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Abstract—Metabotropic glutamate (mGlu) receptors respond to glutamate, the major excitatory neurotransmitter in the mammalian brain, mediating a modulatory role that is critical for higher-order brain functions such as learning and memory. Since the first mGlu receptor was cloned in 1992, eight subtypes have been identified along with many isoforms and splice variants. The mGlu receptors are transmembrane-spanning proteins belonging to the class C G protein–coupled receptor family and represent attractive targets for a multitude of central nervous system disorders. Concerned drug discovery efforts over the past three decades have yielded a wealth of pharmacological tools including subtype-selective agents that competitively block or mimic the actions of glutamate or act allosterically via distinct sites to enhance or inhibit receptor activity. Herein, we review the physiologic and pathophysiological roles for individual mGlu receptor subtypes including the pleiotropic nature of intracellular signal transduction arising from each. We provide a comprehensive analysis of the in vitro and in vivo pharmacological properties of prototypical and commercially available orthosteric agonists and antagonists as well as allosteric modulators, including ligands that have entered clinical trials. Finally, we highlight emerging areas of research that hold promise to facilitate rational design of selective mGlu receptor-targeting therapeutics in the future.

Significance Statement—The metabotropic glutamate receptors are attractive therapeutic targets for a range of psychiatric and neurological disorders. Over the past three decades, intense discovery efforts have yielded diverse pharmacological tools acting either competitively or allosterically, which have enabled dissection of fundamental biological processes modulated by metabotropic glutamate receptors and established proof of concept for many therapeutic indications. We review metabotropic glutamate receptor molecular pharmacology and highlight emerging areas that are offering new avenues to selectively modulate neurotransmission.

I. Introduction

Glutamate is the major excitatory neurotransmitter in the human brain mediating its effects via two distinct receptor classes. Ionotropic glutamate receptors are ligand-gated ion channels that rapidly cause membrane depolarization in response to glutamate. On the other hand, metabotropic glutamate (mGlu) receptors have a modulatory role exerted over a longer time scale including influencing neuronal excitability and synaptic plasticity as well as activity of nonneuronal cells.

The mGlu receptors are a family of eight class C G protein–coupled receptors (Acher et al., 2019; Alexander et al., 2019). They comprise a large extracellular N-terminal domain where glutamate binds, termed the Venus flytrap (VFT) domain, link to 7 transmembrane (7TM) alpha-helical segments and respond to glutamate, the major excitatory neurotransmitter, by metabotropic glutamate receptors and established proof of concept for many therapeutic indications. Metabotropic glutamate receptor variants. The mGlu receptors are transmembrane–coupled receptor family and represent attractive targets for a multitude of central nervous system disorders. Concerned drug discovery efforts over the past three decades have yielded a wealth of pharmacological tools acting either competitively or allosterically via distinct sites to enhance or inhibit receptor activity. Herein, we review the physiologic and pathophysiological roles for individual mGlu receptor subtypes including the pleiotropic nature of intracellular signal transduction arising from each. We provide a comprehensive analysis of the in vitro and in vivo pharmacological properties of prototypical and commercially available orthosteric agonists and antagonists as well as allosteric modulators, including ligands that have entered clinical trials. Finally, we highlight emerging areas of research that hold promise to facilitate rational design of selective mGlu receptor-targeting therapeutics in the future.

Glutamate is the major excitatory neurotransmitter in the human brain mediating its effects via two distinct receptor classes. Ionotropic glutamate receptors are ligand-gated ion channels that rapidly cause membrane depolarization in response to glutamate. On the other hand, metabotropic glutamate (mGlu) receptors have a modulatory role exerted over a longer time scale including influencing neuronal excitability and synaptic plasticity as well as activity of nonneuronal cells. The mGlu receptors are a family of eight class C G protein–coupled receptors (Acher et al., 2019; Alexander et al., 2019). They comprise a large extracellular N-terminal domain where glutamate binds, termed the Venus flytrap (VFT) domain, link to 7 transmembrane (7TM) alpha-helical segments.
domains via a cysteine-rich domain (CRD) (Fig. 1). The mGlu receptors are obligate dimers mediated by an interprotomer disulfide bond at the top of the VFT domains. Structural studies indicate that the bilobed VFT domains adopt a closed conformation upon agonist binding (Kunishima et al., 2000; Tsuchiya et al., 2002; Muto et al., 2007; Monn et al., 2015a,b; Koehl et al., 2019). The CRD transmits the active VFT conformation to the 7TM via interactions with the second extracellular loop of the 7TM (Koehl et al., 2019). When activated the 7TM domains come into closer proximity, with transmembrane domain 6 mediating dimerization between the 7TM domains of the two protomers (El Moustaine et al., 2012; Doumazane et al., 2013; Xue et al., 2015; Koehl et al., 2019; reviewed in Pin and Bettler, 2016). Ultimately, the active 7TM domains couple to intracellular transducers to elicit a cellular response.

The eight mGlu receptor subtypes are commonly divided into three groups based on sequence identity, G protein coupling preferences, and pharmacology. In addition to forming constitutive homodimers, heteromers have been observed among group I members and between group II and III subtypes (Doumazane et al., 2011). The various mGlu receptor subtypes are ubiquitously expressed throughout the brain in neurons and glia, with the exception of mGlu6 receptor, for which expression is restricted to the retina (reviewed in Ferraguti and Shigemoto, 2006). Peripheral mGlu receptors (reviewed in detail by Julio-Pieper et al., 2011) are found in tissues that receive glutamatergic innervation (e.g., heart, gastrointestinal tract, pain circuitry; Pereira and Goudet, 2019) but are also present in some nonexcitatory tissues and organs (e.g., immune cells, liver, kidney). Herein we provide a brief overview of the fundamental biology of the different mGlu subtypes and intracellular signaling, followed by an in-depth discussion of pharmacological agents and therapeutic indications with a focus on central nervous system (CNS) disorders.

II. Group I: Metabotropic Glutamate Receptors 1 and 5

A. Receptor Subtypes and Splice Variants

The group I mGlu receptors include mGlu1 and mGlu5. The mGlu1 receptor gene (GRM1) and its first three splice variants were cloned in rat in 1992 (Pin et al., 1992; Tanabe et al., 1992). In humans, there are seven mGlu1 splice variants (a, b, d, f, g, h) that differ in C terminus length (Sugiyama et al., 1987; Tanabe et al., 1992; Laurie et al., 1996; Makoff et al., 1997; Soloviev et al., 1999; DiRaddo et al., 2013) [Ensembl gene identifier: ENSG00000152822]. In addition, 12 single nucleotide polymorphisms within the GRM1 coding region have been identified in patients with schizophrenia (Frank et al., 2011; Ayoub et al., 2012), suggesting mGlu1 may be a viable therapeutic target for psychosis (Cho et al., 2014b). Spontaneous mutations in GRM1 are also associated with ataxia (Watson et al., 2017). The mGlu5 receptor is encoded by the GRM5 gene [ENSG00000168959], localized in human chromosome 11, and was first cloned in rat in 1992 (Abe et al., 1992) and in human in 1994 (Minakami et al., 1994). Alternative splicing of GRM5 in humans gives rise to two major isoforms that also differ in C-terminal length; the longer of the two, human mGlu5a (equivalent to rat mGlu5b) has a 32-amino acid insertion after residue 876 but is otherwise identical to human mGlu5b (equivalent to rat mGlu5a) (Minakami et al., 1993, 1995). Variations in C-terminal length due to alternative splicing of group I receptors influences surface expression, subcellular localization, dimerization, interactions with intracellular proteins, and ultimately cellular responses (Joly et al., 1995; Mion et al., 2001; Francesconi and Duvoisin, 2002; Kumpost et al., 2008; Tateyama and Kubo, 2008; Francesconi et al., 2009a; Techlovská et al., 2014).
B. Localization and Signal Transduction

The group I mGlu receptors are predominantly found in postsynaptic neurons within the CNS (Fig. 2), increasing neuronal excitability and membrane depolarization when activated. In certain circuits, group I mGlu receptors can be found on presynaptic terminals, acting as autoreceptors to modulate neurotransmitter release (reviewed in Pittaluga, 2016). Furthermore, group I mGlu receptors are also expressed in glial cells (reviewed in Spampinato et al., 2018). The cellular responses resulting from group I mGlu receptor activation are highly complex and context dependent.

Group I mGlu receptors preferentially couple to the Gq/11 family of G proteins, which activate phospholipase C (PLC) β, which hydrolyses phosphatidylinositol 4,5-bisphosphate in the membrane to yield the second messengers: diacylglycerol and inositol 1,4,5-trisphosphate, mobilizing intracellular Ca2+ (iCa2+) stores (Sugiyama et al., 1987) (Fig. 3). Potentiation of mGlu5 receptors increases phosphoinositide hydrolysis in vivo in different mouse brain areas, such as prefrontal cortex, cerebellum, hypothalamus, hippocampus, and striatum (Zuena et al., 2018). Interestingly, endogenous activation of mGlu5 receptors largely accounts for basal phosphoinositide hydrolysis particularly in the prefrontal cortex. Downstream of these second messengers, activation of protein kinase C (PKC) and calcmodulin triggers signaling cascades that ultimately phosphorylate and activate extracellular signal–regulated kinases 1 and 2 (ERK1/2), which regulate gene transcription associated with synaptic plasticity (long-term depression or long-term potentiation) (Servitja et al., 1999, 2003; Kanumilli et al., 2002; Page et al., 2006; Jin et al., 2013a; Hong et al., 2016). Diacylglycerol can be further broken down by diacylglycerol lipase to yield endogenous cannabinoid, 2-arachidonoylglycerol (Jung et al., 2005; Gregg et al., 2012). Beyond Gαq/11-mediated signaling, βγ subunits can enhance or inhibit Ca2+ and K+ channels, including ionotropic glutamate receptors, to modulate neuronal excitability and membrane potential (reviewed in Valenti et al., 2002), through physical interactions mediated by scaffolding proteins (Tu et al., 1999) or indirect mechanisms driven by intracellular effectors (Fig. 3). The βγ subunits can also activate phosphoinositide-3-kinase (PI3K), which in turn activates RAC-alpha serine/threonine-protein kinase (Akt)–dependent signaling cascades implicated in protein synthesis–dependent long-term depression and cell survival (Hou and Klann, 2004; Page et al., 2006; Hullinger et al., 2015; Zhu et al., 2018). In recombinant systems mGlu1 and mGlu5 receptors also couple to Gαs, stimulating adenylyl cyclases (ACs) and increasing cAMP production (Aramori and Nakanishi, 1992; Joly et al., 1995; Francesconi and Duvoisin, 1998, 2000; Nasrallah et al., 2018). In addition, group I receptors signal via G protein–independent mechanisms (e.g., Homer, src kinases, arrestins, transactivation of tyrosine kinases) to activate different kinase cascades that contribute to synaptic plasticity (Iacovelli et al., 2003; Yang et al., 2006; Emery et al., 2010; Kubota et al., 2014; Eng et al., 2016). Downstream of group I receptor activation diverse transcription factors are activated, including cAMP response element-binding protein (Mao and Wang, 2003b), Elk-1 (Mao and Wang, 2003a; Kong et al., 2005, 2009), c-Jun (Jong et al., 2009), and serum response factor (Kumar et al., 2012).

The mechanisms that regulate group I mGlu receptor activity are equally complex (Fig. 3). The C termini of group I receptors contain binding sites for diverse scaffolding proteins that regulate receptor localization and recycling in addition to directly linking group I mGlu receptors to other receptors and channels within the postsynaptic density (Roche et al., 1999; Kitano et al., 2002; Lee et al., 2008; Wang et al., 2009; Hu et al., 2012; Wagner et al., 2015;
Eng et al., 2016; Gulia et al., 2017). Second messenger-activated kinases also provide negative feedback regulating cellular responses (iCa\(^{2+}\) oscillations or receptor desensitization) through phosphorylation of intracellular loops and/or the C terminus (Kawabata et al., 1996; Gereau and Heinemann, 1998; Bhattacharya et al., 2004; Mundell et al., 2004; Bradley and Challiss, 2011; Ko et al., 2012; Jin et al., 2013a,b, 2018; Raka et al., 2015; Uematsu et al., 2015; Vergouts et al., 2017; Yang et al., 2017; Marks et al., 2018). However, not all functional responses are equally influenced. For example, PKC phosphorylation of mGlu1 receptors desensitizes signaling to accumulation of inositol phosphate but not cAMP (Francesconi and Duvoisin, 2000). Select kinases bind the C terminus and/or phosphorylate the receptor altering effector coupling, ability to bind other proteins, or receptor endocytosis (Dale et al., 2000; Sallese et al., 2000; Iacovelli et al., 2003; Pula et al., 2004; Nicodemo et al., 2010; Jin et al., 2017). By example, Presol enhances cyclin-dependent kinase 5 and ERK1/2 phosphorylation of the Homer binding site within the C terminus (Hu et al., 2012), whereas calmodulin and E3 ubiquitin-protein ligase SIAH-1a recognize overlapping sites in the C tail (Ishikawa et al., 1999), with PKC phosphorylation of this site enhancing SIAH-1a but inhibiting calmodulin binding (Ko et al., 2012). Second messenger-dependent kinases are critical for group I mGlu receptor–dependent long-term depression and potentiation by modulating the activity or promoting endocytosis of ionotropic glutamate receptors including N-methyl-D-aspartate (NMDA) and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subtypes (Jia et al., 1998; Snyder et al., 2001; Benquet et al., 2002; Moult et al., 2006; Jin et al., 2013b, 2015; Xu et al., 2013), although PKC-independent mechanisms for NMDA receptor potentiation by group I mGlu receptors have also been reported (Harvey and Collingridge, 1993; Kinney and Slater, 1993; Rahman and Neuman, 1996). In a reciprocal fashion, NMDA receptor-mediated

**Fig. 3.** Signal transduction and regulation of group I mGlu receptors. Overview of group I mGlu receptor scaffolding partners, transducers, downstream effectors, and regulatory proteins; refer to main text for associated primary references. Blue bolded text indicates physiologic consequences linked to specific intracellular responses. The following abbreviations are used: AC, adenylyl cyclase; 2-AG, 2-arachidonoylglycerol; CaMK, Ca\(^{2+}\)/calmodulin-dependent protein kinase; CREB, cAMP response element-binding protein; DAG, diacylglycerol; DGL, diacylglycerol lipase; EGFR, epidermal growth factor receptor; GKAP, guanylate kinase-associated protein; GRK, G protein–coupled receptor kinase; IP\(_3\), inositol 1,4,5-trisphosphate; JNK, c-Jun N-terminal kinase; MEK1/2, mitogen-activated protein kinase kinases 1 and 2; mTOR, mammalian target of rapamycin; NHERF1/2, Na\(^+\)/H\(^+\) exchange regulatory cofactors 1 and 2; PKA, protein kinase A; PLD, phospholipase D; PSD-95, postsynaptic density protein 95 (also known as SAP-90 for synapse-associated protein 90); PP2B, protein phosphatase 2B; PP2A/CaN, protein phosphatase 2A/calcineurin; SGK1, ribosomal protein S6 kinase \(\beta\)-1; SHANK, SH3 and multiple ankyrin repeat domains protein; SIAH-1a, E3 ubiquitin-protein ligase SIAH1A; SRF, serum response factor.
stimulation of protein phosphatase 2A/calcineurin acts to regulate recycling of group I mGlu receptors (Alagarsamy et al., 2005; Pandey et al., 2014), with protein phosphatase 2B playing a similar role (Mahato et al., 2015). It is clear that activation of group I mGlu receptors can trigger a complex network of intracellular effectors that encode the cellular responses that give rise to complex physiologic effects from synaptic plasticity to effector localization and signal transduction. When coexpressed within the same cell population, activation of mGlu1 versus mGlu5 receptors can lead to distinct cellular outcomes (Valenti et al., 2002). Moreover, postsynaptic group I mGlu receptor activation can result in modulation of neurotransmitter release from presynaptic cells via retrograde signaling, for example, endocannabinoids or nitric oxide (Fig. 3) (Maejima et al., 2001; Robbe et al., 2002; Sergeeva et al., 2007; Gregg et al., 2012; Aubrey et al., 2017; Xiang et al., 2019). As a further layer of complexity, both group I mGlu receptors form heteromers and/or larger-order oligomers with other G protein–coupled receptors (GPCRs) (discussed in further detail later), which alters intracellular signaling profiles.

