Progress toward advanced understanding of metabotropic glutamate receptors: structure, signaling and therapeutic indications

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Abstract

The metabotropic glutamate (mGlu) receptors are a group of Class C Seven Transmembrane Spanning/G Protein Coupled Receptors (7TMRs/GPCRs). These receptors are activated by glutamate, one of the standard amino acids and the major excitatory neurotransmitter. By activating G protein-dependent and non G protein-dependent signaling pathways, mGlus modulate glutamatergic transmission in both the periphery and throughout the central nervous system. Since the discovery of the first mGlu receptor, especially the last decade, a great deal of progress has been made in understanding the signaling, structure, pharmacological manipulation and therapeutic indications of the 8 mGlu members.

Keywords

mGlu; 7TMR; pharmacology; allosteric modulation; heterodimer; drug discovery

1. Introduction

Glutamate is not only one of the 23 proteinogenic amino acids, but it is also the major excitatory neurotransmitter in the central nervous system (CNS). The glutamate receptors can be divided into two classes: the ionotropic glutamate receptors and the metabotropic glutamate receptors. While the ionotropic glutamate receptors (AMPA receptors, NMDA receptors and kainate receptors) mediate fast responses elicited by glutamate, the metabotropic glutamate (mGlu) receptors provide a mechanism by which glutamate can transduce environmental cues and modulate synaptic transmission via second messenger

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signaling pathways. Because of their widespread distribution, especially in the CNS, pharmacological manipulation of mGlus may represent ideal therapeutic interventions for a wide range of neurological and psychiatric disorders (Reviewed in (Niswender and Conn 2010, Gregory, Noetzel et al. 2013)).

2. Classification of mGlus

The 7 Transmembrane Spanning Receptors/G Protein Coupled Receptors (7TMRs/GPCRs) account for 4% of the entire protein-coding genome (Bjarnadottir, Gloriam et al. 2006) but represent the targets of approximately 40-50% of medicinal drugs on the market (Thomsen, Frazer et al. 2005). The core function of 7TMRs is to serve as a transducer of signals from the extracellular environment to the intracellular signaling machinery. The 7TMR superfamily can be classified into several classes: the Class A 7TMRs (or Rhodopsin-like receptors) account for almost 85% of the GPCR genes, the Class B 7TMRs (or secretin-like receptors) include 15 receptors and are regulated by peptide hormones, and the Class C 7TMRs, which are characterized by a large extracellular N-terminal domain and contain 22 distinct receptors. The Adhesion, Frizzled, Taste type-2 and other unclassified receptors comprise the rest of the superfamily (Bjarnadottir, Gloriam et al. 2006).

The mGlus belong to the Class C 7TMRs; this class also encompasses calcium sensing receptors, the GABAB receptor, taste receptors and other orphan Class C receptors. Since the cloning of rat mGlu1 in 1991, 8 mGlu subtypes have been cloned thus far, named mGlu1 through mGlu8. Within the family, the eight mGlu subtypes can be further classified into three groups, with an intragroup sequence homology of about 70% and an intergroup sequence homology of about 45% (reviewed in (Conn and Pin 1997)). The classification of mGlu receptors are summarized in Table 1: the group I mGlus include mGlu1 and mGlu5, the group II includes mGlu2 and mGlu3, whereas mGlu4, 6, 7 and 8 comprise the group III mGlus. While the group I mGlus are coupled to Gq, the group II and group III are coupled to Gi/o G proteins.

3. Structure of mGlus

3.1 General structural features of mGlus

As members of the Class C 7TMRs, the mGlus are characterized by a large N-terminal domain, commonly referred to as the Venus Flytrap Domain (VFD). Studies of the crystal structures of the mGlu1, 3 and 7 VFDs reveal that each VFD contains two lobes, which together form a clam shell-like structure, with the glutamate binding site found between the two lobes (Kunishima, Shimada et al. 2000, Tsuchiya, Kunishima et al. 2002, Acher and Bertrand 2005, Muto, Tsuchiya et al. 2007). Besides glutamate, the VFDs of some mGlus also bind other endogenous agonists of mGlus, such as cinnabarinic acid (Fazio, Lionetto et al. 2012) and L-serine-O-phosphate (Klunk, McClure et al. 1991) (Hampson, Huang et al. 1999), as well as magnesium and calcium, which can modulate receptor activity (Kubo, Miyashita et al. 1998, Kunishima, Shimada et al. 2000, Francesconi and Duvoisin 2004). Both structural (Kunishima, Shimada et al. 2000, Tsuchiya, Kunishima et al. 2002, Muto, Tsuchiya et al. 2007) and biochemical data (Romano, Yang et al. 1996) suggest that the VFDs from two distinct mGlu receptors sit back to back and dimerize together. Upon ligand...
binding, large conformational changes lead to closure of the two lobes. Closure of one or both VFDs within each mGlu dimer initiates receptor activation (Kniazeff, Bessis et al. 2004).

Connecting the VFD and the seven transmembrane spanning domain (7TMD) is the cysteine-rich domain (CRD). Based on structural studies with mGlu2, the CRD contains 9 cysteine residues; 8 of them form internal disulfide bonds to stabilize the structure of this domain. In addition, the ninth cysteine forms a disulfide bond linked to the VFD (Muto, Tsuchiya et al. 2007), such that the CRD senses the conformational changes induced by ligand binding and transmits it to the 7TMD. The 7TMD and intracellular loops play important roles in receptor-G protein coupling and receptor modulation. It has been shown that a single mutation in the 7TMD that disrupts the hydrogen-bonding network in TM6 and TM7 induces high constitutive activity of mGlu8 (Yanagawa, Yamashita et al. 2013), suggesting that TM6 and TM7 constrain the receptor in an inactive conformation and that rearrangement between these two helixes is critical for receptor activation. The 7TMD also provides an opportunity to modulate receptor activity at a site other than the traditional agonist-binding VFD. All mGlu small molecule allosteric modulators discovered to date are believed to bind to receptor 7TMDs.

The recently solved crystal structure of the mGlu1 7TMD bound to a negative allosteric modulator has provided us with a more detailed understanding on the structural characteristics and activation mechanisms of the receptors (Wu, Wang et al. 2014). This 2.8Å resolution structure has confirmed a parallel dimer conformation of the mGlu 7TMD, stabilized by cholesterol molecules. Although sharing few identical or even conserved residues with other 7TMRs, such as Class A receptors, the overall fold of the mGlu1 7TMD is highly consistent with those of other families. The discovered binding pocket for the negative allosteric modulator partially overlaps with the binding pocket for orthosteric ligands on Class A receptors. Interestingly, this pocket on mGlu1 is restricted by the second extracellular loop, which may explain the binding of native agonist at the level of the VFD instead of 7TMD.

The intracellular loops of mGlus are involved in G protein coupling and receptor phosphorylation. Specifically, the second intracellular loop is implicated in G protein coupling specificity of the mGlus (Pin, Joly et al. 1994, Gomez, Joly et al. 1996, Havlickova, Blahos et al. 2003), which is usually the function of the third intracellular loop for Class A, rhodopsin-like receptors.

The C-terminal domain of the mGlus is within close proximity to the inner leaflet of the lipid bilayer. Although recent structural studies of the purified intracellular C-terminal domains from mGlu6, 7 and 8 suggest that the C-termini of unliganded mGlus are mediated by linear motifs rather than secondary/tertiary structures (Seebahn, Dinkel et al. 2011), these intracellular tails of mGlus interact with various intracellular proteins, and are subject to alternative splicing (see section 3.1 and 3.2.2), phosphorylation, and SUMOylation (reviewed in (Enz 2012)).
3.2 The dimeric complex of mGlus

As mentioned above, mGlus form stable, covalently linked dimers. Data suggesting constitutive conformation of mGlu homodimers emerged as early as 1996 (Romano, Yang et al. 1996), and was further supported by data obtained from mGlu VFD/CRD crystal structures (Kunishima, Shimada et al. 2000). Evidence from biochemical studies reveals that one or more cysteine residues on the N-terminal extracellular domain mediate the covalent and non-covalent interactions between two mGlu protomers (Ray and Hauschild 2000, Romano, Miller et al. 2001). In addition, several studies have shown that mGlus do not appear to form higher-order oligomers (Brock, Oueslati et al. 2007, Doumazane, Scholler et al. 2011), although this is the case for some other Class C 7TMRs, such as the GABA<sub>B</sub> receptor (Comps-Agrar, Kniazeff et al. 2012).

The activation machinery of mGlu homodimers has been studied in depth, particularly by Jean Philippe Pin's group, using a quality control system adapted from the GABA<sub>B</sub> receptor to generate mGlu dimers bearing specific mutations within one of the protomers. These data suggest that closure of one VFD per dimer is sufficient to activate the receptor, although closure of both VFDs is required to achieve full activity (Kniazeff, Bessis et al. 2004). When one or both VFDs are occupied, the 7TM domain of either protomer can be activated through intersubunit rearrangement (Brock, Oueslati et al. 2007). These findings are consistent with the hypothesis that only a single 7TM domain is turned on upon activation of each homodimeric receptor (Goudet, Kniazeff et al. 2005, Hlavackova, Goudet et al. 2005, Hlavackova, Zabel et al. 2012).

Examples exist for heterodimers of mGlus and Class A 7TMRs in the CNS. Gonzalez-Maeso et al. reported that mGlu<sub>2</sub> receptors interact with 5-HT<sub>2A</sub> receptors through transmembrane helix domains and form functional complexes in brain cortex. Subsequent mutagenesis studies revealed that three residues within transmembrane domain 4 of mGlu<sub>2</sub> are necessary to form the 5-HT<sub>2A</sub>-mGlu<sub>2</sub> receptor heterocomplex (Moreno, Muguruza et al. 2012). Furthermore, hallucinogenic 5-HT<sub>2A</sub> agonists elicit unique responses at 5-HT<sub>2A</sub>/mGlu<sub>2</sub> complexes, which are implicated in the pathogenesis of psychosis (Gonzalez-Maeso, Ang et al. 2008). However, it should be noted that, although the formation of mGlu<sub>2</sub>/5HT<sub>2A</sub> heterocomplexes has been validated by other groups, (Delille et al., 2012) the unique signal transduction pathways mediated by the heterodimeric complex was not replicated. In addition, it was also shown that mGlu<sub>2</sub> can interact with 5-HT<sub>2B</sub>, indicating that complex formation is not specific to the 5-HT<sub>2A</sub>-mGlu<sub>2</sub> pair and challenging the biological relevance of the 5-HT<sub>2A</sub>-mGlu<sub>2</sub> complex.

