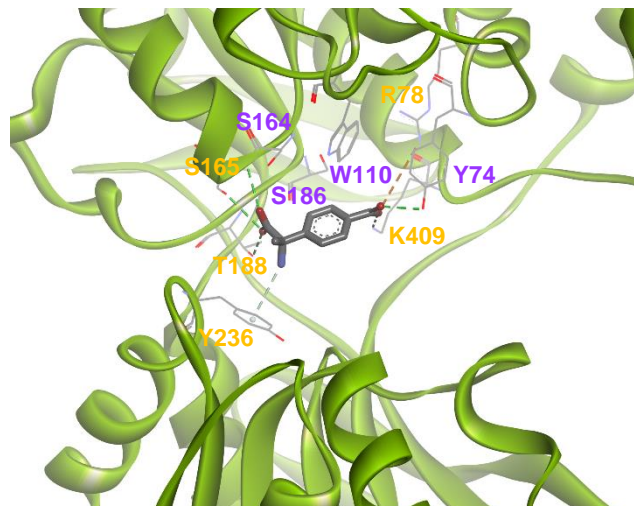


Figure 21. Crystal structure of (*S*)-MCPG bound to mGlu1 glutamate binding site (PDB ID 1ISS) (Tsuchiya et al., 2002). The hydrophobic contact between the α -methyl of (*S*)-MCPG and the phenyl ring of Y236 prevents the VFT closure. The mGlu1 backbone ribbon is colored in green, other labels as in previous figures. The opening angle between the two lobes of the VFT is wider than that observed for the agonist bound structures (Fig. 2-4)

LY3020371

LY3020371 is one of the several antagonists identified when introducing well oriented substituents onto the aminobicyclo[3.1.0]hexane-2,6-dicarboxylate scaffold (LY354740) (Chaki et al., 2004; Chappell et al., 2016). It keeps the group II selectivity and potency of the parent agonist. In addition, a π - π stacking between the aromatic rings of LY3020371 and Tyr216 prevents further closing of the VFT resulting in inactivation of the receptor (Fig. 22). As other antagonists deriving from LY354740, this compound has been demonstrated to exert antidepressant effects via underlying mechanisms that are similar to those of ketamine.

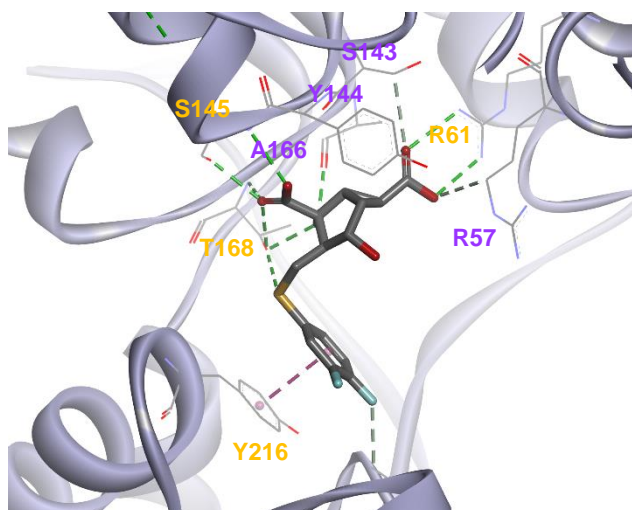


Figure 22. Crystal structure of LY3020371 bound to mGlu2 glutamate binding site (PDB ID 5KZQ) (Chappell et al., 2016). The mGlu2 backbone ribbon is colored in mauve, other labels as in previous

figures. The opening angle between the two lobes of the VFT is wider than that observed for the agonist bound structure (Fig. 10).

3.3 Drug-likeness of orthosteric ligands

All orthosteric ligands described above are polar compounds with mostly negative clogP. Such a property confers excellent aqueous solubility but makes these molecules not drug-like with predicted low bioavailability. It is thus anticipated that these molecules may not cross the blood brain barrier (BBB) to reach their CNS targets. However, all the drugs of this type that have been tested *in vivo* have shown a central effect. Indeed the polarity of these compounds allows a high unbound concentration so that a reduced fraction of the drug needs to pass the BBB to be effective (Cajina et al., 2014). Yet, their major drawback is their lack of oral bioavailability because they cannot cross the gastro-intestinal barrier. The problem can be by-passed using dipeptides as prodrugs (Pak et al., 2017; Patil et al., 2007). The dipeptide are transported by the PEPT1 transporter (Pak et al., 2017) then the drug is released by peptidase/protease hydrolysis (Moulton et al., 2015). The hydrophilicity of the orthosteric mGluR ligands prevents them from metabolization by hepatic cytochrome P450 enzymes. In addition, it also reduces off-target activity in comparison to allosteric modulators (Belhocine et al., 2018).

Altogether, the drug-likeness of orthosteric mGluR ligands may seem limited at first glance, yet these compounds display the required properties for a drug when coupled to a prodrug for oral bioavailability. Such favorable dispositions are in line with the development of Pomaglumedad methionil, which reached phase III in clinical trials.

3.4 Nanobodies

A new generation of mGlu receptor ligands has emerged with the discovery of nanobodies binding to the VFT of mGlu receptors (Scholler et al., 2017b). They mostly bind to the receptor outside the glutamate binding site. They can thus be positive (PAM) or negative (NAM) allosteric modulators. Yet some are also endowed with a full agonist property. DN13 was the first biologics binding at the interface of the mGlu2 homodimer active conformation and enhancing the activation (Fig. 4 and 23) (Scholler et al., 2017b). The docking of DN13 bound to mGlu2 homodimer was confirmed by a cryo-EM structure that was disclosed during the revision of this manuscript (Lin et al., 2021). This PAM proved to be highly potent, selective, and efficient in an animal model of contextual fear memory (Scholler et al., 2017b).