C. Pathophysiology and Therapeutic Potential

A number of reviews provide in depth coverage of the distribution, physiology, and pathophysiological roles of group I mGlu receptors (Ferraguti and Shigemoto, 2006; Niswender and Conn, 2010; Golubeva et al., 2016; Crupi et al., 2019; Pereira and Goudet, 2019). Inhibitors and activators of the individual subtypes are being pursued for a myriad of different psychiatric and neurologic disorders. Briefly, and of relevance to the pharmacological agents reviewed in depth below, distribution of mGlu1 receptors in regions associated with pain perception as well as mGlu1 knockout animal phenotypes suggests mGlu1 inhibitors are potential therapeutic agents for neuropathic pain (Neugebauer, 2002; Schkeryantz et al., 2007). Preclinical studies also indicate mGlu1 receptor inhibitors may have therapeutic benefit in treating seizures, addiction, anxiety, and certain cancers (Namkoong et al., 2006; Dravolina et al., 2017). mGlu5−/− mice have deficits in prepulse inhibition (Brody et al., 2004), impaired learning and memory (Xu et al., 2009; Zeleznikow-Johnston et al., 2018), and reduced propensity for addiction/abuse (Chiamulera et al., 2001) and reverse the majority of fragile X syndrome phenotypes in preclinical models (Dölen and Bear, 2008). As such, selective mGlu5 receptor inhibitors are desired in the setting of depression (Chaki and Fukumoto, 2018) and anxiety (Ferraguti, 2018) as well as neurodevelopmental disorders such as fragile X syndrome (Yamasue et al., 2019). In this respect multiple mGlu5 receptor inhibitors have entered phase 2 clinical trials as discussed in detail later; however, to date none have reached market. Potentiation or activation of both group I receptors offers the promise for treating the positive and cognitive symptoms associated with schizophrenia (Walker and Conn, 2015; Nicoletti et al., 2019).

Beyond neurologic and psychiatric disorders, inhibition of mGlu1 receptors is neuroprotective (in vitro and in vivo) after oxygen-glucose deprivation or ischemic insult (Henrich-Noack et al., 1998; Pellegrini-Giampietro et al., 1999) and may therefore offer a novel intervention for stroke. For multiple preclinical models of neurodegenerative diseases, genetic ablation or pharmacological inhibition of mGlu5 receptors is neuroprotective and treats associated symptoms, for example, acting procognitively in Alzheimer’s disease or Huntington’s disease (reviewed in Ribeiro et al., 2017) or improving motor deficits in amyotrophic lateral sclerosis (reviewed in Battaglia and Bruno, 2018) or Parkinson’s disease (Mazur, 1995; Battaglia et al., 2004; Armentero et al., 2006; Ambrosi et al., 2010; Black et al., 2010; Masilamoni et al., 2011; Fuzzati-Armentero et al., 2015), although mGlu5 receptor activators/potentiators may also treat cognitive symptoms associated with Huntington’s disease (Doria et al., 2013, 2015, 2018). Inhibition of mGlu5 receptors is also indicated for treating neurodegeneration associated with drugs of abuse (Battaglia et al., 2002).

III. Group II: Metabotropic Glutamate Receptors 2 and 3

A. Receptor Subtypes and Splice Variants

The group II members, mGlu2 and mGlu3 receptors, were first cloned in rat in 1992 (Tanabe et al., 1992) and a few years later in human (Flor et al., 1995a; Emile et al., 1996). Encoded by the GRM2 gene [ENSG00000164802] and localized in human chromosome 3 and rat chromosome 8, no splice variants have been described for mGlu2 receptor subtype (Sartorius et al., 2006). In human, the GRM3 gene [ENSG00000198822] encodes the mGlu3 receptor, for which three splice variants are known (Sartorius et al., 2006). The most abundant GRM3 variant lacks exon 4 (GRM3Delta4), encoding a truncated membrane-associated protein that retains the extracellular VFT but lacks the 7TM, which is replaced with a unique 96–amino acid C terminus. mGlu3Delta4 can bind orthosteric ligands and interact with the full-length protein and may thus have a dominant negative effect (Garciabea et al., 2017). Spontaneous mutations in mGlu3 are associated with melanoma (Prickett et al., 2011; Neto and Ceol, 2018), whereas single nucleotide polymorphisms in GRM3 are linked to cognitive performance in individuals with schizophrenia and are postulated to influence pharmacotherapy (reviewed in Maj et al., 2016 and Saini et al., 2017).

B. Localization and Signal Transduction

The group II mGlu receptors are located both pre- and postsynaptically, with mGlu3 receptors also found in...
glial cells throughout the brain (Fotuhi et al., 1994; Testa et al., 1994) (Fig. 2). Using a radiolabeled orthosteric agonist selective for group II receptors, (1R,2S,4R,5R,6R)-2-amino-4-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, in conjunction with knock-out mice for either subtype, mGlu3 receptor levels were found to be generally higher than mGlu2 receptors in forebrain regions but similar within the striatum (Wright et al., 2013). In the thalamus and hippocampus, the two subtypes exhibit distinct and, for some nuclei/circuits, segregated expression patterns (Wright et al., 2013). Dissecting the relative contribution of mGlu2 versus mGlu3 receptor subtypes has presented a major challenge due to the lack of subtype-selective pharmacological tools (discussed in further detail below).

Group II mGlu receptors preferentially couple to G_{i/o} proteins, inhibiting adenylyl cyclase and cAMP production as well as inhibiting guanylate cyclase and cGMP production (Wroblewska et al., 2006) (Fig. 4). On presynaptic terminals, \( \beta \gamma \) subunits modulate ion channel function, inhibiting N-type Ca\(^{2+}\) channels (McCool et al., 1996) and activating G protein–coupled inwardly rectifying potassium channel (GIRK) channels (Knoflach and Kemp, 1998; Sharon et al., 1997), thereby decreasing exocytosis of vesicles containing glutamate (Macek et al., 1998; Flavin et al., 2000; Olivero et al., 2017), GABA (Hayashi et al., 1993; Gereau and Conn, 1995; Salt and Eaton, 1995; Schaffhauser et al., 1998) and dopamine (Feenstra et al., 2003). Interactions between group II mGlu receptor complexes, Akt/glycogen synthase kinase (GSK) 3\( \beta \) (Tyszkiewicz et al., 2004; Xi et al., 2011; Cheng et al., 2013). Furthermore, mGlu3 receptor-dependent long-term depression (LTD) in the prefrontal cortex is mediated by functional cross-talk between mGlu3 and mGlu4 receptors on postsynaptic neurons (Fig. 2). Activation of mGlu3 receptors releases \( \beta \gamma \) subunits to enhance mGlu3 receptor coupling to G_{i/o} (Di Menna et al., 2018). Cross-talk between mGlu3 and mGlu4 receptors is postulated to account for the reported dependence on PLC and PKC for mGlu3 receptor-dependent LTD (Otani et al., 2002; Huang et al., 2007). Recently, however, mGlu4 receptor-dependent LTD was shown to be modulated by mGlu5 receptor interactions with homer and signaling via PI3K, Akt, and GSK3\( \beta \) to result in AMPA receptor internalization, a mechanism disrupted by acute stress (Joffe et al., 2019). Indeed, the cellular context is an important contributor to shaping the cellular responses instigated by group II receptor activation. For example, in astrocytes, mGlu3 receptor activation results in both decreases and increases in cAMP, whereas in neurons the effect on cAMP levels is purely inhibitory (Moldrich et al., 2002). Astrocytic mGlu3-mediated increases in cAMP levels are dependent on iCa\(^{2+}\) levels (mobilization of stores and extracellular influx) and local release of adenosine, which acts at colocated G_s-coupled adenosine A_2A receptors (Moldrich et al., 2002).

With respect to regulation of group II mGlu receptor activity, the cellular context is also a major contributor. Phosphorylation of intracellular loops and/or the C-terminal tail by protein kinase A, PKC, and G protein–coupled receptor kinases have a central role in receptor regulation (Kamiya and Yamamoto, 1997; Macek et al., 1998; Schaffhauser et al., 2000; Cai et al., 2001), uncoupling receptors from G proteins, and promoting interactions with scaffolding proteins such as arrestins, which mediate receptor endocytosis (Iacovelli et al., 2009). The C-terminal tail of group II mGlu receptors binds to multiple scaffolding proteins including protein interacting with C kinase, glutamate receptor-interacting protein, tamalin, Na\(^+/\)H\(^+\) exchange regulatory cofactors 1 and 2, and Ran-binding protein microtubule-organizing center (Hirbec et al., 2002; Kitano et al., 2002; Seebahn et al., 2008; Ritter-Makinson et al., 2017), as well as protein phosphatase 2C, which dephosphorylates mGlu3 receptors (Flajolet et al., 2003). Interactions between group II mGlu receptors with diverse intracellular scaffolding proteins regulates receptor localization and functional responses, which likely governs differences observed between cell types or for neurons from different brain regions. Interestingly, mGlu2, but not mGlu3, receptors are reportedly resistant to homologous desensitization by G protein–coupled receptor kinases with respect to cAMP signaling (Iacovelli et al., 2009), although heterologous mechanisms, for example, due to PKC activation by colocated adenosine A_2 receptors, affect both subtypes (Macek et al., 1998; Lennon et al., 2010). Functional cross-talk between mGlu2 receptors and colocated serotonin receptors 2A (5-HT2A) can also modulate cellular responses to activation of either receptor (Marek et al., 2000; Molinaro et al., 2009; Murat et al., 2019). The interplay of intracellular effectors stimulated by group II mGlu receptors, coupled with regulatory proteins as well as coexpression of...
other cell surface receptors, gives rise to cell type-specific roles for group II mGlu receptors.

C. Pathophysiology and Therapeutic Potential

The expression patterns of group II mGlu receptors (reviewed in Ferraguti and Shigemoto, 2006) coupled with phenotypes of knockout animals suggest that group II receptors are attractive therapeutic targets for psychosis, cognition, anxiety, pain, and addiction (Cross et al., 2018; Mazzitelli et al., 2018; Pereira and Goudet, 2019), although for many indications establishing whether mGlu2 or mGlu3 receptors are the best target in preclinical models of disease has been challenging due to a lack of subtype-selective agents. Despite this shortcoming, both agonists and potentiators of group II receptors have been actively pursued, with multiple agents entering phase 2 or 3 for treating schizophrenia and addiction disorders (Nicoletti et al., 2019). Conversely, group II receptors inhibitors are promising interventions for depression and anxiety and as neuroprotective agents in the setting of ischemia (Celanire et al., 2015; Motolese et al., 2015). However, activation of group II receptors is neuroprotective after excitotoxic insults (Battaglia et al., 2003). In particular, activation of astrocytic mGlu3 receptors confers neuroprotection to various insults (in vitro and in vivo) via paracrine mechanisms (Bruno et al., 1997; Corti et al., 2007; Cippitelli et al., 2010; Caraci et al., 2011), offering a potential therapeutic target for neurodegenerative diseases and psychiatric conditions associated with neuronal death.

IV. Group III: Metabotropic Glutamate Receptors 4, 6, 7, and 8

A. Receptor Subtypes and Splice Variants

Before molecular cloning, group III mGlu receptors were termed L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) receptors due to high sensitivity to this ligand, which depresses synaptic transmission in the brain and in retina (Thomsen, 1997). In the 1990s, molecular cloning revealed that four different receptor subtypes mediated the biologic effects of L-AP4: mGlu4, mGlu6, mGlu7, and mGlu8 receptors. The mGlu4 receptor is...
encoded by the GRM4 gene [ENSG00000124493], which is localized in human chromosome 6, rat chromosome 20, and mouse chromosome 17 (Tanabe et al., 1992; Flor et al., 1995b). Two main splice variants were predicted, termed mGlu$_{4a}$ and mGlu$_{4b}$ (Thomsen et al., 1997), but further studies failed to detect mGlu$_{4b}$ in different rat brain areas, and no splice site consensus sequences that could support its existence were found in human genomic sequence containing the whole GRM4 gene, suggesting mGlu$_{4a}$ corresponds to a recombination artifact (Corti et al., 2002). Another variant lacking the first 128 base pairs, termed taste mGlu$_{4}$, is found in rat taste buds. The corresponding protein is predicted to lack approximately half the extracellular domain, including a large portion of the glutamate-binding domain (Chaudhri et al., 2000). First cloned in 1993, the mGlu$_{6}$ receptor is encoded by GRM6 gene [ENSG00000113262] localized in human chromosome 5 (Nakajima et al., 1993). Two splice variants, termed mGlu$_{6a}$ and mGlu$_{6c}$, are found in both human and rats, which correspond to truncated mGlu$_{6}$ receptors lacking the transmembrane domain and intracellular portions of the receptor (Valerio et al., 2001a,b). The mGlu$_{6a}$ receptor is encoded by the GRM7 gene, localized in rat chromosome 4 and in human chromosome 3 (Okamoto et al., 1994; Saugstad et al., 1994; Makoff et al., 1996). Two main variants, mGlu$_{7a}$ and mGlu$_{7b}$, in rats and humans have different C-terminal tails. The last 16 residues of mGlu$_{7a}$ are substituted by 23 different residues in mGlu$_{7b}$ due to the insertion of an out-of-frame 92–base pair exon (Flor et al., 1997; Corti et al., 1998). Three other isoforms have been described, named v3, v4, and v5 (Schulz et al., 2002). Although mGlu$_{7a}$ and mGlu$_{7b}$ receptor variants are primarily expressed in the CNS, the expression of v3 and v4 isoforms appears to be restricted in nonneuronal tissues (Schulz et al., 2002). The mGlu$_{8}$ receptor, encoded by the GRM8 gene [ENSG00000179603] found in human chromosome 7, was originally cloned in mouse in 1995 (Duvoisin et al., 1995) followed by human (Scherer et al., 1997; Wu et al., 1998) and rat (Saugstad et al., 1997). Three splice variants have been described: mGlu$_{8a}$, mGlu$_{8b}$, and mGlu$_{8c}$ (Corti et al., 1998; Malherbe et al., 1999). The last 16 residues of the C-terminal tails of the mGlu$_{8a}$ and mGlu$_{8c}$ receptors are different, whereas the mGlu$_{8b}$ variant is a truncated receptor lacking the transmembrane domains and intracellular C tail. For each of the group III mGlu receptors the different splice variants show distinct tissue distribution and/or changes in intracellular portions of the receptor, which have the potential to alter signal transduction pathways triggered in response to receptor activation.

B. Localization and Signal Transduction

Most group III mGlu receptors are widely expressed throughout the CNS, with the exception of mGlu$_{4}$, which is mostly restricted to the retina (reviewed in Ferraguti and Shigemoto, 2006). Group III mGlu receptors are also expressed in glial cell types in the brain, with the exception of mGlu$_{7}$ (reviewed in Spampinato et al., 2018). Outside the CNS, group III mGlu receptor expression has been reported in kidney, pancreas, liver, cells from the immune system, and bones, for example (see Julio-Pieper et al., 2011, for review).

In the CNS, mGlu$_{4}$, mGlu$_{7}$, and mGlu$_{8}$ receptors are mainly expressed in the active zone of presynaptic glutamatergic and GABAergic neurons (Kinosita et al., 1996a; Shigemoto et al., 1997; Wada et al., 1998; Corti et al., 2002; Ferraguti et al., 2005; Ferraguti and Shigemoto, 2006) (Fig. 2). Group III receptors can act as autoreceptors reducing glutamate release in the synaptic cleft and as heteroreceptors reducing the release of GABA (Schoepp, 2001). mGlu$_{4}$, mGlu$_{7}$, and mGlu$_{8}$ receptors are preferentially coupled to heterotrimeric $G_{i/o}$ proteins, leading to the inhibition adenyl cyclase production of cAMP via the $G_{o}$ subunit (Fig. 5). On presynaptic terminals, mGlu$_{4}$, mGlu$_{7}$, or mGlu$_{8}$ receptors are well documented to inhibit neurotransmitter release through a direct $\beta\gamma$ subunit–mediated inhibition of N or P/Q type of voltage-gated Ca$_{2+}$ channels (Anwyl, 1999; Perroy et al., 2000; Millán et al., 2002a,b; Capogna, 2004; Martín et al., 2007). In addition, mGlu$_{4}$ and mGlu$_{7}$ receptors also inhibit voltage-gated Ca$_{2+}$ channels via a PKC-dependent mechanism (Perroy et al., 2000; Abitbol et al., 2012), involving interactions between calmodulin and the scaffolding protein, protein interacting with C kinase, mechanism (Perroy et al., 2000; Abitbol et al., 2012), involving interactions between calmodulin and the scaffolding protein, protein interacting with C kinase, for mGlu$_{7}$ receptors (Perroy et al., 2002; Suh et al., 2013). Activation of mGlu$_{4}$, mGlu$_{7}$, or mGlu$_{8}$ receptors can also decrease neuronal excitability by the released $\beta\gamma$ subunits acting on GIRK channels (Dutar et al., 1999; Saugstad et al., 1996). Other studies suggest that group III mGlu receptors may activate background K$^{+}$ channels such as TREK1 and TREK2 (potassium channel subfamily K member 2 and 10), thereby further decreasing neuronal activity (Lesage et al., 2000; Cain et al., 2008). Group III mGlu receptors are also proposed to inhibit neurotransmitter vesicle exocytosis through direct interactions with the release machinery (Chavis et al., 1998; Erdmann et al., 2012). Coupling of group III receptors to PI3K, probably through G$\beta\gamma$ subunits, and mitogen-activated protein kinase is implicated in neuroprotection mechanisms (Lacovelli et al., 2002).