With regards to mGlu heterodimers, the VFD of mGlu<sub>1</sub> can interact with full length mGlu<sub>5</sub> and vice versa (Beqollari and Kammermeier 2010). In addition, a splice variant of mGlu<sub>1</sub> that contains only the VFD functions as a dominant negative to potently block the signaling of full length mGlu<sub>1</sub> or mGlu<sub>5</sub> (Beqollari and Kammermeier 2010). Evidence for full length mGlu heterodimers initially emerged in in vitro expression systems (Doumazane, Scholler et al. 2011, Kammermeier 2012). By using a time-resolved FRET assay, Doumazane et al. demonstrated that group I mGlus can interact with each other, but do not associate with group II and group III mGlu subtypes; in contrast, group II and III mGlu receptors can co-

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assemble within and outside of the two groups. In addition, Kammermeier’s study utilizing injected superior cervical ganglion cells suggests that heterodimerization may alter the pharmacology of mGlus and their modulators. Together, these findings indicate that the functions and signaling of mGlus could be much more diverse and complex than previous estimated.

Recently, the existence and pharmacology of mGlu2/4 heterodimers has been established in native tissues (Yin, Noetzel et al. 2014). Using biochemical and pharmacological approaches, this study confirms that mGlu2 and mGlu4 form a hetero-complex in vitro and extends the finding into rat and mouse brain tissues. In cell lines, as well as at corticostriatal synapses, the mGlu2/4 heterodimer exhibits a distinct pharmacological profile compared to mGlu2 or mGlu4 homodimer populations, with alterations in affinity and efficacy for both mGlu2 and mGlu4 allosteric modulators. Specifically, it has been shown that PHCCC and 4PAM-2, two positive allosteric modulators that bind to the same allosteric pocket, exhibit diminished efficacy at mGlu2/4 heterodimers. In contrast, the potentiation induced by VU0155041 and Lu AF21934, two PAMs that bind to a second allosteric pocket, remains similar at homomers or mGlu2/4 heteromers. This suggests that these allosteric binding pockets may encounter differential conformational changes upon hetero-interaction of the two receptor subunits, and that distinct classes of allosteric modulators can be differentially regulated by mGlu heterodimers. While dramatically shifting our understanding of the functional roles of the mGlu family, these findings also potentially explain the discordant pharmacological findings observed in native brain tissue (Ayala, Niswender et al. 2008, Niswender, Johnson et al. 2010) and provide important clues for rational development of therapeutic reagents that target specific mGlu receptor assemblies.

3.3 Alternative splicing of mGlus

All mGlu subtypes have been discovered to undergo alternative splicing, primarily at the C-terminus, and some of the better characterized splice variants are described below. In human, 8 different splice variants of mGlu1 exist, named mGlu1a, 1b, 1c, 1d, 1e, 1g, 1g-393, 1g-620 and 1h. Two newly identified exons in human GRM1 express a novel splice variant of metabotropic glutamate 1 receptor. mGlu1a is the longest variant and the others result from differential splice site usage, generating distinct isoforms with differing C-termini (reviewed in (Hermans and Challiss 2001)). The splice variant containing only the VFD has been shown to act as a dominant negative, preventing full length mGlu1 isoforms from signaling (Beqollari and Kammermeier 2010). Also within the group I mGlus, mGlu5a and mGlu5b are two splice variants for mGlu5 with similar pharmacological profiles (Minakami, Katsuki et al. 1994, Joly, Gomeza et al. 1995). Three splice variants of mGlu3 exist in human brain due to exon skipping events: GRM3Δ2 (lacking exon 2), GRM3Δ4 (lacking exon 4), and GRM3Δ2Δ3 (lacking exons 2 and 3). Among the three variants, GRM3Δ4 is most abundantly expressed and represents an mGlu3 receptor without a seven-transmembrane domain, which may have unique functions and relate to non-coding single nucleotide polymorphisms (SNPs) in patients with cognitive dysfunctions (Sartorius, Nagappan et al. 2006). As to the group III mGlus, the mGlu4 gene was described as undergoing alternative splicing to generate mGlu4a and mGlu4b (Thomsen, Pekhletsli et al. 1997); however, this result has not been able to be replicated by other
groups (Corti, Aldegheri et al. 2002). Three mGlu6 splice variants exists in human retina, with mGlu6a lacking 97 nucleotides from exon 6 and mGlu6c including 5 nucleotides from intron 5 (Valerio, Ferraboli et al. 2001). Both mGlu7 and mGlu8 can undergo alternative splicing at the C-terminus, resulting in at least 5 splice variants for mGlu7 and 2 variants formGlu8 (Corti, Restituito et al. 1998, Schulz, Stohr et al. 2002). In addition, another splice variant, mGlu8c, contains a 74 nucleotide insertion, resulting in a frame shift and termination of the polypeptide before the seven transmembrane domains (Malherbe, Kratzeisen et al. 1999). As the C-terminal intracellular domain plays important roles in protein-protein interactions and signal transduction, different splice variants may possess distinct profiles with regards to receptor activation, receptor modification and receptor internalization (Enz 2012). With the advances in sequencing technology, the diversity of mGlu splice variants is increasing rapidly. For example, 7 splice variants have been predicted for mGlu4 according to Ensembl genome database, although their existence still needs to be validated experimentally.

Several isoforms of mGlus also exist due to alterations at the N-terminus; for example, Taste mGlu1 and Taste mGlu4 (Chaudhari, Landin et al. 2000), which play roles in detecting the taste of umami. These receptor variants, with approximately 50% of the N-terminus truncated, are expressed in taste buds. Compared to full-length receptors, these N-truncated variants lack much of the glutamate binding domain and thus exhibit lower potency when activated by glutamate (Chaudhari, Landin et al. 2000).

### 3.4 Signaling of mGlus

#### 3.4.1 G protein-dependent signaling—
Classically, group I mGlus are generally coupled to $G_{q/11}$ and activate phospholipase Cβ, which hydrolyses phosphatidylinositides into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol, a pathway leading to calcium mobilization and activation of protein kinase C (PKC). Other effectors downstream of Gq include phospholipase D, ion channels, c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase/extracellular receptor kinase (MAPK/ERK), and the mammalian target of rapamycin (mTOR) pathway (Sayer 1998, Servitja, Masgrau et al. 1999, Page, Khidir et al. 2006, Li, Li et al. 2007) (Figure 1). In addition, evidence has emerged that group I mGlus can also activate $G_s$ and $G_{i/o}$ and their downstream pathways, and that distinct regions on the receptor are responsible for coupling of different G proteins (Francesconi and Duvoisin 1998, McCool, Pin et al. 1998).

As stated previously, the group II and group III mGlu receptors are coupled to $G_{i/o}$ proteins and negatively regulate the activity of adenylyl cyclase. In addition, many ion channels have also been reported to be regulated by $G_{ci}$ and the liberated $G_{i/o}$ subunit (Guo and Ikeda 2005, Niswender, Johnson et al. 2008, Kammermeier 2012). $G_{i/o}$-mediated activation of MAPK and phosphatidylinositol 3-kinase (PI3 kinase) pathways, as supported by the inhibitory effect of pertussis toxin (Iacovelli, Bruno et al. 2002), add another level of complexity to the G protein-mediated signaling of group II and III mGlus.

#### 3.4.2 G protein-independent signaling—
While 7TMRs transduce signals through various cellular pathways, their responsiveness may also be regulated by receptor...
desensitization. When a receptor is stimulated, activated G protein-coupled receptor kinase (GRK) then initiates a combination of events including receptor phosphorylation, arrestin binding, and receptor internalization (reviewed in (Krupnick and Benovic 1998)), providing a feedback mechanism that prevents receptor over-stimulation. For mGlus, such regulation is more thoroughly studied for group I mGlus than the other two groups. For example, internalization of mGlu_1 has been shown to depend on GRK4 and β-arrestin-1 (Dale, Bhattacharya et al. 2001, Iacovelli, Salvatore et al. 2003). Desensitization of mGlu_5 however, seems to be dependent on GRK2 activity, suggesting different mGlu subtypes are regulated by distinct mechanisms (Sorensen and Conn 2003).

Besides regulating receptor desensitization, recruited β-arrestins are also well-known as scaffolding protein for signaling molecules. Reports have shown that active Src is recruited to activated 7TMRs by interaction with β-arrestin, which then results in phosphorylation of downstream molecules and consequently activation of the MAPK cascade (Luttrell, Ferguson et al. 1999). In addition, arrestins also directly facilitate the subcellular localization and activation of two MAPK cascades (the RAF→MEK→extracellular signal-regulated kinases (RAF-MEK-ERK) cascade and the apoptosis signal-regulating kinase→MKK→c-Jun N-terminal kinases (ASK-MKK-JNK cascade)) (Pierce and Lefkowitz 2001), further expanding the dimension and complexity of signal transduction upon GPCR activation. This role of arrestins in mediating signal transduction events has been demonstrated for mGlus as well. For example, activation of mGlu_7 significantly reduces N-methyl D-aspartate receptor (NMDAR)-mediated currents in prefrontal cortex pyramidal neurons in a β-arrestin/ERK signaling pathway-dependent manner (Gu, Liu et al. 2012).