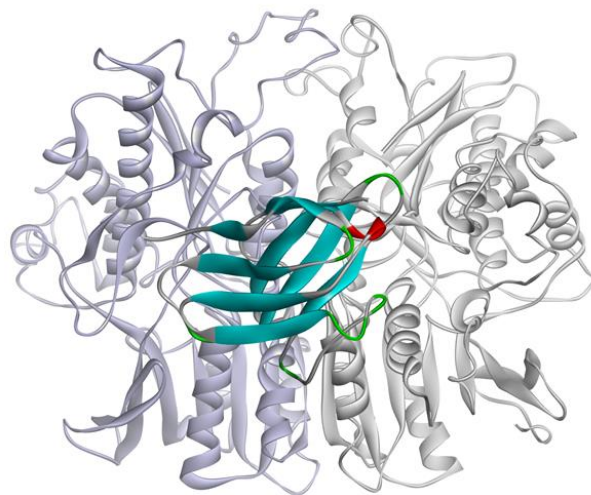


Figure 23. DN13 bound at the interface of the mGlu2 homodimer. New docking to a new mGlu2 receptor crystal structure (PDB ID 4XAQ). Protomer A is colored in mauve, protomer B in gray and DN13 by structural elements (β -sheet cyan, α -helix red, loop green). Docking was performed with Discovery Studio (Biovia Dassault Systèmes)

Very recently, DN45 another nanobody that fully activates mGlu4 homodimer, has been discovered. It does not bind at the dimer interface but with the two lobes of each VFT. Interestingly, DN45 does not activate the heterodimer mGlu2-4, where it acts as a pure positive allosteric modulator (Haubrich et al., 2021)

With the advent of nanobodies as a new type of modulators, a large field of possible development is opened.

3.5 VFT Negative allosteric modulators

Gee et al discovered XAP044 a small molecule blocking mGlu7 activation by interacting with the VFT domain in a non-competitive way ([Gee et al., 2014](#)). The authors demonstrated that XAP044 inhibits stress and anxiety in rodent models. These observations highlight a new type of ligand with therapeutic potential.

The exact mode of action of this molecule is surprisingly not known yet. Understanding the mode of action of XAP044 will certainly reveal a novel way for modulating mGluRs not targeting the hydrophobic 7TM allosteric site.

5. Conclusion

This short survey has reviewed the development of mGluR orthosteric ligands from the initial to latest discoveries. These compounds are mostly group selective, yet it was possible to identify some that are subtype selective. Over the time, several 3D-structures of the glutamate binding domain became available. They allow to interpret the receptor activation mechanism and to rationalize the selectivity of the ligands. Although none of these ligands has reached the market as a drug yet, there are still promises for the numerous diseases that involve glutamate neurotransmission dysfunction.

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Declarations of interest: none

Abbreviations

ACPD, 1-aminocyclopentane-1,3-dicarboxylic acid; ACPT, 1-aminocyclopentane-1,3,4-tricarboxylic Acid; AP4, 2-amino-4-phosphonobutyric acid; 4CPG, 4-carboxyphenylglycine; Acc, Active-closed-closed state; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; ATD, amino-terminal domain; BBB, blood brain barrier; CCG-I, (2*S*,1'*S*,2'*S*)-2-(carboxycyclopropyl)glycine; CNS, central nervous system; CPCCOEt, 7-(hydroxyimino)cyclopropan[*b*]chromen-1 α -carboxylic ethyl ester; CRD, cysteine rich domain; cryo-EM, cryogenic electron microscopy; DHPG, dihydroxyphenylglycine; DS, Discovery Studio; IP3, inositol 1,4,5-triphosphate; LIVBP, Leucine-Isoleucine-Valine Periplasmic Binding Protein; LSP1-2111, [((3*S*)-3-amino-3-carboxy)propyl][(4-hydroxy-5-methoxy-3-nitrophenyl)hydroxymethyl]phosphinic acid; LSP4-2022, [((3*S*)-3-amino-3-carboxy)propyl][(4-(carboxymethoxy)phenyl)hydroxymethyl]phosphinic acid; LSP1-2111, [((3*S*)-3-amino-3-carboxy)propyl][(4-hydroxy-5-methoxy-3-nitrophenyl)hydroxymethyl]phosphinic acid; LY2794193, (1*S*,2*S*,4*S*,5*R*,6*S*)-2-amino-4-[(3-methoxybenzoyl)amino]bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY2812223, (1*R*,2*S*,4*R*,5*R*,6*R*)-2-amino-4-(1*H*-1,2,4-triazol-3-ylsulfanyl)-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY3020371, (1*S*,2*R*,3*S*,4*S*,5*R*,6*R*)-2-amino-3-[(3,4-difluorophenyl)sulfanylmethyl]-4-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY341495, α -9'-xanthenylmethyl-2-(carboxycyclopropyl)glycine; LY354740, (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate; MAP4, 2-amino-2-methyl-4-phosphonobutyric acid; MCPG, α -methyl-4-carboxyphenylglycine; MGS039, (1*R*,2*R*,3*R*,5*R*,6*R*)-2-amino-3-(3,4-dichlorobenzoyloxy)-6-fluorobicyclo-[3.1.0]hexane-2,6-

dicarboxylic acid; NAM, negative allosteric modulator; Quis, quisqualic acid; PAM, positive allosteric modulator; PBP, periplasmic binding protein; PDB, protein data bank; PLC, phospholipase C; Roo, resting-open-open state; 7TM, 7 transmembrane helix domain; VFT, venus flytrap domain; XAP044, 7-hydroxy-3-(4-iodophenoxy)-4*H*-chromen-4-one.

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