Among group III mGlu receptors, the mGlu$_{4}$ receptor is distinct as its expression is mostly restricted to postsynaptic bipolar ON neurons in the retina (Nomura et al., 1994; Vardi et al., 2000), with no strong expression detected in the brain (Nakajima et al., 1993). The mGlu$_{5}$ receptor is critical for glutamate-induced signaling in ON-bipolar cells in the retina during darkness (Nomura et al., 1994; Vardi et al., 2000). Glutamate, released from rod photoreceptors in the dark, activates postsynaptic mGlu$_{6}$ receptors in bipolar ON cells that lead to the closure of a nonselective ion channel, TRPM1-L.
[a long form transcript of transient receptor potential cation channel subfamily M member 1 (TRPM1) expressed solely in the dendritic tip of bipolar ON neurons] (Koike et al., 2010). TRPM1 inhibition results in hyperpolarization of bipolar ON neurons, thus inhibiting the ON pathway into darkness. The signaling cascade involves $G_{o}$ (Koike et al., 2010), $G_{bg}$ (Shen et al., 2012), and other proteins such as the orphan GPCR GPR179 or the interacting protein Nyctalopin (Zeitz et al., 2015) (Fig. 5). Collectively, the intracellular signal transduction and regulatory pathways engaged by group III receptor subtypes have not been as well elucidated as group I and group II counterparts.

C. Pathophysiology and Therapeutic Potential

The phenotypes of mice lacking the group III mGlu receptors have revealed physiologic roles and potential as therapeutic targets in several neurologic disorders. Mice lacking the mGlu₄ receptor present deficits in motor performance, spatial memory, and learning of complex motor tasks (Pekhletski et al., 1996; Gerlai et al., 1998), in accordance with its particularly high expression in the cerebellum (Kinoshita et al., 1996b; Corti et al., 2002). The mGlu₄⁻/⁻ mice also have enhanced seizure-associated vulnerability (Pitsch et al., 2007) and lack the motor stimulant effect of ethanol (Blednov et al., 2004). The sensitivity to strong noxious stimuli of mGlu₄⁻/⁻ mice is altered, and nociceptive behavior in the inflammatory phase of the formalin test is accelerated (Vilar et al., 2013). Knockout phenotypes together with preclinical studies highlight mGlu₄ receptor as a potential therapeutic target in anxiety and depression (Kalinichev et al., 2014), schizophrenia (Wieronska et al., 2012a), epilepsy (Pitsch et al., 2007; Ngomba et al., 2008), neuroinflammation (Fallarino et al., 2010), autism spectrum disorder (Becker et al., 2014), and chronic pain (reviewed in Pereira and Goudet, 2019). In particular, targeting mGlu₄ receptor for the treatment of Parkinson’s disease (PD) has attracted much attention (Célanire and Campo, 2012; Amalric et al., 2013; Charvin, 2018; Volpi et al., 2018). Preclinical studies showed that mGlu₄ receptor activation corrects the imbalance of neurotransmission among the basal ganglia circuitry that is associated with PD (Charvin et al., 2018b), as shown primarily with mGlu₄ potentiation (Marino et al., 2003b) or later with selective agonists (Marino et al., 2003b; Beurrier et al., 2009). Activation or potentiation of mGlu₄ receptors also has neuroprotective effects (Copani et al., 1995; Battaglia et al., 2006). Unfortunately, despite promising preclinical results (Charvin et al., 2017, 2018a), the mGlu₄ receptor potentiator, foliglurax, recently failed to show sufficient efficacy in a phase II clinical trial for PD.

Comparing the phenotypes of mGlu₄, mGlu₇, and mGlu₈ knockout mice indicates the mGlu₄ receptor is most clearly involved in startle and motivational processes, whereas mGlu₇ receptor is involved in hippocampus-dependent spatial learning and fear-related behaviors, and mGlu₈ receptor deletion yields more subtle behavioral changes and influences body weight (Goddyn et al., 2015). The role of mGlu₇ receptors in learning and memory is confirmed by behavioral pharmacology studies (Hikichi et al., 2010a; Klakotskaia et al., 2013). Also, mGlu₇⁻/⁻ mice and mice lacking functional mGlu₇ receptors present an increased susceptibility to seizures

Fig. 5. Signal transduction and regulation of group III mGlu receptors. Overview of group III mGlu receptor scaffolding partners, transducers, downstream effectors, and regulatory proteins; refer to main text for associated primary references. Blue bolded text indicates physiologic consequences linked to specific intracellular responses. The following abbreviations are used: AC, adenylyl cyclase; DAG, diacylglycerol; IP₃, inositol 1,4,5-trisphosphate; PICK1, protein interacting with C kinase; PKA, protein kinase A.
(Sansig et al., 2001; Bertaso et al., 2008). Accordingly, absence seizures can be induced by the pharmacological blockade of the mGlu7 receptor (Tassin et al., 2016), whereas mGlu7 receptor activation protects against epileptogenesis and epileptic seizures (Girard et al., 2019). Widely expressed in the CNS (Kinzie et al., 1997; Shigemoto et al., 1997; Corti et al., 1998; Kinoshita et al., 1998), the mGlu7 receptor is also considered as a potential therapeutic target for anxiety and depression (Cryan et al., 2003) and neurodevelopmental disorders (O’Connor et al., 2010; Palazzo et al., 2016; Fisher et al., 2018). Genetic disruptions in GRM7 are evident in patients with autism spectrum disorders (Liu et al., 2012; Sanders et al., 2012). Furthermore, the potentiation of mGlu7 receptor activity improves cognitive and social deficits, as well as respiratory impairments, in a mouse model of Rett syndrome (Gogliotti et al., 2017).

Interestingly, depending on the brain structure, mGlu7 and mGlu8 receptors play opposing roles in pain (Boccella et al., 2020). For example, mGlu7 receptor activation in periaqueductal gray and amygdala is pronociceptive, whereas mGlu8 receptor activation is antinociceptive (Marabese et al., 2007; Palazzo et al., 2008). Indeed, for mGlu7 receptors, proalgesic or analgesic activity depends on the brain structure and circuits in which the receptor is expressed (see Pereira and Goudet, 2018, for review). For example, activation of mGlu7 receptors in the nucleus accumbens has an antinociceptive effect (Kahl and Fendt, 2016), opposite to the pronociceptive effect when periaqueductal gray mGlu7 receptors are activated (Palazzo et al., 2016). Interestingly, mGlu7 receptor activation also prevents the development of morphine tolerance (Gawel et al., 2018). Additional studies confirmed mGlu8 receptor modulates sensory symptoms associated to neuropathic pain (Rossi et al., 2014). Furthermore, mGlu8 receptor activation in the nucleus tractus solitarius enhances cardiac nociception (Liu et al., 2012). Distinguishing the different physiologic and pathophysiological roles for mGlu7 and mGlu8 receptors has been hampered by a lack of selective pharmacological tools; however, discovery of new pharmacological tools (discussed in further detail later) have aided dissection of different roles and therapeutic indications.

The mGlu8 receptor is one of the least studied mGlu receptor family members, due notably to the lack of selective pharmacological tools. Investigations on the phenotype of genetically modified mice lacking the mGlu8 receptor are thus particularly informative. In various studies, mGlu8−/− mice exhibit anxiety-related phenotypes. However, some studies report an anxiogenic-like phenotype (Linden et al., 2003; Duvoisin et al., 2005, 2011), whereas others find an anxiolytic-like one (Gerlai et al., 2002; Fendt et al., 2010, 2013). Also, mGlu8−/− mice present robust deficits in contextual fear conditioning, novel object recognition, extinction of operant conditioning, and acoustic startle response (Fendt et al., 2010, 2013). mGlu8−/− mice also show enhanced social interaction; however, enhancing mGlu8 receptor activity does not affect social interaction in wild-type mice (Duvoisin et al., 2011). Further studies are required to better understand the role of mGlu8 and to clarify its therapeutic potential.

Outside of the CNS, the mGlu8 receptor plays an important role in visual discrimination in low light conditions (Nomura et al., 1994; Vardi et al., 2000), supported by the mGlu8−/− phenotype (Masu et al., 1995). Mutations in proteins involved in the transmission of the signal between rod photoreceptors and bipolar ON cells have been found in patients suffering from congenital stationary night blindness; these include more than 20 loss-of-function mutations in GRM6 (Dryja et al., 2005; O’Connor et al., 2006; Zeitz et al., 2007, 2015). These mutations affect the normal mGlu8 receptor response to the glutamate released from the photoreceptors, thus impairing signal transmission. The use of an optogenetic tool consisting of a chimera between mGlu8 receptor and melanopsin receptor has been suggested as a potential approach to restore light sensitivity (van Wyk et al., 2015).

V. Orthosteric Ligands

A. Definitions and Mode of Action

By definition, orthosteric ligands act in the same binding pocket as the endogenous ligand, competing to either activate or inhibit mGlu receptor activity. Glutamate and surrogate orthosteric agonists bind in the cleft between the two VFT lobes (Fig. 1). Upon binding, orthosteric agonists stabilize the closed state of the VFT, leading to a change in the relative orientation such that the extracellular domain dimer changes from a “resting” (R) to an active (A) state (Bessis et al., 2002). On the contrary, orthosteric antagonists prevent the full closure of the VFT (Bessis et al., 2000, 2002; Kunishima et al., 2000; Tsuji et al., 2002). Based on crystal structures, the main conformations that define the inactive and active states of mGlus receptors are the resting state Roo where both VFTs are open, and the active states Aco or Acc where one or both VFTs are closed, respectively. The two lobes are distant in the resting state and become closer in the active state (Kunishima et al., 2000; Tsuji et al., 2002). The closure of one VFT (Aco) is sufficient to induce a functional response from the receptor, but the closure of both VFTs (Acc) is necessary to achieve full activation (Kniazeff et al., 2004).

B. Selectivity

The L-glutamate binding site is highly conserved among the mGlu receptor family, resulting in difficulties in identifying compounds with subtype selectivity. Indeed, glutamate binds to all mGlu receptors under
a similar conformation, where the residues participating in direct interactions with its amino acid moiety are fully conserved as well as two residues that interact with the carboxylate moiety (Bertrand et al., 2002; Acher and Bertrand, 2005; Wellendorph and Brauner-Osborne, 2009). Within the orthosteric pocket, several residues that do not directly interact with glutamate are different between the three groups of mGlu receptors, enabling identification of group I, group II, and group III selective ligands (Table 1).

The most commonly used agonists of group I mGlu receptors are 3,5-dihydroxyphenylglycine (DHPG) and quisqualic acid, which are somewhat selective for group I over group II and III receptors (Table 1). Concerning group II mGlu receptors, the classic agonists are (2S,2R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV) (Brabet et al., 1998) and (1S,2S,5R,6S)-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740) (Monn et al., 1997), whereas L-AP4 and ACPT-I (Acher et al., 1997) are most commonly used agonists for group III mGlu receptors (Fig. 6). LY341495 is the most used competitive antagonist, which antagonizes all the subtypes, with higher potency at mGlu2/3 receptors over other subtypes (Kingston et al., 1998) (Fig. 6).

For subtype-selective orthosteric ligands, drug designers have to circumvent the highly conserved binding pocket. One way is to design compounds able to interact with proximal residues to the glutamate binding site that differ between subtypes. By example, newly discovered orthosteric ligands can discriminate between mGlu2 and mGlu3 receptors, for example, (1S,2S,4R,5R,6S)-2-amino-4-methylbicyclo[3.1.0]hexane-2,6-dicarboxylic acid and (1R,2S,4R,5R,6R)-2-amino-4-(1H-1,2,4-triazol-3-ylsulfanyl)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid, are two mGlu2 receptor-selective agonists (Monn et al., 2015a,b), and (1S,2S,4S,5R,6S)-2-amino-4-[(3-methoxybenzoyl)amino]bicyclo[3.1.0]hexane-2,6-dicarboxylic acid is an mGlu3 receptor-selective agonist (Monn et al., 2018). Co-crystallization of the VFT with each of these ligands, coupled with mutagenesis and molecular modeling, revealed that selectivity is due to interactions with amino acids residing at the periphery of the glutamate binding site (Monn et al., 2015a,b, 2018). In a similar fashion, (2S)-2-amino-4-[(4-carboxyamino)phenyl]hydroxy)methyl]hydroxy)methyl)butoanoic acid (LSP4-2022), an mGlu4 receptor-selective orthosteric agonist, binds both to the glutamate binding site and to an adjacent pocket (Goudet et al., 2012) (Fig. 6). This adjacent pocket is thought to be one of the sites of action of Cl− ions, which are potent positive allosteric modulators of mGlu receptors (Acher et al., 2011; Tora et al., 2015). Therefore, LSP4-2022 and related derivatives constitute bitopic ligands that simultaneously target an orthosteric and an allosteric binding site (Selvam et al., 2018). Targeting these two sites in tandem provides the means to overcome the difficulty of designing selective orthosteric drugs.

### C. Biased Agonism

Across the GPCR superfamily, it is becoming increasingly appreciated that the cellular response to receptor activation can differ depending on the ligand used, a phenomenon referred to as biased agonism. Biased agonism is thought to originate from the stabilization of different active conformations by distinct ligands, the balance of which is sampled by measuring different downstream measures of receptor activity. For any definition of biased GPCR agonism, it is critical that biased agonism is quantified relative to both a reference agonist and a reference pathway; this is because the relative efficacy of GPCR agonists is influenced by the stimulus-response coupling of the system. The most commonly applied method to quantify GPCR biased agonism is based on the operational model of agonism (Black and Leff, 1983) and subsequent derivation of transduction ratios (Kenakin et al., 2012). For metabotropic glutamate receptor orthosteric agonists, to date observations of biased agonism are limited to group I receptors. For example, relative to glutamate activation of mGlu1-mediated cytoprotective signaling, quisqualate is biased toward inositol monophosphate (IP1) accumulation in recombinant and native cells (Emery et al., 2012; Hathaway et al., 2015). At mGlu5 receptors, biased agonism for DHPG relative to glutamate arises due to “location bias” as DHPG is impermeable and not actively transported across cell membranes. DHPG is therefore unable to stimulate mGlu5 receptors located on intracellular membranes (Jong et al., 2005). Within different subcellular compartments, mGlu5 receptors interact with a different complement of transducers, giving rise to different forms of synaptic plasticity (Kumar et al., 2012). It remains to be determined whether other mGlu receptors and associated selective ligands also exhibit location bias that contributes to pharmacological differences.