In addition, mGlu receptors also demonstrate the ability to activate signaling cascades through protein-protein interactions. For instance, the C-terminal domains of mGlu_1 and mGlu_5 interact with Homer proteins, a group of scaffolding proteins for multiprotein complexes. Besides interacting with the receptor, Homer proteins demonstrate binding ability with inositol-1,4,5-triphosphate (IP3) receptors, ryanodine receptors, transient receptor channel-1 and 4 (TRPC1, TRPC4), P/Q-type Ca^{2+} channels, Shank, the phosphoinositide 3-kinase (PI3K) enhancer-long (PIKE-L) etc., coupling receptor activation to other signaling components within the cell (Rong, Ahn et al. 2003, Bockaert, Dumuis et al. 2004, Fagni, Ango et al. 2004). mGlu_5 has been found to interact with the NMDA receptor via Homer and other scaffolding proteins and potentiate receptor activity (Tu, Xiao et al. 1999, Attucci, Carla et al. 2001, Pisani, Gubellini et al. 2001). In addition, it has been shown that disruption of mGlu5–Homer interactions selectively blocks mGlu activation of the PI3K-Akt-mTOR pathway (Ronesi and Huber 2008) and contribute to phenotypes of Fmr1 knockout mice, an animal model for Fragile X syndrome (Ronesi, Collins et al. 2012). Interestingly, Homer proteins include long Homer isoforms and short isoforms (Homer1a and Ania), which act as endogenous dominant-negatives and disrupt protein complexes containing the long Homer variant. The ratio of Homer 1a/long Homer bound to mGlu_5 may associate with cognitive aging (Menard and Quirion 2012) and has been shown to be altered in Fmr1 knockout mice (Ronesi, Collins et al. 2012). In addition, genetic deletion of Homer1a rescues several phenotypes in Fmr1 knockout mice, suggesting the importance of Homer proteins in the mechanism of Fragile X syndrome and potential therapeutic intervention for this disease (Ronesi, Collins et al. 2012). Besides the PI3K-Akt-mTOR

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pathway, Homer also links mGlu5 to PIKE-L, which prevents cell apoptosis upon mGlu5 activation (Rong, Ahn et al. 2003). Interestingly, it has been shown that the disruption of Homer-mGlu5 interaction reduces astrocyte apoptosis (Paquet, Ribeiro et al. 2013), suggesting opposite functions of Homer in regulation of cell apoptosis in neurons and astrocytes. Besides the CNS, a point mutation of mGlu1 within the Homer binding region that has been discovered in the somatic cells of lung cancer patients (Esseltine, Willard et al. 2013), indicating important roles of Homer in the periphery.

Another well-studied protein-protein interaction is the mGlu7-protein interacting with C kinase 1 (PICK1) interaction. PICK1 was discovered as a peripheral membrane protein that interacts with protein kinase Cα (PKCα) (Staudinger, Zhou et al. 1995). Besides PICK1, the C-terminus of mGlu7 also interacts with Ca2+-calmodulin, G protein βγ subunits to modulate the activity of voltage-gated Ca2+ channel and negatively regulate neurotransmitter release (Dev, Nakanishi et al. 2001). Interestingly, phosphorylation of the receptor by PKC increases receptor binding to PICK1, which is required for stable surface expression of mGlu7 (Suh, Pelkey et al. 2008, Suh, Park et al. 2013), but, at the same time, inhibits the binding of Gβγ subunits and Ca2+-calmodulin, providing a delicate regulatory machinery. Disruption of the mGlu7-PICK1 interaction has been performed by genetic knock-in of an mGlu7 mutant that does not bind PICK1. The resulting animals exhibited significant defects in hippocampus-dependent spatial working memory and high susceptibility to convulsant drugs (Zhang, Bertaso et al. 2008). Additionally, injection of a competing peptide to rodents also resulted in behavioral symptoms and EEG discharges that are characteristic of absence epilepsy (Bertaso, Zhang et al. 2008). These data indicate that the mGlu7-PICK1 interaction is important for regulating mGlu7 signaling and may underlie certain disease mechanisms, including cognitive disorders and epilepsy.

4. Orthosteric modulation of mGlus

4.1 Non-selective ligands

Glutamate, the endogenous agonist of glutamate receptors (including ionotropic glutamate receptors), activates all mGlu subtypes, each with a distinct binding affinity and potency. Glutamate exhibits the lowest affinity for the group III mGlus and the highest for the group II receptors. As it pertains to synthetic ligands, (±) trans-ACPD (and its active isomer 1S, 3R-ACPD) was the first identified agonist for mGlus with selectivity over ionotropic glutamate receptors and has served as a tool compound to assess the functional involvement of mGlu receptors in the regulation of signaling as a broad group (Palmer, Monaghan et al. 1989, Desai and Conn 1990, Manzoni, Fagni et al. 1990, Schoepp, Johnson et al. 1991, Schoepp, Johnson et al. 1991). Additionally, several amino acid analogs have been shown to be more selective for each of the three subgroups and this as will be discussed in the sections below.

4.2 Orthosteric ligands of group I mGlus

Quisqualic acid demonstrates high affinity and selectivity for mGlu1 and 5 (affinity~56 and 52 nM, respectively) (Ohashi, Maruyama et al. 2002), and was the first identified agonist for Group I mGlus. Mutagenesis studies revealed that the subtype selectivity of this compound
results from a complex interplay of residues shaping the binding pocket of the receptor (Hermit, Greenwood et al. 2004). The use of this compound in native tissue, however, is limited by its activity at AMPA receptors and the subsequent complications that this brings to data interpretation (Watkins, Krogsgaard-Larsen et al. 1990).

3,5-DHPG selectively activates Group I mGlus, with its agonist activity residing exclusively in its S-isomer (Schoepp, Goldsworthy et al. 1994, Baker, Goldsworthy et al. 1995). Importantly, (S)-3,5 DHPG is devoid of activity on both group II and group III mGlus, as well as glutamate transporters (Schoepp, Jane et al. 1999), representing the most selective agonist for group I mGlus thus far, although it lacks the ability to differentiate mGlu$_1$ from mGlu$_5$. Within the group I mGlus, analogs of quisqualic acid and (S)-3,5-DHPG have been shown to be more selective for mGlu$_5$ over mGlu$_1$. A homolog of quisqualic acid, (S)-homois quisqualic acid, is a competitive antagonist at mGlu$_1$ while being a full agonist at mGlu$_5$, displaying some potential to differentiate between the two group I subtypes. However, its selectivity is jeopardized by agonist activity at mGlu$_2$ (Brauner-Osborne and Krogsgaard-Larsen 1998). A DHPG derivative, CHPG, exhibits high selectivity at mGlu$_5$ over mGlu$_1$, although the low potency on mGlu$_5$ (~750 μM) limits the utility of this compound (Doherty, Palmer et al. 1997).

Multiple orthosteric antagonists for group I mGlus have been developed from a phenylglycine scaffold, including (S)-4CPG, (S)-4C3HPG and (S)-MCPG, although they also exhibit some activity at mGlu$_2$ (Schoepp, Jane et al. 1999). Within the 4CPG scaffold, LY367385 is a highly selective antagonist of mGlu$_1$ relative to mGlu$_5$, representing a tool compound to discriminate between the two group I mGlus (Bruno, Battaglia et al. 1999).

4.3 Orthosteric ligands of group II mGlus

DCG IV is potent group II mGlu agonist with affinities of 110 and 150 nM at rat mGlu$_2$ and mGlu$_3$, respectively (Schweitzer, Kratzeisen et al. 2000). However, it also possesses NMDA receptor agonist activity (Ishida, Saitoh et al. 1993) and antagonist activity at group I and group III mGlu receptors at high concentrations (Brabet, Parmentier et al. 1998). Another compound, 2R,4R-APDC, has an EC$_{50}$ value of ~400 nM on cloned human mGlu$_2$ and mGlu$_3$ and is devoid of activity at group I or group III mGlus up to 100μM (Schoepp, Jane et al. 1999).

Compounds with even higher potency come from the heterobicyclic amino acid scaffold, including LY379268, with an EC$_{50}$ of 2.7 nM and 4.6 nM in cells expressing mGlu$_2$ and mGlu$_3$, respectively (Monn, Valli et al. 1999). While this compound shows no activity at ionotropitic glutamate receptors, it does activate mGlu$_4$, mGlu$_6$ and mGlu$_8$ at high concentrations (Monn, Valli et al. 1999). However, the nearly 1000 fold selectivity for mGlu$_2$ and mGlu$_3$ still makes LY379268 a commonly used tool compound for group II mGlus. Although currently no orthosteric agonist can completely differentiate between mGlu$_2$ and mGlu$_3$, an analog of LY354740 was found to exhibit mGlu$_2$ agonist and mGlu$_3$ antagonist activity (Domínguez, Prieto et al. 2005), providing an opportunity to tease apart the functional effects of mGlu$_2$ from mGlu$_3$. 

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EGLU is one of the first compounds that was found to antagonize mGlu2 and mGlu3 with little antagonism on group I or III mGlus (Jane, Thomas et al. 1996). LY341495 is an antagonist at mGlu2 and mGlu3 with high affinity (1.67 nM and 0.75 nM for human receptors, respectively) (Johnson, Wright et al. 1999). However, it also antagonizes group I and group III mGlus at submicromolar or micromolar concentration (Kingston, Ornstein et al. 1998).

4.4 Orthosteric ligands of group III mGlus

L-AP4 and L-SOP have been used as prototypical agonists for group III mGlus, with submicromolar to low micromolar potencies at mGlu4, 6, and 8, but around 200 μM potency and affinity at mGlu7 (Schoepp, Jane et al. 1999, Wright, Arnold et al. 2000). Although these two compounds are highly selective for group III receptors, they activate mGlu4 and mGlu8 with similar potencies. In contrast, (S)-3,4-DCPG exhibits 100-fold selectivity for mGlu8 over mGlu4 (Thomas, Wright et al. 2001) and has been used as a tool compound to study the function of mGlu8 in native systems (Zhai, Tian et al. 2002, Johnson, Jones et al. 2013). Recently, a virtual high throughput screening approach has been used to discover a new variable pocket and develop a group of mGlu4-preferring agonists, including LSP1-2111 and LSP4-2022 (Beurrier, Lopez et al. 2009, Goudet, Vilar et al. 2012). In particular, LSP4-2022 exhibits potencies of 0.1 μM at mGlu4 versus 29 μM at mGlu8, with no activity at the group I and II mGlus up to 100 μM (Goudet, Vilar et al. 2012), making this compound an important tool to selectively activate mGlu4.

MAP4 and MSOP, two analogs of L-AP4 and L-SOP, have been reported to be highly selective antagonists for group III mGlus (Schoepp, Jane et al. 1999). However, these two compound exhibited low potencies in a thallium flux assay where activation of G-protein regulated inwardly rectifying potassium channels (GIRK) was used as a readout of receptor activity (Niswender, Johnson et al. 2008). Besides these two compounds, CPPG antagonizes group III mGlus with at least 30-fold selectivity over other subtypes (Jane, Thomas et al. 1996).

5. Allosteric modulation of mGlus

5.1 Mechanism and advantages of allosteric modulation

Through years of research, orthosteric ligands useful in determining the physiological roles of mGlus have been developed which display group-selectivity. However, as all orthosteric ligands bind to the N-terminal VFD of mGlus, which is evolutionarily designed to bind glutamate, it is difficult to achieve subtype selectivity since the glutamate binding site is highly conserved across all eight subtypes. In addition, brain penetration and pharmacokinetics of these orthosteric ligands can be limited by their amino acid-like properties. Many of these hurdles have been overcome by targeting allosteric binding sites on the receptors. As indicated by the name, allosteric modulators bind to a site other than the endogenous agonist binding site, and provide modulatory effects on the affinity/efficacy of the orthosteric agonist, termed cooperativity. Indeed, the complex structure of mGlus offers a number of possibilities to develop novel allosteric modulators. Because of the greater sequence divergence demonstrated at allosteric sites, a series of subtype-selective allosteric
agonists and positive, negative or silent allosteric modulators (PAM, NAM or SAMs) of mGlus have been identified through high through-put screening campaigns ((Varney, Cosford et al. 1999, Kinney, O’Brien et al. 2005, Mitsukawa, Yamamoto et al. 2005, Niswender, Johnson et al. 2008) and many others), which have greatly advanced studies on mGlu functions and accelerated the development of mGlu reagents as disease therapeutics.

The mechanism of action of allosteric modulators is exemplified by the dataset shown in Figure 2. PAMs potentiate the response to orthosteric agonists by shifting the concentration-response curve of an orthosteric agonist to the left (with or without increasing the maximum response), indicating that the agonist response is being pharmacologically amplified. NAMs are molecules that antagonize the activity of agonists in a noncompetitive fashion through negative cooperativity. SAMs are neutral allosteric modulators that exhibit no apparent effect on agonist responses on their own; however, such compounds can block the binding and subsequent receptor modulation induced by PAMs or NAMs.

Besides improved selectivity, PAMs and NAMs also provide some other advantages when compared to their orthosteric counterparts. First, the biological effects of PAMs and NAMs are dependent on the presence of the endogenous agonist; thus, they have the potential to preserve the spatial and temporal aspects of endogenous signaling. As PAMs which lack allosteric agonist activity will not constantly activate the receptor, they may also reduce the liability of receptor desensitization compared to the direct activation by orthosteric, or even allosteric, agonists. Allosteric modulators also bring a further advantage in that their modulating effect is saturable, thus providing a larger therapeutic window and potentially decreasing the risk of overdose. In addition, these allosteric molecules often possess drug-like properties and better pharmacokinetics and brain penetration compared to orthosteric ligands, an important feature considering the therapeutic indication of mGlus in CNS disorders.

Thusfar, the majority of identified mGlu allosteric modulators bind to the 7TMD region of the receptor. Results from mutagenesis studies indicate that the mGlu allosteric binding site likely corresponds to the orthosteric binding site in Class A 7TMRs (Litschig, Gasparini et al. 1999, Pagano, Ruegg et al. 2000, Malherbe, Kratochwil et al. 2003, Schaffhauser, Rowe et al. 2003, Chen, Goudet et al. 2008, Gregory, Noetzel et al. 2013). Indeed, recent structural studies on mGlu1 suggest that the binding pocket for the NAM FITM is defined by residues on TM2, 3, 5, 6 and 7, which is analogous to the orthosteric site for many Class A receptors (Wu, Wang et al. 2014). In addition, multiple allosteric sites may exist on a given receptor, as examplified by mGlu4 (Drolet, Tugusheva et al. 2011) and mGlu5 (O’Brien, Lemaire et al. 2003, Chen, Nong et al. 2007, Chen, Goudet et al. 2008, Hammond, Rodriguez et al. 2010, Noetzel, Gregory et al. 2013).

The modulating effects induced by allosteric modulators can be quantified using operational models of allosterism (Leach, Sexton et al. 2007, Gregory, Noetzel et al. 2012):

\[
y = \text{basal} + \frac{(E_m - \text{basal})}{(\tau_A [A] (K_A + [B]) + K_A)} \left( \frac{(E_m - \text{basal})}{(\tau_A [A] (K_A + [B]) + [A] [B] K_A + K_A [B] + K_A [A])^n} \right)^n \right]
\]

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where \( A \) and \( B \) are the molar concentration of the orthosteric agonist and the allosteric modulator, respectively; \( K_A \) and \( K_B \) are the equilibrium dissociation constant of the orthosteric agonist and the allosteric modulator, respectively; \( \tau_A \) and \( \tau_B \) quantify the efficacy of the orthosteric agonist and the allosteric modulator, respectively. \( \text{Basal, } E_m \) and \( n \) represent the basal system response, maximal possible system response and the transducer function that links occupancy to response. Importantly, these models have introduced two parameters, \( \alpha \) and \( \beta \), to describe cooperativity of an allosteric ligand on the affinity and efficacy of orthosteric agonist. The operational models of allosterism not only allow quantitative estimation of modulator affinity and cooperativity values, which can be used to guide compound optimization processes, but can be used to derive reliable estimates of modulator affinities when radioligand is not available (Gregory, Noetzel et al. 2012).

Based on molecular pharmacology data, it has been hypothesized that binding of one PAM per mGlu dimer is sufficient to potentiate receptor activity (Goudet, Kniazeff et al. 2005). In contrast, binding of NAMs to both protomers appears to be necessary to inhibit receptor activation (Hlavackova, Goudet et al. 2005). Interestingly, PAMs can directly activate an N-terminal truncated mGlu (Goudet, Gaven et al. 2004, El Moustaine, Granier et al. 2012), suggesting that VFD-CRD region prevents PAMs from activating the receptor until glutamate is bound.

Despite the advantages mentioned above, many allosteric modulators are highly lipophilic, which diminishes their solubility, can affect their pharmacokinetic profile, and potentially increases off-target binding. The shallow structure-activity relationship among classes of allosteric ligands also represents a significant hurdle in the development of allosteric modulators. Additionally, because of the substantial activity alteration generated by minor structural changes, a number of mGlu allosteric modulator classes are susceptible to subtle “molecular switches” (Wood, Hopkins et al. 2011), by which compounds within a series can switch switch from NAM to PAM, PAM to NAM, become silent, or exhibit altered selectivity (Sharma, Kedrowski et al. 2009, Zhou, Manka et al. 2010, Lamb, Engers et al. 2011, Sheffler, Wenthur et al. 2012).

Allosteric compounds have also complicated our understanding of receptor pharmacology. As allosteric modulators potentiate/inhibit the receptor through cooperativity with the orthosteric ligand being used, it is not surprising that “probe dependence” has been reported in some cases, in that modulators have differential effects depending upon the orthosteric ligand that is present (Suratman, Leach et al. 2011, Valant, Felder et al. 2012). Although examples still have yet to be discovered for mGlu receptors, cautions should be taken when choosing the orthosteric ligand for \textit{in vitro} studies. In addition, similar to what has been described with some orthosteric ligands (Urban, Clarke et al. 2007), many allosteric compounds have been found to differentially stimulate multiple signaling cascades downstream of a receptor (Kenakin 2005), a phenomenon often termed “functional selectivity” “biased signaling”, or “ligand directed trafficking” (Mathiesen, Ulven et al. 2005, Zhang, Rodriguez et al. 2005, Urban, Clarke et al. 2007, Marlo, Niswender et al. 2009). Indeed, 7TMRs may adopt multiple structural conformations, and allosteric modulators may stabilize any of them, which can translate into the regulation of some signaling pathways but not others. Biased pharmacology of mGlu4 PAMs has also been...
observed through signal convergence when $G_{q}$-coupled receptors are co-activated (Yin, Zamorano et al. 2013). Although these findings highly complicate the application of allosteric modulators as disease therapeutics, it is conceivable that biased modulators may enhance the therapeutic outcome or avoid adverse effects when modulation/exclusion of a signal pathway is desired.

5.2 Allosteric modulators of group I mGlus

CPCCOEt is the first discovered NAM for mGlu$_{1}$ and serves as a proof-of-concept example for the development of a selective mGlu ligand acting via an allosteric binding site (Litschig, Gasparini et al. 1999). A great number of structurally distinct compounds have now been identified as mGlu$_{1}$-selective NAMs, including Bay 36–7620 (Carroll, Stolle et al. 2001), JNJ16259685 (Lavreysen, Wouters et al. 2004), YM-298198 (Kohara, Toya et al. 2005), FTIDC (Suzuki, Kimura et al. 2007), CFMMC (Fukuda, Suzuki et al. 2009), etc., many of which are potent and active in vivo. Ro 67–7476 and Ro 67–4853 represent two chemical series of selective mGlu$_{1}$ PAMs (Knoflach, Mutel et al. 2001). In addition, VU71, an analog of the mGlu$_{5}$ PAM CDPPB, has also been shown to specifically potentiate mGlu$_{1}$.

Interestingly, none of the three mGlu$_{1}$ PAMs bind to the traditional NAM site (Hemstapat, de Paulis et al. 2006). More recently, a group of 9H-xanthene-9-carboxylic acid oxazol-2-yl-amides were reported as potent mGlu$_{1}$ PAMs with improved pharmacokinetic profiles (Vieira, Huwyler et al. 2009).