### D. Tolerance

Another layer of complexity with regard to orthosteric agonist drug development is the potential for tolerance development. Under normal conditions glutamate is released transiently into the synapse, briefly activating mGlu receptors before active uptake mechanisms, for example, into astrocytes, reduce synaptic glutamate concentrations. However, these clearance mechanisms are not operative for surrogate orthosteric agonists, resulting in sustained receptor activation, which can lead to tolerance development. The potential for tolerance can be exacerbated for neurologic targets given the need for repeated chronic dosing to achieve a therapeutic effect. Tolerance has been noted for group II orthosteric agonists, where LY354740 efficacy for modulating rapid eye movement sleep wanes with repeated dosing (Ahnaou et al., 2015). Similarly, repeated daily dosing of (1R,4R,5S,6R)-4-amino-2-oxabicyclo[3.1.0]...
<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC name</th>
<th>Mechanism of Action</th>
<th>Selectivity (pKᵢ or pEC₅₀/pIC₅₀)</th>
<th>In vivo activity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Glutamate</td>
<td></td>
<td>Endogenous agonist</td>
<td>rR1: 6.5; hR2: 5.1; hR7: 3.2</td>
<td>Major excitatory neurotransmitter</td>
<td>1</td>
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<td>Nonselective</td>
<td>(1S, 3R)-ACPD (1S,3R)-1-aminoctocyclopentane-1,3-dicarboxylic acid</td>
<td>Group I and II agonist</td>
<td>rR1: 5.5; rR5: 5.7</td>
<td>Neurotoxicity; anti-Parkinsonian</td>
<td>2</td>
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<tr>
<td>ACPT-II</td>
<td>(1R,3R,4S)-1-aminoctocyclopentane-1,3,4-tricarboxylic acid</td>
<td>Pan-mGl antagonist</td>
<td>rR1a: 3.9; rR2: 4.1</td>
<td>Group II/III antagonist</td>
<td>3</td>
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<tr>
<td>CPG</td>
<td>(RS)-α-cyclopropyl-4-phosphonophenylglycine</td>
<td>Group II/III antagonist</td>
<td>rR2: 4.7; rR3: 7.3</td>
<td>Neuroprotection</td>
<td>4</td>
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<tr>
<td>(S)-MCPG</td>
<td>(S)-α-methyl-4-carboxyphenylglycine</td>
<td>Nonselective mGl antagonist</td>
<td>rR1: 3.8; rR5: 3.7</td>
<td>Spatial learning; antipsychotic-like</td>
<td>5</td>
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<tr>
<td>LY341495</td>
<td>(2S)-2-amino-2-[(1S,2S)-2-carboxy-cyclop-1-yl]-3-(xanth-9-yl)</td>
<td>Group II antagonist but blocks all</td>
<td>hR1: 5.2; hR2: 8.6-8.7; hR3: 8.4; hR4: 4.7; hR5: 7.3; hR7: 6.7-6.5; hR7: 6.3; hR8: 7.2</td>
<td>Antidepressant; memory; hypnosis; withdrawal</td>
<td>6</td>
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<tr>
<td>Group I mGl receptor</td>
<td></td>
<td>Group I mGl antagonist</td>
<td>rR1: 4.4-4.0; rR5: 4.3</td>
<td>Epilepsy; spatial memory; pain; neuroprotection</td>
<td>7</td>
</tr>
<tr>
<td>AIDA</td>
<td>(RS)-1-aminoindan-1,5-dicarboxylic acid</td>
<td>mGl2 antagonist</td>
<td>rR1: 3.8; rR5: 3.4</td>
<td>Neuroprotection; pain; epilepsy</td>
<td>8</td>
</tr>
<tr>
<td>(R,S)-CHPG</td>
<td>(RS)-2-chloro-5-hydroxyphenylglycine</td>
<td>mGl4 antagonist</td>
<td>rR1: 6.0; rR5a: 5.4</td>
<td>Anxiety; memory; pain; epilepsy</td>
<td>9</td>
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<tr>
<td>LY367385</td>
<td>(S)-3,5-dihydroxyphenylglycine</td>
<td>mGl4 antagonist</td>
<td>rR1: 5.1; rR7: &lt;4</td>
<td>Neuroprotection; antidepressant</td>
<td>10</td>
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<tr>
<td>L-Quisqualic acid</td>
<td>(L)-(+)-(α-amino-3,5-dioxo-1,2,4-oxadiazolidine-2-propanoic acid</td>
<td>Group I and AMPA agonist</td>
<td>rR1: 7.5</td>
<td>Epilepsy; neurotoxicity</td>
<td>11</td>
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<tr>
<td>Group II mGl receptor</td>
<td></td>
<td>group II agonist</td>
<td>hR2: 7.2-6.4; hR3: 7.9</td>
<td>Antipsychotic; neuroprotection; anticonvulsant</td>
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<td>DCG-IV</td>
<td>(2S,2′R,3′R)-2′-(2′,3′-dicarboxycyclopropyl)glycine</td>
<td>mGl2 antagonist</td>
<td>hR2: 8.1</td>
<td>Antipsychotic; clinical trials for bipolar disorder (phase 1) and dementia-related psychosis and/or agitation and aggression (phase 2)</td>
<td>13</td>
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<tr>
<td>LY2812223 (MP-101, prodrug: LY2979165)</td>
<td>ammonium (1R,2S,4R,5R,6R)-4-[(4H-1,2,4-triazol-3-yl)thio]-2-(1S)-2-amino propanamido-2-carboxycyclohexane-6-carboxylic acid</td>
<td>mGl2 antagonist</td>
<td>hR2: 9.9-8.6; hR3: 8.9-8.2</td>
<td>Anxiolytic; antidepressant; antipsychotic; neuroprotection</td>
<td>14</td>
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<td>LY354470</td>
<td>(1S,2S,5R,6S)-2-aminothiol[3.1.0]hexane-6,2-dicarboxylic acid</td>
<td>mGl2 antagonist</td>
<td>hR2: 7.0; hR3: &lt;5</td>
<td>Antipsychotic effects</td>
<td>15</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>
<td>mGl2 antagonist</td>
<td>hR2: 7.6</td>
<td>Alcohol-seeking; antipsychotic; antipsychotic; clinical trials for produrg: LY2140023 for psychosis (phase II) and posttraumatic stress disorder (III)</td>
<td>16</td>
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<tr>
<td>LY541850</td>
<td>(1S,2S,5R,6S)-2-amino-4-methyl thiol[3.1.0]hexane-6,2-dicarboxylic acid</td>
<td>mGl2 antagonist</td>
<td>hR2: 7.6</td>
<td>Alcohol-seeking; antipsychotic; antipsychotic; clinical trials for produrg: LY2140023 for psychosis (phase II) and posttraumatic stress disorder (III)</td>
<td>17</td>
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<tr>
<td>Pomaglutemad (LY404039)</td>
<td>4-amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic acid 2,2-dioxide</td>
<td>mGl2 antagonist</td>
<td>hR2: 7.6</td>
<td>Alcohol-seeking; antipsychotic; antipsychotic; clinical trials for produrg: LY2140023 for psychosis (phase II) and posttraumatic stress disorder (III)</td>
<td>18</td>
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<tr>
<td>Group III mGl receptor</td>
<td></td>
<td>Group III agonist</td>
<td>rR4: 5.5; rR6: 5.3; rR7: 3.6; rR8: 5.3</td>
<td>Neuroprotective; anxiolytic; antidepressant; analgesic</td>
<td>19</td>
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<td>ACPT-I</td>
<td>(1S, 3R,4S)-1-aminoctocyclopentane-1,2,4-tricarboxylic acid</td>
<td>mGl4 partial agonist</td>
<td>rR4a &lt; 4</td>
<td>Neuroprotection, off-target effects in mGl4 knockout mice</td>
<td>20</td>
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<tr>
<td>Cinnaricin acid</td>
<td>2-amino-3-oxo-3H-phenoxazine-1,9-dicarboxylic acid</td>
<td>Group III agonist</td>
<td>rR4: 6.7; hR6: 6.1</td>
<td>Neuroprotection, analgesic, Anti-Parkinsonian</td>
<td>21</td>
</tr>
<tr>
<td>L-AP4</td>
<td>L-(-)-2-amino-4-phosphonobutyric acid</td>
<td>Group III agonist</td>
<td>rR4: 7.2; rR7: 8.7; hR6: 6.1; rR8: 8.7</td>
<td>Neuroprotection; anti-Parkinsonian; antipsychotic; antiepileptic</td>
<td>22</td>
</tr>
<tr>
<td>L-thioAP4</td>
<td>L-(-)-2-amino-4-thiophosphonobutyric acid</td>
<td>Group III agonist</td>
<td>rR4: 7.2; rR7: 6.7; hR6: 5.7; rR8: 4.3</td>
<td>Epilepsy; ethanol consumption and relapse; morphine rewarding effect</td>
<td>23</td>
</tr>
<tr>
<td>L-SOP</td>
<td>O-phospho-L-serine</td>
<td>Endogenous agonist</td>
<td>rR4: 7; rR8: 5.4</td>
<td>Analgesic; antidepressant; antipsychotic-like</td>
<td>24</td>
</tr>
<tr>
<td>LSP1-2111</td>
<td>(2S)-2-amino-4-hydroxy[hydroxyhydroxy(4-hydroxy-3-methoxy-5-nitrophenyl)methyl]phosphoryl]butanoic acid</td>
<td>Group III agonist</td>
<td>rR4: 6; rR6: 5.5; rR7: 4; rR8: 4.7</td>
<td>Anti-Parkinsonian; antipsychotic; anxiolytic</td>
<td>25</td>
</tr>
<tr>
<td>LSP2-1916</td>
<td>(2S)-2-amino-4-[(4-carboxymethyl)phenyl]hydroxy[methyl]hydroxy]phosphoryl]butanoic acid</td>
<td>mGl4 antagonist</td>
<td>rR4: 7; rR8: 5.4</td>
<td>Alcohol-seeking; antipsychotic; antipsychotic; clinical trials for produrg: LY2140023 for psychosis (phase II) and posttraumatic stress disorder (III)</td>
<td>26</td>
</tr>
<tr>
<td>LSP4-2022</td>
<td>(2S)-2-amino-4-((4-carboxymethyl)phenyl)hydroxy[methyl]hydroxy]phosphoryl]butanoic acid</td>
<td>mGl4 antagonist</td>
<td>rR4: 7; rR8: 5.4</td>
<td>Alcohol-seeking; antipsychotic; antipsychotic; clinical trials for produrg: LY2140023 for psychosis (phase II) and posttraumatic stress disorder (III)</td>
<td>27</td>
</tr>
</tbody>
</table>

(continued)
hexane-4,6-dicarboxylic acid (LY379268) results in loss of efficacy as an analgesic (Jones et al., 2005) and antipsychotic-like activity to inhibit phencyclidine- or amphetamine-induced hyperlocomotion (Galici et al., 2005). However, tolerance is not consistently observed with chronic LY379268 dosing and can differ between behavioral paradigms (Cartmell et al., 2000; Anderson et al., 2014; Battaglia et al., 2015; Halberstadt et al., 2019). Whether tolerance development will prove to limit therapeutic efficacy of mGlu receptor orthosteric agonists remains to be seen.

### E. Orthosteric Ligands in the Clinic: Success and Failure

The most successful mGlu receptor discovery campaigns focused on orthosteric ligands targeting group II

### Table 1—Continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC name</th>
<th>Mechanism of Action</th>
<th>Selectivity (pKi or pEC50/pIC50)</th>
<th>In vivo activity</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>(RS)-PPG</td>
<td>(RS)-4-phosphonophenylglycine</td>
<td>Group III agonist</td>
<td>hR4a: 5.3; hR6: 5.3; hR7b: 3.7; hR8a: 6.7</td>
<td>Anticonvulsant; neuroprotective</td>
<td>26</td>
</tr>
<tr>
<td>MSOP</td>
<td>(RS)-α-methylserine-O-phosphate</td>
<td>Group III antagonist</td>
<td></td>
<td>Anxiolytic</td>
<td>27</td>
</tr>
</tbody>
</table>

IUPAC, International Union of Pure and Applied Chemistry; pKi, negative logarithm of the equilibrium dissociation constant.

Where affinity estimates were unavailable pIC50 (antagonists) or pEC50 (agonists) values are reported indicated by italics.

1) Pin et al., 1999; Meldrum, 2000; Mutel et al., 2000. 2) Zalewska and Winiarska, 1997; Henrich-Noack and Reymann, 1999; Mutel et al., 2000; Conti et al., 2002. 3) Acher et al., 1997. 4) Toms et al., 1996; Naples and Hampson, 2001. 5) Watkins and Collingridge, 1994; Bordin et al., 1996; Kim and Vezina, 1998; Mutel et al., 2000. 6) Kingston et al., 1998; Ornstein et al., 1998; Liechti and Markou, 2003; Podkowa et al., 2016; Witkín et al., 2016. 7) Pelleri et al., 1995; Moroni et al., 1997; Thomsen and Dalby, 1998; Christoffersen et al., 1998; Neugebauer et al., 1999; Mutel et al., 2000; Rao et al., 2000. 8) Young et al., 1995, 1997; Doherty et al., 1997; Chapman et al., 2000; Mutel et al., 2000; Bao et al., 2001. 9) Schoepf et al., 1994; Sekiyama et al., 1996; Mutel et al., 2000; Zalewska-Wińska and Winiarska, 2000; Nadlewska et al., 2002; Barton and Shannon, 2005; Ghiba et al., 2008; also see Winiarska and Car (2002) for a review. 10) Clark et al., 1997; Bruno et al., 1999; Car and Winiarska, 2006. 11) Shinozaki and Shibuya, 1974; Fukuda et al., 1985; Silverstein et al., 1986; Holmes et al., 1993; Litman et al., 1995; Hinoi et al., 2000; Mutel et al., 2000. 12) Bruno et al., 1994; Miyamoto et al., 1997; Attwell et al., 1998; Baret et al., 1998; Cartmell et al., 1998; Tomita et al., 2000; Yoshikawa et al., 2009. 13) Monn et al., 2015a; Felder et al., 2017; McColm et al., 2017; https://clinicaltrials.gov/ct2/show/NCT01383967; https://clinicaltrials.gov/ct2/show/NCT0344249. 14) Bond et al., 1997; Helton et al., 1997; Moom et al., 1997; Schoepf et al., 1997; Koniecny et al., 1998; Cartmell et al., 1999; Schreiber et al., 2000; Tizzano et al., 2002; Grillon et al., 2003; Marek et al., 2006. 15) Bond et al., 1999, 2000; Cartmell et al., 1999, 2000; Kingston et al., 1999; Monn et al., 1999; Clark et al., 2002; Greco et al., 2005; Matrisian et al., 2008; Woolley et al., 2008; Cippitelli et al., 2010; Di Liberto et al., 2010. 16) Hanna et al., 2013. 17) Rödd et al., 2000; Rorick-Kehn et al., 2007a,b; Fell et al., 2008; Lebois, 2008; Seeman, 2013; Annes et al., 2015; https://clinicaltrials.gov/ct2/show/NCT01487083; https://clinicaltrials.gov/ct2/show/NCT02234687. 18) Acher et al., 1997; Tatarczyńska et al., 2002; Palucha et al., 2004; Kik et al., 2007; Lopez et al., 2007; Goudet et al., 2008; Palucha-Poniewiera et al., 2008; Domín et al., 2014, 2016, 2018. 19) Throne et al., 2002, 2014, 2017; 20) Thomsen et al., 1992; Trombley and Westbrook, 1992; Fuden et al., 1997; Thomsen, 1997; Pizzi et al., 2000; Naples and Hampson, 2001; Chen and Pan, 2005; Zhou et al., 2006; Lopez et al., 2007; Vernon et al., 2007; Park et al., 2019. 21) Selvam et al., 2007. 22) Nicoletti et al., 1986; Thomsen and Suzdak, 1993; Tizzano et al., 1995; Faden et al., 1997; Tatarczyńska et al., 2001; MacLounes et al., 2004; Artibière et al., 2009. 23) Beurrier et al., 2009; Cesari et al., 2010, 2012a; Commare et al., 2015; Selvam et al., 2018. 24) Hajasova et al., 2018; Lebourgeois et al., 2018; Girard et al., 2019. 25) Goudet et al., 2012; Vilar et al., 2013; Podkowa et al., 2015; Wozniak et al., 2017; Zussy et al., 2018. 26) Bigge et al., 1989; Gasparini et al., 1999a. 27) Chojnacka-Wójcik et al., 1996, 1997; Thomas et al., 1996.

Fig. 6. Structures of select orthosteric ligands of mGlu receptors. Representative orthosteric ligands for mGlu receptors. Antagonists are labeled with black text, and agonists are in blue. Detailed in vitro and in vivo pharmacological profiles are listed in Table 1.
receptors. Multiple group II orthosteric agonists have reached phase II or phase III trials for psychiatric indications. LY354740/eglumegad was well tolerated and showed anxiolytic efficacy in humans (Grillon et al., 2003; Schoepp et al., 2003), with further development focused on a prodrug formulation (LY544344) to improve bioavailability (Rorick-Kehn et al., 2006). However, trials for generalized anxiety disorder were discontinued due to concerns regarding convulsions in animals (Dunayevich et al., 2008). Another group II mGlu receptor-selective agonist, LY2140023/pomaglumetad [prodrug for 4-amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic acid 2,2-dioxide], improved both negative and positive symptoms in patients with schizophrenia in a randomized phase II clinical trial (Patil et al., 2007). Subsequent phase III trials failed to report significant antipsychotic efficacy for all the patients involved, terminating further development (Kim et al., 2014) and posttraumatic stress disorder related to traumatic brain injury (Perez-Garcia et al., 2018). Despite successful discovery efforts for subtype-selective and drug-like orthosteric ligands for the group II mGlu receptors in particular, the majority of mGlu receptor discovery programs are pursuing allosteric modulators as reviewed below.

VI. Allosteric Modulators

A. Definitions, Quantification, and Identification

Since glutamate is recognized by two different receptor families (ionotropic and metabotropic receptors) as well as transporters, there remains a concern that orthosteric compounds will suffer from lack of selectivity due to high conservation of glutamate binding sites across different proteins. As such, many discovery programs have focused efforts on identification and development of allosteric modulators. Allosteric modulators interact with sites that are topographically distinct from the orthosteric site, such that a receptor may be simultaneously bound by both an orthosteric and an allosteric ligand (Fig. 1A). For the most part, allosteric sites are located in regions of receptors that show greater sequence divergence across subtypes and therefore offer greater selectivity. An allosteric modulator may enhance or inhibit the binding and/or efficacy of an orthosteric ligand, with the magnitude and direction described as "cooperativity." An allosteric modulator that enhances orthosteric ligand affinity or efficacy is referred to as a positive allosteric modulator (PAM), whereas an inhibitor is a negative allosteric modulator (NAM). In addition, allosteric ligands may also bind to a receptor but have no net effect on either affinity or efficacy of an orthosteric ligand; referred to as neutral allosteric ligands (NALs). Furthermore, allosteric ligands may also possess intrinsic efficacy as either positive or inverse agonists in addition to, or exclusive of, cooperativity with an orthosteric ligand. By example, a PAM with intrinsic agonist activity is referred to as a PAM agonist or ago-PAM.