Selective allosteric modulators have also been developed for mGlu$_{5}$. SIB-1757 and SIB-1893 were the first mGlu$_{5}$ selective allosteric antagonists to be discovered. Further structural modification of the same scaffold led to the discovery of the now widely used compounds MPEP and MTEP, two mGlu$_{5}$ NAMs with improved potency, selectivity, and pharmacokinetic profile (Gasparini, Lingenhohl et al. 1999, Cosford, Tehrani et al. 2003, Lea and Faden 2006). Ongoing discovery and development work lead to a number of novel mGlu$_{5}$ NAMs, many of which are in clinical development for multiple indications. An early mGlu$_{5}$ NAM, fenobam, was evaluated in a small clinical trial in Fragile X syndrome (FXS) patients and exhibited efficacies in half of the patients (Berry-Kravis, Hessl et al. 2009). Currently, the mGlu$_{5}$ NAMs ADX48621 (Dipraglurant) from Addex and AFQ056 (Mavoglurant) from Novartis are under Phase II/III clinical trials and demonstrate efficacy in Parkinson’s disease levodopa-induced dyskinesia (PD-LID) and FXS (AddexPharmaceuticalsPressRelease, Berg, Godau et al. 2011, Jacquemont, Curie et al. 2011). Besides FXS, RO4917523 (RG7090), an mGlu$_{5}$ NAM from Roche is being evaluated in Phase II clinical trial for treatment-resistant depression. Preclinically, novel mGlu$_{5}$ NAMs from the (3-cyano-5-fluorophenyl)biaryl series demonstrated efficacious in operant sensation seeking test, an mouse model of addiction (Lindsley, Bates et al. 2011), further expanding potential indications and encouraging continued research for mGlu5 NAMs.

Several mGlu$_{5}$ PAMs have been derived from multiple chemical series and more than one binding site exists for mGlu PAMs. VU-29, CDPPB and DFB and other compounds from those series interact with the common “MPEP” site (O’Brien, Lemaire et al. 2003, Chen, Nong et al. 2007), whereas CPPHA and the subsequently discovered NCFP interact with the receptor at a distinct allosteric site (Chen, Goudet et al. 2008) (Noetzel, Gregory et al. 2013).
A benzamide scaffold, as exemplified by VU0357121, has been suggested to bind to a non-MPEP, non-CPPHA site on mGlu5 (Hammond, Rodriguez et al. 2010), raising the possibility of the existence of at least three PAM binding sites. Along with the discovery of PAMs, DCB (a DFB analog) and VU0365396 (a benzamide series analog) appear to be SAMs, with neutral activity at MPEP and non-MPEP sites (O’Brien, Lemaire et al. 2003, Hammond, Rodriguez et al. 2010), representing useful tool compounds to further investigate the allosteric binding sites on mGlu5.

5.3 Allosteric modulators of group II mGlus

Several NAMs from a dihydrobenzo[1,4]diazepin-2-one series have been reported to antagonize both mGlu2 and mGlu3 in a non-competitive fashion (Hemstapat, Da Costa et al. 2007, Woltering, Adam et al. 2008, Woltering, Wichmann et al. 2008). Recently, selective NAMs for mGlu3, ML289 and ML337, have also been discovered via a “molecular switch” from an mGlu5 PAM scaffold, where small modification to the structure changes its mode of pharmacology (Sheffler, Wenthur et al. 2010, Sheffler, Wenthur et al. 2012, Wenthur, Morrison et al. 2013).

BINA, LY487379 (along with CBiPES) and THIIC represent three chemical scaffolds of highly selective mGlu2 PAMs that lacks activity at mGlu3 (Johnson, Barda et al. 2005, Galici, Jones et al. 2006, Fell, Witkin et al. 2011). Additionally, a group of mGlu2 SAMs have been identified through FRET-based binding assays and slight modification of these SAMs yields three mGlu2 NAMs with mGlu3 PAM activity, indicating that identification of SAMs is a useful approach to discover novel mGlu allosteric modulators (Schann, Mayer et al. 2010). Mutagenesis studies have revealed that amino acids that are essential for mGlu2 PAM activity are dispensible for NAMs, and the converse has also been reported (Schaffhauser, Rowe et al. 2003, Rowe, Schaffhauser et al. 2008, Lundstrom, Bissantz et al. 2011). These data suggest that mGlu2 PAMs and NAMs may bind to different allosteric pockets, although binding studies using appropriate allosteric radioligands are required to validate this hypothesis.

5.4 Allosteric modulators of group III mGlus

Allosteric modulation has also been demonstrated to be a successful approach to selectively modulate mGlu4, 7 and 8. An mGlu1 partial antagonist, PHCCC, was the first identified PAM for mGlu4 with no activity on 6 of the other mGlu subtypes except for mGlu6 (Maj, Bruno et al. 2003, Marino, Williams et al. 2003, Beqollari and Kammermeier 2008). In in vitro mGlu4 assays, PHCCC exhibits no agonist activity by itself but increases the potency of glutamate at mGlu4 and acts as a proof-of-concept compound for targeting mGlu4 as a potential therapeutic strategy for disorders such as Parkinson’s disease (Marino, Williams et al. 2003). Further optimization of the PHCCC scaffold has been challenging (Niswender et al., 2008, Williams et al 2010); however, a high-throughput screening campaign led to the identification of VU0155041 and VU0080421 as examples of non-PHCCC scaffold mGlu4 PAMs (Niswender, Johnson et al. 2008, Niswender, Lebois et al. 2008). Recently, many other mGlu4 PAMs, including 4PAM-2, VU0364770, ADX88178, LuAF21934 and others (Drolet, Tugusheva et al. 2011, Jones, Bubser et al. 2012, Le Poul, Bolea et al. 2012, Bennouar, Uberti et al. 2013), have emerged with significant improvements in potency,
selectivity and pharmacokinetic profile compared to PHCCC. Particularly, VU0364770, ADX88178 and LuAF21934 exhibit high potency with good pharmacokinetic profiles for use as tool compounds; each of these ligands shows in vivo efficacy in animal models of PD and other disorders. Interestingly, VU0155041 also exhibits allosteric agonist activity at mGlu₄ when tested in vitro (Niswender, Johnson et al. 2008), suggesting an alternative mechanism of action compared to PHCCC. Indeed, data obtained from radioligand binding assays has demonstrated that VU0155041 binds to a unique allosteric site on mGlu₄ which is different from the binding site of PHCCC and 4PAM-2 (Drolet, Tugusheva et al. 2011); such differences may underlie the diverse performance of mGlu₄ PAMs in mGlu₂/₄ expressing tissues, such as the corticostriatal synapses (Yin, Noetzel et al. 2014).

Allosteric compounds for mGlu₇ suffer from pharmacological complications. AMN082 was discovered in a high through-put screen and is an orally active, brain-penetrant allosteric agonist of mGlu₇ that directly activate the receptor by binding to the transmembrane domain (Mitsukawa, Yamamoto et al. 2005). Although AMN082 inhibits cAMP accumulation and stimulates GTPγS binding in cells expressing mGlu₇, it was subsequently shown to have no effect on mGlu₇ in activating a promiscuous G protein or the GIRK potassium channels (Suzuki, Tsukamoto et al. 2007, Ayala, Niswender et al. 2008). Furthermore, it does not activate mGlu₇ at the Shaffer collateral-CA1 synapse in hippocampal slices (Ayala, Niswender et al. 2008). In addition, AMN082 and its major metabolite exhibit physiologically relevant binding affinity at several transporters, such as the serotonin transporter, the dopamine transporter and the norepinephrine transporter (Sukoff Rizzo, Leonard et al. 2011). With these caveats, the physiological and pharmacological effects of AMN082 in vivo should ideally be confirmed using mGlu₇ knockout mice versus wildtype mice. When administered orally, AMN082 has been shown to induce a robust increase in stress hormone levels in wildtype mice, but not in mGlu₇ knockout animals (Mitsukawa, Yamamoto et al. 2005), supporting a role of mGlu₇ in conditions involving chronic stress. In addition, intraperitoneal injection of AMN082 induced antidepressant-like behavior in wildtype animals but not mGlu₇ knockout littermates when they were examined in a tail suspension test (Palucha, Klak et al. 2007). However, it should be noted that AMN082 significantly decreased spontaneous locomotor activity and induced body tremors in both mGlu₇ knockout mice and wildtype animals, suggesting phenotypes that are mediated by an off-target effect(s). Similar to AMN082, a selective mGlu₇ NAM, termed MMPIP, also exhibits pathway dependence in different assays. It has been shown that MMPIP inhibits mGlu₇-mediated calcium mobilization through Gα₁₅ (Suzuki, Tsukamoto et al. 2007, Niswender, Johnson et al. 2010)); however, its potency is much lower in affecting mGlu₇-mediated inhibition of cAMP accumulation or activation of GIRK channels (Suzuki, Tsukamoto et al. 2007, Niswender, Johnson et al. 2010). In addition, it appears to be unable to block mGlu₇-modulated neurotransmission-at the Shaffer collateral-CA1 synapse (Niswender, Johnson et al. 2010). Therefore, caution should be taken in terms of the pathway dependence and selectivity when utilizing these two compounds. More recently, ADX71743 has been discovered to be a potent, selective and brain-penetrant NAM for mGlu₇. Administration of ADX71743 in vivo resulted in an anxiolytic-like effect in the marble burying and elevated plus maze tasks, suggesting that inhibition of mGlu₇ is a promising approach for the treatment of anxiety disorders (Kalinichev, Rouillier et al. 2013).
AZ12216052 is the best characterized selective mGlu8 PAM with an EC$_{50}$ of 1 μM (Duvoisin, Pfankuch et al. 2010). This compound is systemically available upon intraperitoneal administration, and has been used in animal models for CNS diseases, such as anxiety (Duvoisin, Pfankuch et al. 2010, Duvoisin, Villasana et al. 2011). However, activity of AZ12216052 was retained in mGlu8 knockout animals (Duvoisin, Villasana et al. 2011), suggesting that the compound may have off target effects that mediate some of its anxiolytic profile.

6. Therapeutic indications of mGlus

6.1 CNS-related diseases

6.1.1 Schizophrenia and Alzheimer's disease—Schizophrenia is a debilitating psychiatric illness that affects approximately 1% of the population. Three clusters of symptoms exist for schizophrenia patients, these are classified as positive symptoms (hallucinations, delusions, paranoia), negative symptoms (social withdrawal and anhedonia) and cognitive impairments (attention, memory and problem solving deficits) (AmericanPsychiatricAssociation 2000, Nuechterlein, Barch et al. 2004). A number of neurotransmission systems are disturbed in schizophrenia patients; the most well studied is the dopaminergic system. A dopamine hypothesis has been proposed to link altered brain function to the misfiring of dopaminergic neurons based on the fact that amphetamines, which have been shown to exacerbate the psychotic symptoms in schizophrenia, increase dopamine release (Laruelle, Abi-Dargham et al. 1996), and drugs that block dopamine receptor function reduce psychotic symptoms. Although current therapies targeting the dopamine D$_2$ receptor (typical antipsychotics) are available, multiple adverse drug effects can occur with these treatments and the therapeutic effects on the negative symptoms or cognitive deficits are limited (Liberman and Glick 2004, Liberman 2005, Pramyothin and Khaodhiar 2010). Atypical antipsychotics are as effective as the typical antipsychotics, although their affinity for dopamine D$_2$ receptor is much lower (Jones and Pilowsky 2002), suggesting that the dopamine hypothesis may not be sufficient to explain the mechanism of schizophrenia. On the other hand, modulation of the glutamatergic pathway appears to be an attractive alternative approach for schizophrenia treatment (reviewed in (Herman, Bubser et al. 2012)). For example, the NMDA receptor antagonists phencyclidine (PCP), ketamine and MK-801 have been reported to induce psychotomimetic effects in healthy human subjects and exacerbate symptoms in schizophrenia patients (Lahti, Koffel et al. 1995, Adler, Malhotra et al. 1999). In contrast, agonists at the glycine site of the NMDA receptor which enhance receptor function have been shown to exhibit beneficial effects in schizophrenia patients (Heresco-Levy, Ermilov et al. 2004, Heresco-Levy and Javitt 2004).

As development of direct-acting NMDA receptor agonists has been limited by the potential risk of side effects such as excitotoxicity and seizures, mGlu5 has been found to interact with the NMDA receptor via scaffolding proteins and to potentiate receptor activity, providing an alternative approach to indirectly modulate NMDA receptor activity (Attucci, Carla et al. 2001, Pisani, Gubellini et al. 2001). Consistent with this, a number of selective mGlu5 PAMs have been demonstrated to potentiate mGlu5 as well as NMDA receptor activity in brain slices (O'Brien, Lemaire et al. 2004, Rodriguez, Nong et al. 2005, Chen,
Nong et al. 2007) and, as such, represent promising therapeutic agents for schizophrenia patients. Furthermore, mGlu5 PAMs, such as CDPPB, ADX47273, VU0360172 and LSN2463359, exhibit antipsychotic-like and procognitive effects in rodent models of schizophrenia (Kinney, O’Brien et al. 2005, Liu, Grauer et al. 2008, Rodriguez, Grier et al. 2010, Gastambide, Cotet et al. 2012), making allosteric potentiation of mGlu5 a promising avenue to pursue. However, cautions should be taken regarding side effects induced by mGlu5 potentiation, which may limit the development of mGlu5 PAMs as treatment for schizophrenia. For example, administration of 4 structurally distinct mGlu5 PAMs developed at Merck and Co. demonstrated significant neurotoxicity in wildtype animals but not mGlu5 knockout mice, indicating a mechanism-based side effect induced by mGlu5 potentiation (Parmentier-Batteur, Hutson et al. 2013). Similarly, VU0422465, an allosteric agonist-PAM of mGlu5, was shown to induce epileptiform activity and behavioral convulsions in rodents. In contrast, VU0361747, an mGlu5 PAM that does not show allosteric agonist activity in vitro, demonstrated robust antipsychotic efficacy without inducing adverse behavioral effects (Rook, Noetzel et al. 2013). These data suggest that development of mGlu5 PAMs without agonist activity may be required to limit toxicity. Additionally, it has been proposed that mGlu5 PAMs with lower levels of cooperativity may be preferred to limit neurotoxic side effects.

Beyond mGlu5, the group II mGlus are also expressed in regions potentially involved in schizophrenia and cognitive function, such as the prefrontal cortex and hippocampus (Wright, Johnson et al. 2013). Group II mGlu agonists (or their prodrugs) including LY379268, LY354740 and LY2140023, have shown to exhibit antipsychotic-like effects in both animal models and human subjects of schizophrenia in the positive and negative symptom domains of the disease (Cartmell, Monn et al. 1999, Patil, Zhang et al. 2007). Studies using knockout animals suggest that the therapeutic effect of group II mGlu agonists may rely on their activity at mGlu2 instead of mGlu3 (Spooren, Gasparini et al. 2000, Fell, Svensson et al. 2008), and selective mGlu2 PAMs have been shown to have robust antipsychotic-like efficacy in animal models (Galici, Echemendia et al. 2005, Galici, Jones et al. 2006, Benneyworth, Xiang et al. 2007). It has been recently discovered that mGlu2 can heterodimerize with the serotonin 5-HT2A receptor (Gonzalez-Maeso, Ang et al. 2008) and reduce serotonin-stimulated glutamate release at the thalamocortical synapse, a synapse that is implicated in schizophrenia (Marek, Wright et al. 2001, Benneyworth, Xiang et al. 2007), indicating that the interaction between mGlu2 and serotonin 5-HT2A receptor might play a role in mediating the antipsychotic effects of mGlu2 activation.

Besides schizophrenia, psychosis has also been associated with Alzheimer’s disease, the most common form of dementia that is characterized by deposition of toxic β-amyloid protein. Interestingly, activation of the group II mGlus using DCG IV triggers production and release of Alzheimer’s β-amyloid (1-42) from isolated intact nerve terminals (Kim, Fraser et al. 2010). In addition, LY566332, an mGlu2 PAM, amplifies Aβ-induced neurodegeneration, which can be blocked by the mGlu2/3 receptor antagonist LY341495 (Caraci, Molinato et al. 2011). Based on results of these preclinical studies, selective mGlu2 NAMs are now being pursued for cognition enhancement and antipsychotic activity in Alzheimer’s disease.
6.1.2 Fragile X syndrome—Fragile X Syndrome (FXS) is the most common monogenic form of autism (Garber, Visootsak et al. 2008). FXS is caused by the expansion of CGG repeats and subsequent methylation and silencing of the gene encoding fragile X mental retardation protein (FMRP) (Fu, Kuhl et al. 1991, Pieretti, Zhang et al. 1991). FMRP act as a translational repressor in neuronal dendrites that regulates translation of the proteins that promote long-term depression (LTD). Therefore, group I mGlu-dependent LTD is excessive in mouse models of FXS (Huber, Gallagher et al. 2002, Bear, Huber et al. 2004).

Specifically, mGlu5 has been suggested to play a role in the cognitive impairments observed in patients with FXS (Dolen, Osterweil et al. 2007) and antagonists of mGlu5 have been shown to have beneficial effects in FXS models. Specifically, acute treatment with the mGlu5 NAM CTEP corrects elevated hippocampal LTD, protein synthesis, and audiogenic seizures in mouse model (Michalon, Sidorov et al. 2012). In addition, chronic treatment using CTEP is able to rescue cognitive deficits in the Fmr1 knockout mouse, suggesting that pharmacological intervention could correct FXS symptoms even after the disease phenotype is established (Michalon, Sidorov et al. 2012). Several selective mGlu5 NAMs are now in clinical trials for FXS patients (www.ClinicalTrials.gov) and have shown promising therapeutic effects (Berry-Kravis, Hessl et al. 2009). Interestingly, in a recent clinical trial which evaluated the effect of mGlu5 NAM AFQ056 in male FXS patients, heterogeneity was discovered in terms of patient response to AFQ056 treatment: within 25 patients, 7 patients with full FMR1 promoter methylation and no detectable FMR1 messenger RNA exhibited significant improvement whereas no effect was detected in the remaining 18 patients who exhibited partial promoter methylation (Jacquemont, Curie et al. 2011). This study, if confirmed with a larger patient group, would suggest a beneficial effect of blocking the mGlu5 receptor in FXS patients with full promoter methylation and provide important criteria in patient selection.

6.1.3 Anxiety—In addition to potential efficacy in treating FXS, mGlu5 NAMs also represent potential therapeutics for anxiety disorders. As mentioned previously, mGlu5 activation potentiates NMDA receptor activity and an increase in NMDA receptor function has been associated with anxiety (Riaza Bermudo-Soriano, Perez-Rodriguez et al. 2012). Therefore, antagonists of mGlu5 may possess anxiolytic activity and have been tested in both preclinical and clinical studies. The early mGlu5 NAM MPEP exhibited anxiolytic efficacy in several rodent models of anxiety (Spooren, Vassout et al. 2000, Tatarczynska, Klodzinska et al. 2001). Another potent and selective mGlu5 NAM, fenobam, demonstrated anxiolytic efficacy in clinical trials (Pecknold, McClure et al. 1982, Porter, Jaeschke et al. 2005), providing further validation for targeting mGlu5 for anxiety disorders.

In addition to mGlu5 antagonism, activation of group II mGlus has potential as a novel approach for the treatment of anxiety disorders through modulation of glutamatergic transmission. Several orthosteric agonists, including LY354740 and LY404039, demonstrate anxiolytic efficacy in rodent models and/or human subjects (Schoepp, Wright et al. 2003, Rorick-Kehn, Johnson et al. 2007, Dunayevich, Erickson et al. 2008). While studies using mGlu5-selective compounds are quite limited, BINA and CBiPES, two selective mGlu2 PAMs, also exhibit anxiolytic effects in rodent behavioral models, such as stress-induced
hyperthermia and elevated plus maze tasks (Johnson, Barda et al. 2005, Galici, Jones et al. 2006).