In addition to potential for increased subtype selectivity, allosteric modulators offer a number of advantages over their orthosteric counterparts. Cooperativity between two ligands is saturable, offering the potential for greater safety in an overdose. Allosteric modulators that have no intrinsic efficacy and are quiescent in the absence of endogenous ligand also provide scope to fine-tune receptor activity in a spatiotemporal fashion, exerting potentiation or inhibition only where, and when, the endogenous ligand is present. For these reasons, discovery programs in industry and academia alike have sought allosteric modulators of mGlu receptors as potential novel therapeutics for a wide array of CNS disorders. However, discovery of allosteric modulators can be associated with considerable challenges with respect to quantification and validation.

Allosteric modulator binding is defined by the law of mass action, where the equilibrium dissociation constant, commonly defined as $K_B$, describes the affinity of...
an allosteric modulator for its site. However, the simultaneous binding of an allosteric modulator and orthosteric ligand gives rise to different receptor conformations than can be achieved by the binding of each ligand individually, altering ligand affinity as defined by cooperativity. To quantify cooperativity, the simplest scheme is the allosteric ternary complex model (ATCM) (Fig. 1B; Gregory et al., 2010b), which describes the reciprocal change in ligand affinity when a receptor is simultaneously bound by both an allosteric and orthosteric ligand, defined by the cooperativity factor $\alpha$. The ATCM is limited to describing allosteric interactions at the level of receptor binding, and for many mGlu allosteric modulators it is apparent these ligands have effects on receptor activity in addition to, or independent of, affinity.

To quantify the full scope of effects an allosteric ligand may have on receptor activity, multiple alternative pharmacological models have been proposed that can accommodate allosteric ligand intrinsic efficacy and efficacy modulation (Slack and Hall, 2012; Hall, 2013; Roche et al., 2014; Hall and Giraldo, 2018; Gregory et al., 2020). A challenge in applying these models is the inclusion of many parameters, which can prohibit fitting to experimental data. In this respect, the most widely adopted framework for quantification of pharmacological activity is an operational model of allostery (Fig. 1C), which combines the Black and Leff operational model of agonism with the ATCM (Leach et al., 2007; Gregory et al., 2012). Within this framework, the influence of an allosteric modulator on orthosteric agonist efficacy is accounted for by $\beta$, an experimentally derived scaling factor. Application of this model therefore allows for delineation of the influence of an allosteric modulator on affinity independently of efficacy. This provides an important distinction given that allosteric modulators can have differential effects, which may be in opposing directions, on affinity versus efficacy. The operational model of allostery also allows for intrinsic allosteric agonism, defined by $\tau$, but cannot account for inverse agonism.

Accurate quantification of allosteric ligand pharmacology requires appropriately designed experimental paradigms. The definitive experiment to unambiguously demonstrate an allosteric mechanism of action is a kinetic binding paradigm. The simultaneous binding of an allosteric ligand may enhance or slow the dissociation rate ($K_{d,0}$) of the orthosteric radioligand from the receptor, or vice versa. Interaction studies using radiolabeled orthosteric ligands can be used to quantify modulation of affinity ($\alpha$) as well as ligand affinity for the free receptor (Gregory et al., 2010b). However, it is important to note that the magnitude and direction of cooperativity between two ligands depends on the chemotypes present, a phenomenon known as “probe dependence.” This is an important consideration when extrapolating pharmacological profiles of allosteric ligands based on interactions with a radiolabeled orthosteric antagonist or from a surrogate orthosteric agonist, which is often required in native cells/tissues. Radiolabeled allosteric ligands have been described for multiple subtypes, which can also be used to quantify affinity for the receptor for unlabeled ligands at a common allosteric site (Cosford et al., 2003; Lavreyens et al., 2003; O’Brien et al., 2018) or provide evidence for additional allosteric sites that are conformationally linked such that there is cooperativity between the two allosteric sites.

The vast majority of allosteric ligands for mGlu receptors have been identified and validated using functional assays. The most commonly used approach involves generating modulator titration curves in the presence of either an $-EC_{50}$ agonist concentration for PAM identification, or an $EC_{80}$ to identify NAMs. The potencies and relative maximum response (for PAMs) or inhibitory effect (for NAMs) from these titration curves are routinely used to drive discovery programs (Lindsley et al., 2016). However, these parameters represent composite values encompassing $\alpha$, $\beta$, $K_B$, and $\tau$ and are also influenced by the concentrations of orthosteric agonist used, orthosteric agonist intrinsic efficacy, and the stimulus-response coupling of the system under investigation (Lindsley et al., 2016). Modulator potencies curves can be analyzed in parallel with an agonist concentration-response curve to estimate $K_B$ and a composite $\alpha\beta$ value where the maximum degree of potentiation or inhibition does not reach the limit of the system (Gregory et al., 2012, 2019). However, to quantify the interaction between a modulator and orthosteric agonist, the most robust approach is to perform full agonist concentration-response curves in the absence and presence of increasing concentrations of modulator. Despite the limitations in the most commonly applied screening approaches, drug discovery programs for small molecule synthetic allosteric ligands of mGlu receptors have been largely successful. In addition, there is increasing evidence for endogenous allosteric modulators for mGlu receptor family members.

B. Endogenous Allosteric Modulators

The greater class C GPCR family also includes the calcium-sensing receptor and GPRC6A, two receptors that are known to respond to multiple endogenous ligands including amino acids and cations (Leach and Gregory, 2017). It is perhaps therefore not surprising that divalent and trivalent cations, including Ca$^{2+}$, can directly activate mGlu$_1$, mGlu$_3$, and mGlu$_7$ receptors (Kubo et al., 1998; Miyashita and Kubo, 2000a,b; Jiang et al., 2014). Furthermore, extracellular Ca$^{2+}$ potentiates binding/function of orthosteric ligands at mGlu$_1$ (Saunders et al., 1998; Jiang et al., 2014). In addition, negatively charged chloride ions activate mGlu$_3$, mGlu$_4$, mGlu$_6$, and mGlu$_8$ receptors and potentiate glutamate...
prion protein interacts with the mGlu5 receptor acting as a coreceptor for amyloid β oligomers, although the precise binding interactions within mGlu5 receptors are unknown (Um et al., 2013). Beyond the extracellular domains, molecular dynamics studies have proposed that lipids can interact with mGlu5 7TM (Dalton et al., 2017). Furthermore, cholesterol membrane content enhances mGlu5 signaling to ERK1/2 phosphorylation mediated via a cholesterol recognition/interaction amino acid consensus motif in transmembrane domain 5 (Kumari et al., 2013). The existence of endogenous allosteric modulators for the mGlu receptors is often overlooked during discovery and validation of synthetic small molecule allosteric modulators.

C. Small Molecule Allosteric Modulators

Concerted discovery efforts from both industrial and academic researchers have yielded a wealth of chemically and pharmacologically diverse small molecule allosteric modulators for the mGlu receptor family (Tables 2–5). The majority of small molecule mGlu receptor allosteric modulators identified to date interact with a common pocket within the 7TM domains. This binding pocket is in a location analogous to the biogenic amine orthosteric site of class A GPCRs, largely lined by residues in transmembrane domains 3, 5, 6, and 7. To date, six X-ray crystal structures of the mGlu1 or mGlu5 receptor 7TM domains have been solved with NAMs occupying this common allosteric site (Doré et al., 2014; Wu et al., 2014; Christopher et al., 2015, 2019). A wealth of previous mutagenesis data indicate that this pocket is shared across the mGlu receptor family, and indeed for all class C GPCRs, and can be engaged by both NAMs and PAMs (see Leach and Gregory, 2017, for review). Here we focus on the pharmacological profiles of prototypical and well validated commercially available allosteric modulators for mGlu receptors.

D. Group I PAMs, NAMs, NALs

The first disclosed mGlu receptor allosteric modulator was ethyl (7Z)-7-hydroxyimino-1,7a-dihydrocyclopropa[b]chromene-1a-carboxylate (CPCCOEt) (Annoura et al., 1996; Litschig et al., 1999), a negative allosteric modulator of mGlu1 receptor. CPCCOEt has low micromolar affinity for mGlu1 receptors (Lavreysen et al., 2003) and negatively modulates glutamate efficacy but has neutral cooperativity with respect to [3H]glutamate affinity (Litschig et al., 1999). Moreover, CPCCOEt has poor selectivity between group I mGlu receptors (Table 2), negatively modulating mGlu5 receptor activation with a similar apparent K_B (Hellyer et al., 2018). The discovery of CPCCOEt was followed by EM-TBPC and BAY-36-7620, which showed species differences in mGlu1 receptor NAM activity with considerably higher affinity for the rat versus human receptor (Malherbe et al., 2003; Cho et al., 2014a). Similar to CPCCOEt, BAY-36-7620 has neutral cooperativity with respect to [3H]quisqualate affinity but inhibits orthosteric agonist efficacy (Carroll et al., 2001; Lavreysen et al., 2003). Since the discovery of these early tool compounds, a wealth of structurally diverse mGlu1 receptor NAMs have been disclosed that have therapeutic efficacy in preclinical models for analgesia, antipsychotic-like activity, anxiety, addiction, and cancer and as anticonvulsants (Table 2). For diverse scaffolds (Fig. 7, e.g., A-841720 and 1-(3,4-dihydro-2H-pyran[2,3-b]quinolin-7-yl)-2-phenylethanone), the higher affinity for rat over human (>10-fold) persisted (Cho et al., 2014a).

Breakthrough chemotypes represented by FTIDC and JNJ16259685 have similar nanomolar affinities for the rat and human receptors and >100-fold selectivity as NAMs for mGlu1 over mGlu5 receptors (Lavreysen et al., 2003, 2004; Suzuki et al., 2007a). Despite ultimate identification of high affinity, in vivo efficacious mGlu1 receptor NAMs, further development has stalled due to on-target mediated adverse effects such as cognitive impairments from multiple scaffolds (Steckler et al., 2005b; Schröder et al., 2008).

On the other hand, mGlu1 receptor PAMs have been relatively unexplored, although they may be a promising therapeutic strategy for schizophrenia by restoring function of naturally occurring mutations (Garcia-Barrantes et al., 2015b). The first mGlu1 receptor PAMs included diverse chemotypes, for example, 2-(4-fluoro-phenyl)-1-(4-methylphenyl)sulfonylpyrrolidine (RO 67-7476) and ethyl N-[2,2-di(phenyl)acetyl]carbamate, identified from high-throughput screening, which enhanced orthosteric agonist affinity and functional responses at rat mGlu1 receptor without intrinsic agonist activity (Knoflach et al., 2001) but were not suitable for in vivo studies. Similar to multiple mGlu1 receptor NAM scaffolds, RO 67-7476 lacks the ability to potentiate glutamate at human mGlu1 receptors (Knoflach et al., 2001). A subsequent study suggested these mGlu1 receptor PAMs may have intrinsic efficacy for ERK1/2 and cAMP accumulation; however, this agonist activity could be blocked by both orthosteric and allosteric antagonists, raising the possibility that the apparent intrinsic agonism may be attributable to potentiation of ambient glutamate (Sheffler and Conn, 2008).

Of note, both RO 67-7476 and ethyl N-[2,2-di(phenyl)acetyl]carbamate were unable to completely displace binding of the radiolabeled mGlu1 receptor NAM [3H]1-(3,4-dihydro-2H-pyran[2,3-b]quinolin-7-yl)-2-phenylethanone (Hemstapat et al., 2006), suggesting these compounds recognize a different site within the 7TM domain. Subsequent discovery efforts identified 3-chloro-N-[3-chloro-4-(4-chloro-1,3-dihydro-1,3-dioxo-2H-isooindol-2-yl)
TABLE 2
Pharmacology of commercially available mGlu1 allosteric ligands

<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC name</th>
<th>Mechanism of Action</th>
<th>Selectivityα (pK_B, pEC50/pIC50)</th>
<th>In vivo activity</th>
<th>Ref(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-841720</td>
<td>3-(azepan-1-yl)-9-(dimethylamino)pyrindo</td>
<td>NAM</td>
<td>rR1: 9.0; hR1: 8.0; rR5: 6.7</td>
<td>Analgesic; disrupts locomotion and cognition</td>
<td>1</td>
</tr>
<tr>
<td>BAY-36-7620</td>
<td>[1,2]thieno[3,4-d]pyrimidin-4-one</td>
<td>NAM</td>
<td>rR1: 8.0; hR1: &lt;5</td>
<td>Anticonvulsive, cognitive impairments</td>
<td>2</td>
</tr>
<tr>
<td>CFMTI</td>
<td>5-(1-(2,4-difluorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-2-phenyl-2-pyridinecarboxamide (VU0483605)</td>
<td>NAM</td>
<td>rR1: 8.4-8.1; hR1: 8.4-7.5; hR5: 5.8</td>
<td>Analgesic</td>
<td>5</td>
</tr>
<tr>
<td>CFCCOEt</td>
<td>2-cyclopropyl-5-(1-(2-fluoropyridin-3-yl)-5-methyltriazol-4-yl)-1,7a-dihydrocyclopropa[β]chromene-1-carboxylate</td>
<td>NAM</td>
<td>rR1: 5.3-4.9; hR1: &lt;4</td>
<td>Antituromogenic (melanoma), analgesia, memory impairment, reverses morphine tolerance; neuroprotective (trauma)</td>
<td>4</td>
</tr>
<tr>
<td>DFMTI (MK-5435)</td>
<td>5-(1-(2,4-difluorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-2-isopropylsphingosine-1-one</td>
<td>NAM</td>
<td>rR1: 7.8</td>
<td>Analgesic</td>
<td>6</td>
</tr>
<tr>
<td>DM-PPP</td>
<td>4,10-(2R,3,3-dimethylbutan-2-yl)-2,0-propyl-3,5-dimethyl-1H-pyrrrole-2,4-dicarboxylate</td>
<td>NAM</td>
<td>NAM</td>
<td>Not suitable for in vivo dosing</td>
<td>7</td>
</tr>
<tr>
<td>EM-TBPC</td>
<td>1-ethyl-2-methyl-6-oxo-1,2,4,5-tetrahydro-9-benzazepin-3-yl pyrimidine-9-carboxylate</td>
<td>NAM</td>
<td>rR1: 8.2; hR1: low</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>FITM, [19F]FTM</td>
<td>4-fluoro-N-methyl-N-[4-(3,5-dimethyl-1H-pyrrrole-2-yl)benzamide]</td>
<td>NAM</td>
<td>hR1: 6.6; rR5: 5.2</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>FPTQ</td>
<td>6-1-(2-fluoropropyl-5-methyl-1H-1,2,3-triazol-4-yl)quinoline</td>
<td>NAM</td>
<td>hR1: 7.9</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>FTIDC</td>
<td>4-[1-(2-fluoropropyl-5-methyltriazol-4-yl)N-methyl-N-propan-2-yl-3,6-dihydro-2H-pyridine-1-carboxamide</td>
<td>NAM</td>
<td>hR1: 7.7-7.8; rR1: 7.7; rR5: 7.8-7.5</td>
<td>Antipsychotic-like, antiabuse/addiction, anxiety, cognitive impairment, no tolerance with repeat dosing, protective in retinal neurodegeneration</td>
<td>11</td>
</tr>
<tr>
<td>JNJ18259685</td>
<td>3,4-dihydro-2H-pyrano[2,3-b]quinolin-7-yl-(4-methylcyclohexyl)ethanone</td>
<td>NAM</td>
<td>hR1: 7.7; hR5: 5.8-5.4</td>
<td></td>
<td>12</td>
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<tr>
<td>LY456066</td>
<td>2-[4-(2,3-dihydro-1H-inden-2-ylamino)-5,6,7,8-tetrahydroquinazolin-2-yl]sulfanyl(ethanol)</td>
<td>NAM</td>
<td>hR1: 7.7; hR5: &lt;5</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>LY456236</td>
<td>6-methoxy-N-(4-methoxyphenyl)quinazolin-4-amine hydrochloride</td>
<td>NAM</td>
<td>rR1: 5.9; hR1: 6.9-5.5</td>
<td>Analgesic, anticonvulsant</td>
<td>14</td>
</tr>
<tr>
<td>R214127, [1H]R214127</td>
<td>1-(3,4-dihydro-2H-pyrano[2,3-b]quinolin-4-yl)-2-phenylethanone</td>
<td>NAM</td>
<td>rR1: 9.8-9.6; hR1: &lt;5</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>RO 67-7476</td>
<td>2-(4-fluorophenyl)-1-(4-methylphenyl)sulfonylpyrrolidine</td>
<td>PAM</td>
<td>hR1: 6.8-6.7; hR5: &lt;5</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>RO 01-6128</td>
<td>ethyl N-[2,2-di(phenyl)acetylcarbamate]</td>
<td>PAM</td>
<td>hR1: 6.7-6.6; hR5: &lt;5</td>
<td></td>
<td>17</td>
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<tr>
<td>VU4083605</td>
<td>3-chloro-N-(3-chloro-4-(4-chloro-1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)phenyl)-2-pyridinecarboxamide</td>
<td>PAM</td>
<td>hR1: 6.8; hR5: 6.0; hR4: &lt;5; rR5(NAL): 6.5</td>
<td>Antiipsychotic-like; analgesic, no motor impairments</td>
<td>18</td>
</tr>
<tr>
<td>YM298198</td>
<td>6-amino-N-cyclohexyl-N,3-dimethylthiazolo[3,2-a]benzimidazole-2-carboxamide</td>
<td>NAM</td>
<td>hR1: 6.9; hR5: 7.7; hR5: &lt;5.2</td>
<td></td>
<td>19</td>
</tr>
</tbody>
</table>

IUPAC, International Union of Pure and Applied Chemistry; MoA, mechanism of action; pKB, negative logarithm of the equilibrium dissociation constant for an allosteric ligand; pEC50, negative logarithm of the agonist or PAM concentration required to give the half-maximal response (activation or potentiation); pIC50, negative logarithm of the NA concentration required to give the half-maximal inhibition.