### 6.1.4 Parkinson’s disease

Parkinson’s disease (PD) is a debilitating neurodegenerative disorder characterized by movement symptoms including tremor, rigidity, bradykinesia and postural instability, as well as disturbances in sleep, depression and cognitive impairments (Jankovic 2008, Johnson, Conn et al. 2009). The pathology of PD stems from severe degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc), a brain structure that plays important roles in the basal ganglia to control motor function (Surmeier and Sulzer 2013). Within the basal ganglia, dopamine released from SNc neurons delicately controls the balance of between the “direct pathway” and the “indirect pathway”, which oppose each other in controlling motor output in the basal ganglia. In PD patients, however, the loss of dopaminergic neurons leads to an overall increase of activity in the indirect pathway, which ultimately inhibits motor function in PD patients (reviewed in (Johnson, Conn et al. 2009)). Thus, according to this model, a rebalancing of the basal ganglia circuitry is predicted to alleviate disease symptoms.

The current gold standard treatment for PD is dopamine replacement therapy using L-DOPA, the precursor of dopamine. However, long-term treatment with L-DOPA results in “wearing-off” of efficacy and development of side effects, such as dyskinesias and psychiatric complications (Chen and Swope 2007). In addition, no treatment is available to delay the progression of the disease. The mGlu4 receptor is highly expressed presynaptically at the first synapse in the indirect pathway (the striatopallidal synapse) (Bradley, Standaert et al. 1999), thus providing an exciting alternative approach to rebalance the basal ganglia circuitry for PD treatment. Administration of the group III mGlu agonists L-AP4 or L-SOP, and recently the more mGlu4-selective agonists LSP1-2111 and LSP4-2022, has been shown to reduce GABAergic transmission at the striatopallidal synapse and demonstrate efficacy in several rodent PD models, including haloperidol-induced catalepsy and 6-OHDA-induced motor deficits (Wittmann, Marino et al. 2001, Matsui and Kita 2003, Valenti, Marino et al. 2003, MacInnes, Messenger et al. 2004, Macinnes and Duty 2008, Beurrier, Lopez et al. 2009, Goudet, Vilar et al. 2012). Recently, numerous highly selective mGlu4 PAMs have been developed from different chemical series and exhibit robust efficacy in preclinical rodent models. Administration of either PHCCC or VU0155041, two mGlu4 PAMs that bind to distinct binding sites on the receptor, reversed parkinsonian behavior in PD animal models, such as reserpine-induced akinesia as well as haloperidol-induced catalepsy (Marino, Williams et al. 2003, Niswender, Johnson et al. 2008). In another example, VU0364770, a systemically active mGlu4 PAM, produced a reversal of forelimb asymmetry induced by unilateral 6-hydroxydopamine (6-OHDA) lesion of the median forebrain bundle either alone or in combination with L-DOPA (Jones, Bubser et al. 2012). In contrast, Lu AF21934 alone exhibited no effect in the akinesia induced by unilateral 6-OHDA lesion of the SNc unless a sub-threshold dose of L-DOPA was co-administered. Similarly, ADX88178 alone had no impact on forelimb akinesia induced by a bilateral 6-OHDA lesion. However, coadministration of ADX88178 with a low dose of L-DOPA enabled a robust reversal of the forelimb akinesia deficit. The difference between PAMs in 6-OHDA lesion models could result from distinctions in the lesion protocols used by the different
research groups. It is also possible that the discrepancy is mediated by different pharmacological profiles of mGlu4 PAMs. Detailed studies of PAMs using the same lesion procedure will be required to elucidate the mechanism for these differences.

Besides a symptom-alleviating effect, mGlu4 PAMs also possess other potential benefits, such as potential disease-modifying efficacy and a lack of L-DOPA-mediated side effects, such as dyskinesia. PHCCC and VU0155041 has been shown to reduce dopaminergic cell death (Battaglia, Busceti et al. 2006, Betts, O’Neill et al. 2012), possibly due to reduced excessive excitatory drive onto dopamine neurons, and provide the potential to slow disease progression. In addition, LuAF21934, a selective mGlu4 PAM related to the VU0155041 series, has been shown to decrease the incidence of L-DOPA-induced dyskinesia (Bennouar, Uberti et al. 2013), further making mGlu4 an attractive target for PD treatment.

Despite the similar anti-parkinsonian effect observed with PHCCC and VU0155041, their differential responses at the corticostriatal synapses, presumably due to the presence of mGlu2/4 heteromers, are worth investigation with regard to additional benefits and adverse effects. Corticostriatal synapses have been shown to be overactive in dopamine-depleted animals (Picconi, Centonze et al. 2004, Centonze, Gubellini et al. 2005), contributing to the loss of spines in striatal medium spiny neurons in PD (Garcia, Neely et al. 2010). In addition, the deregulated plasticity (such as long-term depression and depotentiation) at corticostriatal synapses may underlie the mechanism of L-DOPA-induced dyskinesias (Picconi, Centonze et al. 2003, Picconi, Bagetta et al. 2011). Therefore, VU0155041-like mGlu4 PAMs that potentiate mGlu4-containing heteromers may potentially provide additional therapeutic effects, such as restoring morphology of striatal neurons and reversing L-DOPA-induced dyskinesias. In contrast, PHCCC-like PAMs with selectivity for homodimers might be preferable if potentiating mGlu2/4 signaling proves to engender side effects.

6.1.5 Other CNS disorders—Besides the disorders mentioned above, targeting mGlus also represents potential therapeutic approaches for other CNS diseases. For example, activation of mGlu4 inhibits the release of neuroinflammatory chemokines and increases the recovery from experimental autoimmune encephalomyelitis (Besong, Battaglia et al. 2002), a model for multiple sclerosis. This anti-inflammatory effect of mGlu4 has been postulated to be mediated by its activity in dendritic cells and T cells (Fallarino, Volpi et al. 2010). Furthermore, activation of mGlu2,3 or inhibition of mGlu5 has demonstrated efficacy in decreasing drug seeking behavior in rodents, providing therapeutic potential in drug abuse (Laruelle, Abi-Dargham et al. 1996, Bossert, Gray et al. 2006, Peters and Kalivas 2006, Liechti, Lhuillier et al. 2007, Lu, Uejima et al. 2007). Compounds targeting mGlus are also under preclinical and clinical development for depression, gastroesophageal reflux disorder and migraine, among others (reviewed in (Gregory, Noetzel et al. 2013)).

6.2 Peripheral diseases

6.2.1 Pain sensation—Besides their synaptic and extrasynaptic expression in the CNS, mGlus also have a widespread distribution in the periphery and non-neural tissues and regulate a variety of physiological and pathological processes (reviewed in (Jones and
Particularly, several mGlu subtypes are involved in the pain sensation process ((Varney and Gereau 2002, Parmentier-Batteur, Hutson et al. 2013)). mGlu5 is expressed on the peripheral terminals of sensory neurons and the selective mGlu5 NAM, MPEP, demonstrates an inhibitory effect on inflammatory hyperalgesia (Rook, Noetzel et al. 2013). The group I mGlus also play a role in the generation of mechanical hyperalgesia following peripheral nerve injury. In a rodent spinal nerve lesion model, the group I mGlu receptor antagonist DL-amino-3-phosphonopropionic acid significantly delayed the onset of pain-induced paw withdrawal threshold reduction (Paquet, Ribeiro et al. 2013).

The group II and group III mGlus, on the other hand, negatively modulate nociceptive behavior. In the same model mentioned above, Jang et al. demonstrated that intraplantar injection of the group II mGlu receptor agonist APDC also delayed the onset of mechanical hyperalgesia (Paquet, Ribeiro et al. 2013), suggesting that peripheral group II mGlu receptors inhibit the induction of neuropathic pain. In addition, the injection of either APDC (a group II mGlu agonist) or L-AP4 (a group III mGlu agonist) into the arthritic rodents negatively modulates nociceptive behavior during both the induction and maintenance phases of carrageenan-induced arthritic pain (Lee, Park et al. 2013).

6.2.2 Congenital stationary night blindness—The mGlu6 subtype is expressed exclusively in the ON-bipolar cells in the retina and several mutations of mGlu6 lead to congenital stationary night blindness, a disorder characterized by myopia and impairment of night vision (Mathiesen, Ulven et al. 2005, Zeitz, Forster et al. 2007). These mutations have been found to interfere with proper protein trafficking to the cell surface (Zeitz, Forster et al. 2007), or disturb Gα-mediated signaling downstream of mGlu6 activation (Mathiesen, Ulven et al. 2005), suggesting that Gα-coupling of mGlu6 is essential for its function in retinal ON bipolar cells.

7. Concluding Remarks

The important functions of mGlus are becoming increasingly appreciated with a focus in the CNS, but also in the peripheral organs and tissues. During the last decade, a number of selective ligands, both orthosteric and allosteric, have been discovered that have expanded our understanding of receptor function and can be applied to clinical studies. In addition, novel findings in receptor biology, such as the crystal structure of the mGlu 7TMD and the heterodimerization of receptors, opens new avenues for the development of mGlu-based therapeutics.

Acknowledgments

This work was supported by grant NS078262 and a Basic Research Grant from the International Rett Syndrome Foundation.

Glossary

<p>| 2R,4R-APDC | (2R,4R)-4-Aminopyrrolidine-2,4-dicarboxylate |
| 6-OHDA | 6-hydroxydopamine |</p>
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
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<tbody>
<tr>
<td>7TMR/GPCR</td>
<td>Seven Transmembrane Spanning/G Protein Coupled Receptor</td>
</tr>
<tr>
<td>ADX88178</td>
<td>5-methyl-N-(4-methylpyrimidin-2-yl)-4-(1H-pyrazol-4-yl)thiazol-2-amine</td>
</tr>
<tr>
<td>AFQ056</td>
<td>(3S,5S,7aR)-methyl 5-hydroxy-5-(m-tolylethyl)octahydro-1H-indole-1-carboxylate</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>AMN082</td>
<td>N1,N2-dibenzhydylethane-1,2-diamine</td>
</tr>
<tr>
<td>BINA</td>
<td>biphenyl-indanone A</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CBIPES</td>
<td>N-[4’-cyano-biphenyl-3-yl]-N-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride</td>
</tr>
<tr>
<td>CDPPB</td>
<td>3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide</td>
</tr>
<tr>
<td>CHPG</td>
<td>(RS)-2-chloro-5-hydroxyphenylglycine</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPCCOEt</td>
<td>7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester</td>
</tr>
<tr>
<td>CRD</td>
<td>cysteine-rich domain</td>
</tr>
<tr>
<td>CPPG</td>
<td>(RS)-α-Cyclopropyl-4-phosphonophenylglycine</td>
</tr>
<tr>
<td>CPPHA</td>
<td>N-[4-chloro-2-[[1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl]methyl][phenyl]-2-hydroxybenzamide</td>
</tr>
<tr>
<td>DCG-IV</td>
<td>(2S,1’R,2’R,3’R)-2-(2,3-dicarboxycyclopropyl)glycine</td>
</tr>
<tr>
<td>DCPG</td>
<td>(S)-3,4-dicarboxyphenylglycine</td>
</tr>
<tr>
<td>DFB</td>
<td>[(3-fluorophenyl)methylene]hydrazone-3-fluorobenzaldehyde</td>
</tr>
<tr>
<td>DHPG</td>
<td>(S)-3,5-dihydroxyphenylglycine</td>
</tr>
<tr>
<td>FITM EGLU</td>
<td>(2S)-α-Ethylglutamic acid</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>FITM</td>
<td>4-fluoro-N-[4-[6-(isopropylamino)pyrimidin-4-yl]thiazol-2-yl]-N-methylbenzamide</td>
</tr>
<tr>
<td>FMRP</td>
<td>fragile X mental retardation protein</td>
</tr>
<tr>
<td>FXS</td>
<td>Fragile X syndrome</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GIRK</td>
<td>G protein coupled inwardly rectifying potassium channel</td>
</tr>
<tr>
<td>JNJ16259685</td>
<td>(3,4-dihydro-2H-pyran-2,3[b quinolin-7-yl] (cis-4-methoxycyclohexyl) methanone</td>
</tr>
<tr>
<td>L-AP4</td>
<td>L-(+)2-Amino-4-phosphonobutyric acid</td>
</tr>
<tr>
<td>Code</td>
<td>Name</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>L-3,4-dihydroxyphenylalanine (levodopa)</td>
</tr>
<tr>
<td>L-SOP</td>
<td>L-serine-O-phosphate</td>
</tr>
<tr>
<td>LSP1-2111</td>
<td>(2S)-2-amino-4-[(hydroxy[hydroxy(4-hydroxy-3-methoxy-5-nitro-phenyl)methyl] phosphoryl]butanoic acid)</td>
</tr>
<tr>
<td>LSP4-2022</td>
<td>(2S)-2-amino-4-(((4-(carboxymethoxy)phenyl)(hydroxy)methyl)(hydroxy)phosphoryl)butanoic acid Lu AF21934 (1S,2S)-N1-(3,4-dichlorophenyl)cyclohexane-1,2-dicarboxamide</td>
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<tr>
<td>LY2140023</td>
<td>(1R,4S,5S,6S)-2-thiabicyclo[3.1.0]-hexane-4,6-dicarboxylic acid,4-[(2S)-2-amino-4-(methylthio)-1-oxobutyl]amino-,2,2-dioxide monohydrate</td>
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<td>LY341495</td>
<td>2S-2-amino-2-(1S,2S-2-carboxycyclopropyl-1-yl)-3-(xanth-9-yl)propanoic acid LY354740 2-amino-bicyclo[3.1.0]hexane 2,6-dicarboxylate</td>
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<tr>
<td>LY379268</td>
<td>(1R,4R,5S,6R)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid</td>
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<td>LY404039</td>
<td>(−)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid</td>
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<td>LY487379</td>
<td>2,2,2-trifluoro-N-[4-(2-methoxyphenoxy) phenyl]-N-(3-pyridinylmethyl)ethanesulfonamide</td>
</tr>
<tr>
<td>LY566332</td>
<td>N-4’-cyano-biphenyl-3-yl)-N-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen activated protein kinase</td>
</tr>
<tr>
<td>MCPG</td>
<td>(S)-a-methyl-4-carboxyphenylglycine</td>
</tr>
<tr>
<td>mGlu</td>
<td>metabotropic glutamate receptor</td>
</tr>
<tr>
<td>MMPIP</td>
<td>6-(4-methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo [4,5-c]pyridine-4(5H)-one hydrochloride</td>
</tr>
<tr>
<td>MPEP</td>
<td>2-methyl-6-(phenylethynyl)-pyridine</td>
</tr>
<tr>
<td>MTEP</td>
<td>3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine</td>
</tr>
<tr>
<td>mTOR</td>
<td>mammalian target of rapamycin</td>
</tr>
<tr>
<td>NAM</td>
<td>negative allosteric modulator</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PAM</td>
<td>positive allosteric modulator</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson's disease</td>
</tr>
<tr>
<td>PHCCC</td>
<td>N-phenyl-7-(hydroxylimino)cyclopropa[b]chromen-1a-carboxamide</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphatidylinositol 3-kinase</td>
</tr>
</tbody>
</table>
SAM  silent allosteric modulator
SIB-1757  6-methyl-2-(phenylazo)-3-pyridinol
SIB-1893  2-methyl-6-(2-phenylethenyl)pyridine
THIIC  N-(4-((2-(trifluoromethyl)-3-hydroxy-4-(isobutyryl)phenoxy)methyl)benzyl)-1-methyl-1H-imidazole-4-carboxamide
VFD  Venus flytrap domain
VU0155041  (±)-cis-2-(3,5-Dicholorphenylcarbamoyl)cyclohexanecarboxylic acid
VU0364770  N-(3-chlorophenyl)picolinamide
VU29  4-nitro-N-(1,3-diphenyl-1-H-pyrazol-5-yl)benzamide

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HIGHLIGHTS

- Detailed review of the biology of mGlu receptors
- Summary of the pharmacological tools and therapeutic merits of mGlu receptors
- Review of mGlu structural features and evidence for heteromeric conformations
Figure 1.
The group I, II and III mGlu receptors induce signal transduction through both G protein-dependent and independent pathways. mGlu$_{2/4}$ heterodimerization differentially regulates the efficacies of mGlu$_4$ PAMs binding to separate allosteric pockets, whereas the expression, signaling and pharmacology of other mGlu heteromers remain unexplored. GKAP, guanylate kinase-associated protein; PSD-95, postsynaptic density protein 95; NMDAR, N-methyl-D-aspartate receptor; PI3K, phosphatidylinositol 3-kinase; PIKE-L, phosphatidylinositol 3-kinase enhancer-long; AKT, protein kinase B; mTOR, mammalian target of rapamycin; ERK, extracellular signal-regulated kinase; ASK, apoptosis signal-regulating kinase; JNK, c-Jun N-terminal kinases; PIP2, Phosphatidylinositol 4,5-bisphosphate; DAG, diacyl-glycerol; IP3, inositol 1,4,5-trisphosphate; ER, endoplasmic reticulum; PKC, protein kinase C; AC, adenylate cyclase; PKA, protein kinase A.
Figure 2.
Mechanism of action of allosteric modulators. Instead of binding to the glutamate binding site on the N-terminal VFD, mGlu PAMs and NAMs bind to the 7TMD on the receptor and induce selective modulating effects, which can be blocked by SAMs. PAMs potentiate the response to orthosteric agonists by shifting the dose response curve of an orthosteric agonist to the left, whereas NAMs progressively antagonize the activity of agonists through negative cooperativity in a noncompetitive fashion.
Table 1

Classification, G protein coupling, splice variants and selective ligands for mGlu subtypes.

<table>
<thead>
<tr>
<th>Classification</th>
<th>G protein coupling</th>
<th>Receptor subtypes</th>
<th>Splice variants</th>
<th>Selective ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td>G&lt;sub&gt;q/11&lt;/sub&gt;</td>
<td>mGlu&lt;sub&gt;1&lt;/sub&gt;</td>
<td>mGlu&lt;sub&gt;1a-h&lt;/sub&gt;, mGlu&lt;sub&gt;1p20&lt;/sub&gt;, Taste mGlu&lt;sub&gt;1&lt;/sub&gt;</td>
<td>LY367385 (orthosteric agonist) Bay 36–7620 (NAM) Ro 67–4853 (PAM) VU71 (PAM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mGlu&lt;sub&gt;5&lt;/sub&gt;, mGlu&lt;sub&gt;5a,b&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td>G&lt;sub&gt;11&lt;/sub&gt;</td>
<td>mGlu&lt;sub&gt;2&lt;/sub&gt;</td>
<td>mGlu&lt;sub&gt;2&lt;/sub&gt;</td>
<td>LY487379 (PAM) BINA (PAM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GRM3&lt;sub&gt;Δ2&lt;/sub&gt; GRM3&lt;sub&gt;Δ2Δ3&lt;/sub&gt;</td>
<td>ML337 (NAM)</td>
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<tr>
<td><strong>Group III</strong></td>
<td>G&lt;sub&gt;11&lt;/sub&gt;</td>
<td>mGlu&lt;sub&gt;4&lt;/sub&gt;</td>
<td>mGlu&lt;sub&gt;4a,b&lt;/sub&gt;, Taste mGlu&lt;sub&gt;4&lt;/sub&gt;</td>
<td>LSP1-2111 (orthosteric agonist) LSP4-2022 (orthosteric agonist) PHCCC (PAM) VU0155041 (PAM) ADX88178 (PAM) Lu AF21934 (PAM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mGlu&lt;sub&gt;6&lt;/sub&gt;, mGlu&lt;sub&gt;6a,c&lt;/sub&gt;</td>
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