αWhere affinity estimates were unavailable pIC50 (NAMs) or pEC50 (PAMs) values are reported indicated italics.

βA scaffold hop from an mGlu4 PAM/mGlu1 NAM chemo-type (Cho et al., 2014b); however, the selectivity of VU4083605 as an mGlu1 PAM is based on cooperativity, as it has similar affinity for mGlu5 receptors, albeit with neutral cooperativity with mGlu5 receptor orthosteric agonist efficacy (Hellyer et al., 2018). Recent medicinal chemistry efforts have yielded the first CNS penetrant mGlu1 receptor PAMs (Garcia-Barrantes et al., 2015a, 2016a,b; Yohn et al., 2018), paving the way forward to establish therapeutic potential for schizophrenia and beyond.

Although CPCCOEt was the first mGlu1 receptor NAM disclosed, the mGlu15 receptor NAM fenobam was discovered earlier (Itil et al., 1978), but its mechanism...
<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC name</th>
<th>Mechanism of Action</th>
<th>Selectivity$^a$ (pKᵦ or pEC₅₀/pIC₅₀)</th>
<th>In vivo activity (clinical data)</th>
<th>Ref$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADX47273</td>
<td>(S)-(4-fluorophenyl)-(3-[3-(4-fluoro-phenyl)-1,2,4-oxadiazol-5-y1]-piperidin-1-yl)methanone</td>
<td>PAM</td>
<td>rR5: 5.5-5.2; hR5: 7.1</td>
<td>Cognition enhancement, antipsychotic-like</td>
<td>1</td>
</tr>
<tr>
<td>CDPPB</td>
<td>3-cyano-N(1,3-diphenyl-1H-pyrazol-5-yl)benzamide</td>
<td>PAM</td>
<td>rR5: 7.4-5.9; hR5: 7.1</td>
<td>Antipsychotic-like, neuroprotective in Alzheimer’s disease and Huntington’s disease models, cognition enhancement, promote addiction recovery, tolerance development</td>
<td>2</td>
</tr>
<tr>
<td>CPPHA</td>
<td>N-[4-chloro-2-(phthalimidomethyl)phenyl]salicylamide</td>
<td>PAM</td>
<td>rR1: 5.5; rR5: 6.9-5.5; hR5: 6.5-6.3; NAM at hR4: 4.9; and rR8: 5.1</td>
<td>Not suitable for in vivo use</td>
<td>3</td>
</tr>
<tr>
<td>CTEP</td>
<td>2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethyl)pyridine</td>
<td>NAM inverse agonist</td>
<td>hR5: 7.8; rR5: 8.0; mR5: 7.9; A₂AR: 5.6; L-type Ca channel: 5.6</td>
<td>Huntington’s disease, chronic stress, Alzheimer’s disease, deficits in FMR1$^{-/-}$; anxiolytic</td>
<td>4</td>
</tr>
<tr>
<td>DFB</td>
<td>3,3’-difluorobenzaldazine</td>
<td>PAM</td>
<td>hR5: 5.6; rR5: 5.5-5.3</td>
<td>Cognition enhancement</td>
<td>5</td>
</tr>
<tr>
<td>Dipraglurant (ADX48621)</td>
<td>6-fluoro-2-(4-[2-pyridinyl]-3-butyn-1-yl)imidazole</td>
<td>PAM</td>
<td>hR5: 7.5</td>
<td>Antidyskinesia; PD-LID</td>
<td>6</td>
</tr>
<tr>
<td>Fenobam[3H]</td>
<td>[N-(3-chlorophenyl)]-N’-(4,5-dihydro-1-methyl-4-oxo-1H-imidazole-2-yl)urea</td>
<td>NAM inverse agonist</td>
<td>hR5: 7.4-7.3; rR5: 7.5-7.2; A₂AR; MAO-B</td>
<td>Analgesia, anibause/addiction (cocaıne, methamphetamine) but appetite/sucrose effects, anxiolytic; autism spectrum disorder behaviors (FMR1$^{-/-}$); cognitive deficits in wild-type mice; psychoactive/stimulant</td>
<td>7</td>
</tr>
<tr>
<td>LSN2463359</td>
<td>N-(1-methylthyl)-5-(pyridin-4-ylethynyl)pyridine-2-carboxamide</td>
<td>PAM</td>
<td>rR5: 6.4; hR5: 7.5</td>
<td>Antipsychotic-like, wake- promoting, cognition enhancement</td>
<td>8</td>
</tr>
<tr>
<td>Mavoglurant (AFQ056)</td>
<td>methyl [(3R,8S,5aR)-4-hydroxy-4-[2-(3-methylphenyl)ethynyl]-3,3a,5,6,7,7a-hexahydro-2H-indole-1-carboxylate]</td>
<td>NAM</td>
<td>hR5: 8.3-7.6; rR5: 7.2</td>
<td>Autism spectrum disorder behaviors (FMR1$^{-/-}$), sleep-wake modulation, GERD, PD-LID, chorea in Huntington’s disease (Reilmann)</td>
<td>9</td>
</tr>
<tr>
<td>5-MPEP</td>
<td>5-methyl-2-[phenylethynyl]pyridine</td>
<td>NAL</td>
<td>rR5: 6.7-6.0</td>
<td>Not suitable for in vivo use</td>
<td>10</td>
</tr>
<tr>
<td>M-5MPEP</td>
<td>2-[2-(3-methoxyphenyl)ethyl]n-5-methylpyridine</td>
<td>NAM</td>
<td>rR5: 7.0-6.2</td>
<td>Antiaaddiction/abuse (cocaıne), anxiolytic, antidepressive, no psychotomimetic-like effects</td>
<td>11</td>
</tr>
<tr>
<td>MPEP/[3H]</td>
<td>2-methyl-6-[phenylethynyl]pyridine</td>
<td>NAM inverse agonist</td>
<td>hR5: 8.8-8.2; rR5: 8.8-8.0</td>
<td>Autism spectrum disorder behaviors (VPA, BTBR, ENU2 mouse models); cognitive impairment; psychostimulant, analgesia, enhances alcohol sedation/hypnosis; anti-Parkinssonian; antiaddiction (alcohol, cocaine), reverse morphine tolerance, PD-LID, antiepileptic (as an adjunct), sleep modulation</td>
<td>12</td>
</tr>
<tr>
<td>MTEP</td>
<td>3-([2-methyl-4-thiazolyl]ethynyl)pyridine</td>
<td>NAM inverse agonist</td>
<td>hR5: 8.3-7.9; rR5: 8.3-7.6</td>
<td>Anti-obesseive compulsive disorder (Sapap3$^{-/-}$), anti-Parkinssonian and neuroprotective; psychostimulant; antiaddiction (methamphetamine, alcohol, cocaine)</td>
<td>13</td>
</tr>
<tr>
<td>Raseglurant (ADX10059)</td>
<td>2-[3-(fluorophenyl)ethyl]n-4,6-dimethyl-3-pyriridinamine</td>
<td>NAM</td>
<td>R5: ~8.0</td>
<td>GERD, migraine</td>
<td>14</td>
</tr>
<tr>
<td>VU29</td>
<td>N-(1,3-diphenyl-1H-pyrazol-5-yl)-4-nitrobenzamide</td>
<td>PAM</td>
<td>rR5: 7.6-6.2</td>
<td>Cognition enhancement</td>
<td>15</td>
</tr>
<tr>
<td>VU0357121</td>
<td>4-butoxy-N’[2,4-difluorophenyl]benzamide</td>
<td>PAM</td>
<td>rR5: 5.7</td>
<td>Not suitable for in vivo dosing</td>
<td>16</td>
</tr>
<tr>
<td>VU0360172</td>
<td>N-cyclobutyl-6-[2-(3-fluorophenyl)ethynyl]-3-pyridinocarboxamide hydrochloride</td>
<td>PAM</td>
<td>rR5: 7.0-6.6</td>
<td>Antiepileptic; neuroprotective; antipsychotic-like</td>
<td>17</td>
</tr>
</tbody>
</table>

(continued)
of action was not elucidated until over 20 years later (Porter et al., 2005). Indeed, all of the subtypes, allosteric ligand discovery against mGlu5 receptors has proven to be the most fruitful with a wealth of pharmacologically and structurally diverse ligands identified including NAMs, PAMs, and NALS (Table 3). Prototypical mGlu5 receptor NAMs based on an acetylene core, 2-methyl-6-(phenyl-ethyl)-pyridine (MPEP) and 3-(2-methyl-4-thiazolyl) ethynyl(pyridine (MTEP), as well as fenobam, have demonstrated the therapeutic potential for mGlu5 receptor inhibition for addiction, depression, anxiety, neurodegenerative disorders, and autism spectrum disorders (Table 3). Often referred to as “full NAMs,” these ligands have high negative cooperativity with respect to orthosteric agonist efficacy, completely abolishing agonist responses at saturating concentrations, but are neutral with respect to glutamate affinity (Gregory et al., 2012; Sengmany et al., 2019). The relatively high affinity of MPEP and fenobam presented the opportunity to generate radiolabeled versions (Cosford et al., 2003; Porter et al., 2005), which facilitated discovery and validation of novel mGlu5 receptor NAMs. Of note, many full NAMs have inverse agonist activity (Porter et al., 2005; Sengmany et al., 2019). It has been postulated that the combination of high negative cooperativity and inverse agonism contributes to on-target adverse effect liability of mGlu5 receptor NAMs, including cognitive impairments (Porter et al., 2005). Subsequent efforts have identified high affinity mGlu5 receptor NAMs [e.g., 3-azabicyclo[3.1.0]hexan-3-yl]-5-[2-(3-fluorophenyl)ethyl]pyridin-2-yl)methanone, (R)-5(4R,5R)-rel-5-(2-chlorophenyl)-4-(5-(phenethyl)pyridin-3-yl)oxazolidin-2-one] with suitable properties for in vivo studies (Gregory et al., 2010a; Haas et al., 2017).

Molecular switches within mGlu5 receptor NAM scaffolds (Fig. 7) have also yielded PAMs and PAM agonists, with advanced compounds from the biaryl

TABLE 3—Continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC name</th>
<th>Mechanism of Action</th>
<th>Selectivity&lt;sup&gt;a&lt;/sup&gt; (pEC50 or pEC50/pIC50)</th>
<th>In vivo activity (clinical data)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>VU0409551 (JNJ-46778212)</td>
<td>6,7-dihydro-2-(phenoxymethyl)oxazolo[5,4-c]pyridin-5-(4H)-yl-(fluorophenyl)methanone</td>
<td>PAM</td>
<td>r5R: 6.6-6.4; r5R: 7.1; MAO-B: 5.2</td>
<td>Cognition in Huntington’s disease condition; reverse deficits in serine racemase; —/— antipsychotic-like; cognition enhancement</td>
<td>18</td>
</tr>
<tr>
<td>VU0409106</td>
<td>3-fluoro-N-(4-methyl-2-thiazolyl)-5-(4-pyrimidinyl)benzamide</td>
<td>NAM inverse agonist</td>
<td>R5: 7.6-7.9</td>
<td>Anxiety</td>
<td>19</td>
</tr>
</tbody>
</table>

<sup>a</sup>Where affinity estimates were unavailable pEC50 (NAMs) or pEC50 (PAMs) values are reported indicated by italics.
TABLE 4
Pharmacology of commercially available group II mGlul allosteric ligands

<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC name</th>
<th>Mechanism of Action</th>
<th>Selectivity[a] (pK&lt;sub&gt;a&lt;/sub&gt; or pEC&lt;sub&gt;50&lt;/sub&gt;/pIC&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>In vivo activity</th>
<th>Ref&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD8529</td>
<td>7-methyl-5-[(3-piperazin-1-ylmethyl)-1,2,4-oxazolidin-5-yl]-2-[(trifluoromethoxy)phenyl]methyl]-3H-isindol-1-one</td>
<td>R2 PAM</td>
<td>hR2: 6.4</td>
<td>Addiction (alcohol, nicotine, methamphetamine)</td>
<td>1</td>
</tr>
<tr>
<td>BINA</td>
<td>4-[2-(cyclopentyl)-6,7-dimethyl-1-oxo-2,3-dihydroindene-5-yl]oxymethyl]phenyl]benzoic acid</td>
<td>R2 PAM</td>
<td>agonist</td>
<td>hR2: 8.4-6.2; rR2: 7; rR5(NAL): 4.7</td>
<td>Anxiolytic, antipsychotic-like, sleep-wake modulation, addiction (cocaine), cognition, S&lt;sub&gt;2&lt;/sub&gt; negative symptoms, mania</td>
</tr>
<tr>
<td>CPIBES</td>
<td>N-[4-cyano-biphenyl-3-yl]-N-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride</td>
<td>R2 PAM</td>
<td>hR2: 7.0</td>
<td>Antipsychotic-like, locomotor effects, antipanic</td>
<td>3</td>
</tr>
<tr>
<td>JNJ-46281222/</td>
<td>3-cyclopentyl)methyl-7-(3-phenylpiperidin-1-yl)methyl]-8-(trifluoromethyl)[1,2,4]triazolo[4,3-a]pyridine</td>
<td>R2 PAM</td>
<td>hR2: 8.8-9.3</td>
<td>No reported in vivo activity</td>
<td>4</td>
</tr>
<tr>
<td>LY2389575</td>
<td>(S)-1-(5-bromopyrimidin-2-yl)-N-(2,4-dichlorobenzyl)pyrrolidin-3-amine methanesulfonate hydrate</td>
<td>R3 NAM</td>
<td>hR3: 6.7-5.4; hR2: 4.8</td>
<td>No reported in vivo activity</td>
<td>5</td>
</tr>
<tr>
<td>JNJ-42153605</td>
<td>3-cyclopentyl)methyl-7-(3-phenylpiperidin-1-yl)-8-(trifluoromethyl)[1,2,4]triazolo[4,3-a]pyridine</td>
<td>R2 PAM</td>
<td>hR2: 7.8-6.6</td>
<td>Sleep/wake modulation, antipsychotic-like, locomotor effects, anticonvulsant</td>
<td>6</td>
</tr>
<tr>
<td>JNJ-40411813</td>
<td>(ADX71149)</td>
<td>1-butyl-3-chloro-4-(4-phenyl-1-piperidinyl)-2(HH)pyridinone</td>
<td>R2 PAM</td>
<td>hR2: 7.2-6.8; 5-HT&lt;sub&gt;2A&lt;/sub&gt; (antag): 6</td>
<td>Antipsychotic-like, locomotor effects, anticonvulsant, sleep modulation</td>
</tr>
<tr>
<td>LY487379 (4-MPTPS)</td>
<td>2,2,2-trifluoro-N-[4-(2-methoxyphenyl)phenyl]-N-[3-pyridinylmethyl]-ethanesulfonamide</td>
<td>R2 PAM</td>
<td>hR2: 7.6-3</td>
<td>Anxiolytic, antipsychotic-like, cognition</td>
<td>8</td>
</tr>
<tr>
<td>ML289 (VU463597)</td>
<td>[3(R)-3-hydroxy(methyl)-1-pipridinyl][4-[2-(4-methoxyphenoxy)]phenyl]methylane</td>
<td>R3 NAM</td>
<td>hR3: 6.2-5.8</td>
<td>Centrally active, no reported in vivo efficacy</td>
<td>9</td>
</tr>
<tr>
<td>ML337</td>
<td>[2-fluoro-4-[2-(4-methoxyphenyl)ethyl]phenyl][3(R)-3-hydroxy-1-piperidinyl]methane</td>
<td>R3 NAM</td>
<td>hR3: 7.1; rR5(NAL): 5.7</td>
<td>No reported in vivo activity</td>
<td>10</td>
</tr>
<tr>
<td>MNI-137</td>
<td>4(S)-bromo-5-oxo-3,4,5,6-tetrahydro-1,6-benzodiazocin-2-yl)pyridine-2-carbonitrile</td>
<td>R2/N3 NAM</td>
<td>inverse agonist</td>
<td>hR2: 7.5-6.2; hR2: 8.1-7.1; rR3: 7.7</td>
<td>No reported in vivo activity</td>
</tr>
<tr>
<td>Ro64-5229</td>
<td>(Z)-1-[2-cyclopehlyoxy-2-(2,6-dichlorophenyl)ethyl]-1H-1,2,4-triazole</td>
<td>R2 NAM</td>
<td>hR3: 7.0</td>
<td>No reported in vivo activity</td>
<td>12</td>
</tr>
<tr>
<td>TASP0433864</td>
<td>(2S)-2-[4-(1,1-dimethylthethyl)phenoxymethyl]-2,3-dihydro-5-methylimidazole</td>
<td>R2 PAM</td>
<td>hR2: 7.1-6.7; hR2: 6.6; hR3: &lt;5; 5-HT&lt;sub&gt;2A&lt;/sub&gt;: 5.4</td>
<td>Antipsychotic-like</td>
<td>13</td>
</tr>
</tbody>
</table>

IUPAC, International Union of Pure and Applied Chemistry; MAO-B, monoamine oxidase B; p<sub>K<sub>a</sub></sub>, negative logarithm of the equilibrium dissociation constant for an allosteric ligand; pEC<sub>50</sub>, negative logarithm of the agonist or PAM concentration required to give the half-maximal response (activation or potentiation); pIC<sub>50</sub>, negative logarithm of the NAM concentration required to give the half-maximal inhibition; * indicates a known off target activity.

[a] Where affinity estimates were unavailable pIC<sub>50</sub> (NAMs) or pEC<sub>50</sub> (PAMs) values are reported indicated by italics.

[b] 1) Caprioli et al., 2015; Justinova et al., 2015; Augier et al., 2016; Li et al., 2016; Doornbos et al., 2017; 2) Galici et al., 2006; Benneyworth et al., 2007; Ahnaou et al., 2009; Hackler et al., 2010; Jin et al., 2010; Hikichi et al., 2013; Farinha et al., 2015; Doornbos et al., 2016, 2017; Kawaura et al., 2016; Panaccione et al., 2017; Perez-Benito et al., 2017; Hellyer et al. 2018; O'Brien et al., 2018; 3) Johnson et al., 2005, 2013; Fell et al., 2015; Benveniga et al., 2018; 4) Farinha et al., 2015; Doornbos et al., 2016; Perez-Benito et al., 2017; 5) Caraci et al., 2011; Scheffer et al., 2012; 6) Cid et al., 2012; Megens et al., 2014; Ahnaou et al., 2015; Metcalf et al., 2017. 7) Cid et al., 2014; Lavedreyes et al., 2015; Ahnaou et al., 2016a; Metcalf et al., 2017. 8) Johnson et al., 2005; Galici et al., 2005; Harich et al., 2007; Nikiforuk et al., 2010; Wiersnau et al., 2012b; Farina et al., 2015; Lundstrom et al., 2016. 9) Sheffer et al., 2012. 10) Wenzhur et al., 2014; Hellyer et al. 2018. 11) Hemstapat et al., 2007; Yin et al., 2014; O'Brien et al., 2018. 12) Kolaczewski et al., 1999; Gutzeit et al., 2013. 13) Hiyoshi et al., 2014.

Acetylene scaffold, such as N-cyclobutyl-6-[2-(3-fluorophenyl)ethyl]-3-pyridinecarboxamide hydrochloride (VU0360172) and N-[1-methyl-5-(4-pyridin-4-ethylanil-3-yl)-phenyl]benzamidamide (CDPPB), N-[4-chloro-2-(phthalimidomethyl)phenyl]salicylamide (CPPHA) were identified from high-throughput screening using functional assays (O'Brien et al., 2003, 2004; Lindsley et al., 2004; Liu et al., 2008). Structurally diverse mGlul receptor PAM scaffolds compete for the common allosteric site within the 7TM used by MPEP (Gregory et al., 2013b, 2014); however, select PAMs [e.g., CPPHA and 4-butoxy-N-(2,4-difluorophenyl)benzamidine] are thought to interact with distinct but as yet unknown site(s) within the 7TM (O'Brien et al., 2004; Chen et al., 2008; Hammond et al., 2010; Noetzel et al., 2013). Mechanistically, mGlul receptor PAMs are largely considered to potentiate mGlul receptor activity in response to glutamate via efficacy modulation (Gregory et al., 2012); however, probe dependence can dictate the nature of these allosteric interactions with multiple PAMs reported to enhance [3H]quisqualate binding (Bradley et al., 2011; Koehl et al., 2019) as well as show different magnitudes of cooperativity depending on the orthosteric agonist used (Sengmany et al., 2017). In this respect the largest magnitude of potentiation observed...
in mGlu$_5$ receptor functional assays is ~20-fold shift in glutamate potency for 1-(4-(2,4-difluorophenyl)piperazin-1-yl)-2-((4-fluorobenzyl)oxy)ethan-1-one (DPFE) and 5-fluoro-2-[(3S,6R)-1-[(4-fluorophenyl)carbonyl]-6-methylpiperidin-3-yl]-1,2,4-oxadiazol-5-yl)pyridine (Gregory et al., 2013a; Parmentier-Batteur et al., 2014). Indeed, the magnitude of cooperativity was the best predictor of in vivo efficacy of an mGlu$_5$ receptor PAM series using the amphetamine-induced hyperlocomotion assay, when total and free brain and plasma concentrations were determined from the same rats (Gregory et al., 2019). Select mGlu$_5$ receptor PAMs have intrinsic agonist efficacy; however, in some cases this is linked to high receptor reserve in recombinant systems (Noetzel et al., 2012). However, this is not always the case, with some compounds, for example, DPFE, showing intrinsic efficacy in low expression and native cell systems (Gregory et al., 2013a; Sengmany et al., 2017), and may

### TABLE 5
Pharmacology of commercially available group III mGlu allosteric ligands

<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC name</th>
<th>Mechanism of Action</th>
<th>Selectivity ($p$K$<em>A$, $p$EC$</em>{50}$/pIC$_{50}$)</th>
<th>In vivo activity</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADX71743</td>
<td>6-(2,4-dimethylphenyl)-2-ethyl-4,5,6,7-tetrahydro-1,3-benzoxazol-4-one</td>
<td>R7 NAM inverse</td>
<td>hR7: 7.2-6.4; rR7: 7.1</td>
<td>Antipsychotic-like; anxiolytic, procognitive; analgesic</td>
<td>1</td>
</tr>
<tr>
<td>ADX8178</td>
<td>5-methyl-N-(4-methylpyrimidin-2-yl)-4-(1H-pyrazol-4-yl)thiazol-2-amine</td>
<td>R4 PAM agonist</td>
<td>hR4: 7.4; rR4: 8.0-7.9; R8 (PAM): 5.7; hA$_{AR}$: 5.7</td>
<td>Anti-inflammatory, anxiolytic; antidepressant, antipsychotic-like; anti-Parkinsonian</td>
<td>2</td>
</tr>
<tr>
<td>AMN-082</td>
<td>N,N'-dibenzyldihydroxy-1,3-diamine dihydrochloride</td>
<td>R7 agonist</td>
<td>hR7: 7.2-7.1; NET: 5.9; *metabolite SERT/NET/DAT: 6.5-5.5</td>
<td>Antidepressant, motor effects, antiaaddiction (alcohol, cocaine, opiates)</td>
<td>3</td>
</tr>
<tr>
<td>AZ12216052</td>
<td>2-[[4-(bromophenyl)methyl]sulfonyl]-N-[(4-(butan-2-yl)phenyl]acetamide</td>
<td>R8 PAM</td>
<td>hR8: 6; rR8:5.4; R5 (NAL agonist): 5.4</td>
<td>Anxiolytic, analgesia in neuropathic pain</td>
<td>4</td>
</tr>
<tr>
<td>Lu AP21934</td>
<td>(1S,2R)-2-[(aminooxy)methyl]-N-(3-chloro-4-fluorophenyl)-1H-pyrazolo[4,3-b]pyridin-3-amine</td>
<td>R4 PAM</td>
<td>rR4: 5.9</td>
<td>Antipsychotic-like</td>
<td>5</td>
</tr>
<tr>
<td>MPPiP</td>
<td>6-(4-methoxyphenyl)-5-methyl-3-(4-pyridyl)-1-isoxazolo[4,5-c]pyridin-4(5H)-one</td>
<td>R7 NAM inverse</td>
<td>hR7: 7.6-6.7; hR7: 6.5-6.2</td>
<td>Analgesia; symptomatic relief in neuropathic pain model (antidepressive, anxiolytic, cognition); impaired cognition and social interaction</td>
<td>6</td>
</tr>
<tr>
<td>PHCC</td>
<td>N-phenyl-7-(hydroxymino)cyclopropa[c]benzoxen-1a-carboxamide</td>
<td>R4 PAM</td>
<td>hR4: 5.6; R5; hR5: &lt;5;</td>
<td>Anti-Parkinsonian; impulsivity</td>
<td>7</td>
</tr>
<tr>
<td>TCN238</td>
<td>(E)-4-(2-phenylethynyl)-2-pyrimidinamine</td>
<td>R4 PAM agonist</td>
<td>rR4: 5.3; hR4: 6.1</td>
<td>Anti-Parkinsonian; neuropathic pain; anxiolytic; autism spectrum disorder symptoms (OPRM1$^*$); bladder cancer</td>
<td>8</td>
</tr>
<tr>
<td>VU0155041</td>
<td>(1R,2S)-2-[[3,3-dichlorophenyl]carboxamido]cyclohexane-1-carboxylic acid</td>
<td>R4 PAM agonist</td>
<td>rR4: 7; hR4: 6.6</td>
<td>Anti-Parkinsonian</td>
<td>9</td>
</tr>
<tr>
<td>VU0361737</td>
<td>N-(4-chloro-3-methylphenyl)-2-pyridinecarboxamide</td>
<td>R4 PAM</td>
<td>hR4: 7.7</td>
<td>Not suitable for in vivo dosing</td>
<td>10</td>
</tr>
<tr>
<td>VU0364770</td>
<td>N-(3-chlorophenyl)pyroli nidine</td>
<td>R4 PAM</td>
<td>hR4: 6; rR4: 6.5; MAO-A: 5.1; MAO-B: 6.1; rR5(NAM): 4.7; hR6(PAM): 5.2</td>
<td>Anti-Parkinsonian</td>
<td>11</td>
</tr>
<tr>
<td>VU0418506</td>
<td>N-(3-chloro-4-fluorophenyl)-4H-pyrazol[4,3-b]pyridin-3-amine</td>
<td>R4 PAM</td>
<td>rR4: 7; hR4: 7.3;</td>
<td>Anti-Parkinsonian</td>
<td>12</td>
</tr>
<tr>
<td>VU0422896</td>
<td>N-(4-chloro-3-methylphenyl)oxazol-5(4H)-one</td>
<td>Pan-III PAM</td>
<td>R4: 7.1-6.5; R7: 7.0-6.3; R8: 6.8-6.2</td>
<td>Rescues deficits in Rett models</td>
<td>13</td>
</tr>
<tr>
<td>VU6005649</td>
<td>3-(2,3-difluoro-4-methoxyphenyl)-2,5-dimethyl-7-(3 trifluoromethyl)pyrazol-1,5-c-pyrimidine</td>
<td>R7 PAM</td>
<td>R7: 6.2; R8(PAM): 5.6; NK1 (antag): 6.2</td>
<td>Procognitive</td>
<td>14</td>
</tr>
<tr>
<td>XAP044</td>
<td>7-hydroxy-4-(3-iodophenyl)(7-oxo-1a,4H)chromene-4-carboxamide</td>
<td>R7 NAM</td>
<td>hR7a: 5.6; hR7b: 5.5; R5: &lt;4.7; R8: &lt;4.5</td>
<td>Symptomatic relief in neuropathic pain model (antidepressive, analgesic)</td>
<td>15</td>
</tr>
</tbody>
</table>

DAT, dopamine transporter; IUPAC, International Union of Pure and Applied Chemistry; MAO-A, monoamine oxidase A; MAO-B, monoamine oxidase B; NRT, norepinephrine transporter; OPRM1, gene encoding the mu opioid receptor; pK$_A$, negative logarithm of the equilibrium dissociation constant for an allosteric ligand; pEC$_{50}$, negative logarithm of the agonist or PAM concentration required to give the half-maximal response (activation or potentiation); pIC$_{50}$, negative logarithm of the NAM concentration required to give the half-maximal inhibition; SERT, serotonin transporter. *indicates a known off target activity.

*Where affinity estimates were unavailable pIC$_{50}$ (NAMs) or pEC$_{50}$ (PAMs) values are reported indicated by italics.

1) Kalinichev et al., 2013; Moloney et al., 2015; Cieslik et al., 2018. 2) Le Poul et al., 2012; Yin et al., 2013; Kalinichev et al., 2014; Pennazhagan et al., 2016; Volpi et al., 2016. 3) Mitsuoka et al., 2005; Palucha et al., 2007; Saling et al., 2008; Li et al., 2009, 2010; Sukoff Rizzo et al., 2011; Bahi et al., 2012; O'Connor and Cryan, 2013; Palucha-Poniewier and Pile, 2013; Jenda et al., 2015; Podkowa et al., 2018. 4) Davison et al., 2010, 2011; Rossi et al., 2014; Hellyer et al., 2015. 5) Schwaitska et al., 2013; Yin et al., 2014. 6) Suzuki et al., 2007a; Hikichi et al., 2010a; Palazzo et al., 2015; Cieslik et al., 2018. 7) Annoura et al., 1996; Maj et al., 2003; Marino et al., 2003b; Stachowicz et al., 2004, 2006; Iacovelli et al., 2006; Klak et al., 2007; Beqalari and Kammermeier, 2008; Goudet et al., 2008; Ngomba et al., 2009; Szczurowska and Mars, 2012; Yin et al., 2013, 2014; Postiainen et al., 2015; Hellyer et al., 2018. 8) Felt and Gerlach, 2010; Isherwood et al., 2017. 9) Niwender et al., 2008b; Davison et al., 2011; Wang et al., 2011; Betts et al., 2012; Yin et al., 2013, 2014; Becker et al., 2014; Zhang et al., 2019. 10) Engers et al., 2009. 11) Engers et al., 2010. 12) Jones et al., 2012; Iderberg et al., 2015. 13) Engers et al., 2016; Niwender et al., 2016. 14) Jahan-Sakrikar et al., 2014; Glogiotti et al., 2017. 15) Abe et al., 2017. 16) Gee et al., 2014; Palazzo et al., 2015.
also be dependent on measure of receptor activation, where mGlu5 receptor PAMs often activate mGlu5-ERK1/2 phosphorylation at concentrations that do not elicit iCa2+ mobilization responses (Gregory et al., 2012; Rook et al., 2013). Despite the complexity in pharmacology, successful discovery efforts for multiple centrally active mGlu5 receptor PAMs have established proof-of-concept for procognitive and antipsychotic efficacy of mGlu5 potentiators (Table 3). However, on-target adverse effect liability has been associated with multiple scaffolds, which has been attributed in part to intrinsic agonist activity and/or magnitude of cooperativity (Rook et al., 2013; Parmentier-Batteur et al., 2014). Recent studies have challenged these conclusions (Rook et al., 2015b; Sengmany et al., 2017), suggesting that biased pharmacology of mGlu5 receptor PAMs may be linked to adverse versus therapeutic effects (discussed in detail below).

E. Group II PAMs and NAMs

Discovery and validation of group II mGlu receptor allosteric ligands has benefited from the availability of radiolabeled orthosteric agonists and antagonists owing to the generally higher affinity of orthosteric ligands for group II mGlu receptors (Table 1). Indeed, multiple different mGlu2 receptor-selective PAMs have been disclosed and established proof-of-concept for mGlu2 receptor potentiation as a viable therapeutic intervention for anxiety, psychosis, and addiction (Table 4). The first mGlu2 receptor-selective PAM was 2,2,2-trifluoro-N-[4-(2-methoxyphenoxy)phenyl]-N-(3-pyridinylmethyl)ethanesulfonamide (LY487379; also referred to as 4-MPPTS) (Johnson et al., 2003), which enhances radiolabeled orthosteric agonist ([3H]LY354740 and [3H]DCG-IV) binding (Schaffhauser et al., 2003; Lundström et al., 2016), orthosteric agonist affinity (Johnson et al., 2005), and functional activity, although LY487379 shows probe dependence as it is neutral with respect to affinity of LY379268 (a high affinity orthosteric agonist) (Johnson et al., 2005). Furthermore, LY487379 has neutral cooperativity with respect to orthosteric antagonist binding (Schaffhauser et al., 2003; Lundström et al., 2016), orthosteric agonist affinity (Johnson et al., 2005), and functional activity, although LY487379 shows probe dependence as it is neutral with respect to affinity of LY379268 (a high affinity orthosteric agonist) (Johnson et al., 2005). Furthermore, LY487379 has neutral cooperativity with respect to orthosteric antagonist binding (Schaffhauser et al., 2003; Johnson et al., 2005), suggesting LY487379 preferentially interacts with the active

Fig. 7. Structures of select allosteric modulators of group I mGlu receptors. Representative allosteric modulators for mGlu1 (A) and mGlu5 (B) demonstrating the structural diversity for compounds that recognize the common allosteric site within the 7TM. In both panels NAMs are labeled with black text, and PAMs are in blue. Detailed in vitro and in vivo pharmacological profiles for each compound including subtype selectivity are listed in Tables 2 and 3. The following abbreviation is used: RO 01-6128, ethyl N-[2,2-diphenylacetyl]carbamate.

Fig. 8. Structures of select allosteric modulators of group II and III mGlu receptors. Structurally diverse chemotypes allosterically modulate group II (A) and group III (B) mGlu receptors. NAMs are labeled with black text, and PAMs are in blue. Detailed in vitro and in vivo pharmacological profiles for each compound including subtype selectivity are listed in Tables 4 and 5. The following abbreviation is used: LY2389575, (3S)-1-(5-bromopyrimidin-2-yl)-N-(2,4-dichlorobenzyl) pyrrolidin-3-amine methanesulfonate hydrate.
receptor state. A similar pharmacological profile has been noted for mGLu2 receptor-selective PAMs from different chemotypes [including 4-[2-(2-cyclopentyl-6,7-dimethyl-1-oxo-2,3-dihydroinden-5-yl)oxy]methyl]phenyl]benzoic acid (BINA), 5-[7-chloro-2-((S)-1-cyclopropyl-ethyl)-1-oxo-2,3-dihydro-1H-isooindol-5-yl]isoazole-3-carboxylic acid dimethylamide, and 8-chloro-3-(cyclopropylmethyl)-7-[4-(3,6-difluoro-2-methoxyphenyl)piperidin-1-yl]-[1,2,4] triazolo[4,3-a]pyridine-7-one, which also potentiate glutamate binding and efficacy but are neutral with respect to orthosteric antagonist binding (O’Brien et al., 2018), although the recently disclosed mGLu2 receptor modifier, (S)-2-[1,1-dimethyl-indan-5-yl]oxymethyl]-2,3-dihydro-oxazolo[3,2-a]pyridine-7-one, has even more pronounced probe dependence, potentiating glutamate affinity but inhibiting [3H]LY341495 binding (Griebel et al., 2016). Further insights into the mechanism of action of mGLu2 receptor PAMs have been elucidated using radiolabeled mGLu2 receptor PAMs [2,2,2-trifluoro-N-(3-pentan-2-yloxy)phenyl]-N-(pyridin-3-ylmethyl)ethanesulfonamide, compared with orthosteric radioligands, radiolabeled mGLu2 receptor PAMs recognize fewer binding sites, which is thought to indicate occupation of a single 7TM domain within the dimeric receptor as well as preferential binding to active receptor conformations since orthosteric agonists can increase the number of mGLu2 receptor PAM binding sites (Lundström et al., 2009, 2011, 2016; Lavreysen et al., 2013; Doornbos et al., 2016; O’Brien et al., 2018). Most mGLu2 receptor PAMs have intrinsic agonist efficacy (Table 4) with the maximal degree of potentiation observed for glutamate potency of between 10- and 30-fold (Johnson et al., 2005; Galici et al., 2006; Lavreysen et al., 2013; Campo et al., 2011; O’Brien et al., 2018). In addition to increased selectivity over mGLu3 relative to orthosteric agents, mGLu2 receptor PAMs may also provide improved therapeutic efficacy owing to reduced capacity for induction of tolerance compared with group II receptor orthosteric agonists (Ahnaou et al., 2015). In contrast to successful mGLu2 receptor PAM discovery campaigns, to date, mGLu3 receptor-selective PAMs have remained elusive.

Discovery efforts for group II mGLu receptor NAMs have yielded both mGLu2 and mGLu3 receptor subtype-selective ligands, although there is less structural diversity available when compared with mGLu2 receptor PAMs (Fig. 8; Table 4). Negative allosteric modulators of group II mGLu receptors have demonstrated efficacy for improving cognitive deficits and reversing behaviors in preclinical models for depression and anxiety (Woltering et al., 2010; Campo et al., 2011; Goeldner et al., 2013; Engers et al., 2015, 2017) and are neuroprotective under ischemic insult (Motoles et al., 2015). Mechanistically, group II receptor NAMs [including 4-(8-bromo-5-oxo-3,4,5,6-tetrahydro-1,6-benzodiazoicin-2-yl)pyridine-2-carbonitrile (MNI-137), 4-[2-(2,6-dimethylpyridin-4-yl)phenyl]-7-methyl-8-(trifluoromethyl)-1,3-dihydro-1,5-benzodiazezip-2-one, 5-[2-(7-trifluoromethyl)-5-[4-(trifluoromethyl)phenyl]pyrazolo[1,5-a]pyrimidin-3-yl]ethyl)pyridin-2-amine (decogurant), and related compounds] are neutral with respect to glutamate affinity, primarily acting as negative modulators of glutamate efficacy (Hemstap et al., 2007; Campo et al., 2011; O’Brien et al., 2018). Akin to observations with group II receptor PAMs, select NAMs have demonstrated probe dependence with respect to modulation of orthosteric agonist affinity, where ligands related to 4-[3-(2,6-dimethylpyridin-4-yl)phenyl]-7-methyl-8-(trifluoromethyl)-1,3-dihydro-1,5-benzodiazezip-2-one or decogurant are NAMs with respect to [3H]LY354740 binding (Woltering et al., 2008; Lundström et al., 2011). For the majority of group II, mGLu receptor NAMs, including [2-fluoro-4-[2-(4-methoxyphenyl)ethyl]phenyl]|3R)|-3-hydroxy-1-piperidinyl)methanone (ML337), MNI-137, and decogurant, are full NAMs, completely abolishing the functional response to orthosteric agonists at both mGLu2 and mGLu3 receptors (Caraci et al., 2011; Wenthur et al., 2014; O’Brien et al., 2018). However, MNI-137 has differing degrees of negative cooperativity in functional assays of mGLu2 receptor activity, in some instances showing full blockade but in others limited negative cooperativity (Hemstap et al., 2007; Yin et al., 2014; O’Brien et al., 2018). Furthermore, both the reported selectivity and inhibitory activity of mGLu2 receptor NAM (3S)-1-(5-bromopyrimidin-2-yl)-N-(2,4-dichlorobenzyl) pyrrolidin-3-amine methanesulfonate hydrate differs depending on the response measured (Caraci et al., 2011; Sheffler et al., 2012). In this respect, it is worth noting that for many ligands and series there has been limited pharmacological profiling to fully discern mechanism of action. Subtype-selective mGLu2 receptor [e.g., 6-((2S,6R)-2,6-dimethylmorpholinyl)methyl]-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 4-(2-fluoro-4-methoxyphenyl)-7-(2-(2-methylpyrimidin-5-yl)ethyl)quinoline-2-carboxamide, 4-(6-bromo-5-oxo-3,4,5,6-tetrahydro-1,6-benzodiazoicin-2-yl) pyridine-2-carbonitrile] or mGLu3 receptor [(3R)-3-(hydroxymethyl)-1-pipridinyl][4-[2-(4-methoxyphenyl)ethyl]phényl]methanone, (S)-1-(4-fluorophenyl)-4-(2-phenoxypropoxy)pyridin-2(1H)-one] NAMs have been reported (Kolczewski et al., 1999; Sheffler et al., 2012; Felts et al., 2015; Walker et al., 2015; Engers et al., 2017). However, the group II receptor selectivity of ML337 was recently demonstrated to be in part attributable to cooperativity, as this ligand is an NAL at mGLu3 (Hellyer et al., 2018). Whether other reportedly subtype-selective NAMs are also due to cooperativity rather than affinity remains to be elucidated.

Both selective and pan-group II receptor NAMs from diverse scaffolds interact with a common or overlapping site with that used by mGLu2 receptor PAMs within the 7TM domain (Schaffhauser et al., 2003; Rowe et al., 2008; Lundström et al., 2011, 2016; O’Brien et al., 2018). Select amino acid residues within this common site can differentially influence group II receptor NAM versus
PAM activity (Hemstapat et al., 2007; Lundström et al., 2011; Perez-Benito et al., 2017). These differential effects may be attributable to differential effects on cooperativity or affinity whereby distinct ligand-receptor interactions may contribute to active versus inactive receptor conformations. However, for some scaffolds [e.g., 8-chloro-3-(cyclopropylmethyl)-7-(4-(3,6-difluoro-2-methoxyphenyl)piperidin-1-yl)-[1,2,4]triazolo[4,3-3-(cyclopropylmethyl)-7-(4-(3,6-difluoro-2-methoxyphenyl) conformations. However, for some scaffolds [e.g., 8-chloro-6-((2S,6R)-2,6-dimethylmorpholinomethyl)-1-(4-fluorophenyl))-4-oxo-1,4-dihydroquinoiline-3-carboxamide, 4-(2-fluoro-4-methoxyphenyl)-7-(2-(2-methylpyrimidin-5-yl) ethyl)quinoiline-2-carboxamide] allosteric interactions have been observed with a mGlu2 receptor PAM radioligand, indicative of multiple allosteric sites within the 7TM (O'Brien et al., 2018) or possibly more complex interactions due to the dimeric nature of mGlu receptors as has been noted for other class C GPCR allosteric modulators (Gregory et al., 2018).

F. Group III PAMs and NAMs

A list of the some of the commercially available allosteric modulators of group III mGlu receptors is provided in Table 5. The first identified group III mGlu receptor-selective allosteric modulator was N-phenyl-7-(hydroxymino)cyclopropan[b]chromen-1a-carboxamide (PHCCC) (Maj et al., 2003; Marino et al., 2003b) (Fig. 8). PHCCC acts as an mGlu4 receptor PAM, increasing potency and efficacy of glutamate or L-AP4 in cell-based assays. PHCCC is closely related to the mGlu1 receptor-selective NAM CPCCOEt (Annoura et al., 1996) (Fig. 7). Although it has weak potency and poor solubility, PHCCC provided a very useful tool to demonstrate the therapeutic potential of targeting mGlu4 receptors in Parkinson's disease and paving the way to drug candidates (Charvin, 2018). Indeed, PHCCC potentiated the inhibitory effect of L-AP4 on transmission at the striatopallidal synapse and reversed akinesia in rats (Marino et al., 2003a,b). PHCCC also reduces hyperalgesia in rat models of chronic pain (Goudet et al., 2008). Subsequently, a new mGlu4 receptor PAM named (1R,2S)-2-[(3,5-dichlorophenyl)carbamoyl]cyclohexane-1-carboxylic acid (VU155041) was discovered (Christov et al., 2011), which is more potent and more soluble than PHCCC. Interestingly, VU155041 is an mGlu4 receptor allosteric agonist (PAM agonist), contrary to the pure PAM profile of PHCCC. PHCCC and VU155041 do not compete for the same site (Niswender et al., 2008a). Accordingly, two partially overlapping 7TM binding pockets have been identified in mGlu4 receptors, a shallow and a deep pocket (Rovira et al., 2015). Analysis of the pharmacological properties and binding modes of several mGlu4 receptor PAMs, revealed the intrinsic efficacy and cooperativity of mGlu4 PAMs (both affinity and efficacy modulation of L-AP4 and glutamate) correlate with the binding mode (Rovira et al., 2015). PAMs with intrinsic allosteric agonism bind in the shallow pocket, analogous to the pocket of natural agonists of class A GPCRs, whereas PAMs exhibiting the highest cooperativity with orthosteric agonists bind into a deeper pocket, corresponding to that of mavoglurant in the mGlu5 receptor 7TM crystal structure (Doré et al., 2014) and pointing toward a site topographically homologous to the Na+ binding pocket of class A GPCRs. In preclinical studies, VU0155041 improves symptoms of Parkinson’s disease (Christov et al., 2011), chronic pain (Wang et al., 2011), and autistic-like syndromes (Becker et al., 2014). Foliglurax (PXT002331) is a derivative of PHCCC with good water solubility and high brain exposure after oral administration (Charvin et al., 2017). It is a potent and selective mGlu4 receptor PAM displaying strong anti-Parkinsonian activity in rodent preclinical models of Parkinson’s disease (Charvin et al., 2017) as well as in primates (Charvin et al., 2018a); however, it recently failed to show efficacy in a phase II clinical trial. Several mGlu4 receptor PAMs also exhibit PAM activity on mGlu6 receptors; as yet no selective mGlu4 receptor allosteric modulators have been described.

The first allosteric modulator acting at the mGlu7 receptor to be described was N,N‘-dibenzhydrolyethane-1,2-diamine dihydrochloride (AMN082) (Mitsukawa et al., 2005). Since there was a lack of pharmacological tools to study mGlu7 receptor, this mGlu7 PAM agonist attracted much interest. However, AMN082 presents off-target effects, as it retains activity in mGlu1 receptor knockout mice (Ahnaou et al., 2016b). Indeed, AMN082 is rapidly metabolized in vivo, with the major metabolite being a potent monoamine transporter inhibitor (Sukoff Rizzo et al., 2011). Thus, preclinical results obtained with AMN082 have to be carefully interpreted, since its actions may not be driven solely by mGlu7 receptors. Selective mGlu7 receptor NAMs have been described and may be more adequate for investigating the role of mGlu7 receptor in vivo: 6-(4-[(methoxy)phenyl]-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-c]pyridin-4H-one (MMPiP) (Suzuki et al., 2007b), 6-(2,4-dimethylphenyl)-2-ethyl-4,5,6,7-tetrahydro-1,3-benzoxazol-4-one (ADX71743) (Kalinichev et al., 2013), and 7-hydroxy-3-(4-iodophenox)-4H-chromen-4-one (XAP044) (Gee et al., 2014). Interestingly, the inhibitory activity of MMPiP is context dependent, where MMPiP may not antagonize mGlu7 receptor activity in all cellular contexts. Indeed, MMPiP is unable to block agonist-mediated responses at the Schaffer collateral-CA1 synapse, where mGlu7 receptor is known to modulate neurotransmission (Niswender et al., 2010). ADX71743 is a bioavailable and brain penetrant mGlu7 NAM that induces a robust anxiolytic effect in rodents (Kalinichev et al., 2013). Most small allosteric modulators described so far act via a binding pocket located within the transmembrane domain; however, XAP044 mediates its action through an interaction with the extracellular domain of mGlu7 receptor (Gee et al., 2014). The exact binding pocket of XAP044 is not known at the moment, with chimeric mGlu7/mGlu6 receptors used to map its action to the

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extracellular domains (Gee et al., 2014). Recent efforts aiming to identify novel mGlu7 receptor PAM scaffolds have turned to cheminformatics-based approaches; however, to date these have yielded low potency potentialitters (Tresadern et al., 2017).

Only few mGlu5 receptor allosteric modulators have been identified. 2-[[4-(Bromophenyl)methyl]sulfanyl]-N-[4-(butan-2-yl)phenyl]acetamide (AZ12216052) is an mGlu5 receptor PAM agonist of glutamate at mGlu5 receptors (Duvoisin et al., 2010). In vivo, AZ12216052 is anxiolytic in apolipoprotein E−/− mice (Duvoisin et al., 2011). Another useful pharmacological tool for mGlu5 receptor is VU6005649, a brain penetrant PAM of mGlu7 and mGlu5 receptors that displays in vivo efficacy in a mouse contextual fear conditioning model (Abe et al., 2017). The pan–group III receptor PAM, N-[3-chloro-4-[[5-chloro-2-pyridinyl]oxy]phenyl]-2-pyridinecarboxamide (VU0422288), which has similar affinity for mGlu4, mGlu5, and mGlu5 receptors (Jalan-Sakrikar et al., 2014), rescues deficits (synaptic plasticity and behavioral phenotypes) in a mouse model of Rett syndrome (Gogliotti et al., 2017). VU0422288 also shows probe dependence with respect to both its apparent affinity and magnitude of positive cooperativity (Jalan-Sakrikar et al., 2014). Whether targeting one or multiple of the group III receptor subtypes will best treat this neurologic disorder remains to be explored with subtype-selective pharmacological agents.

G. Allosteric Modulators Progressing to Clinical Trials

With respect to clinical translation of promising preclinical efficacy for mGlu receptor allosteric modulators, mGlu5 NAMs have demonstrated the most progress with multiple agents reaching phase II trials for a variety of indications. Prior to elucidation of its mechanism of action, fenobam was assessed in a small double-blind placebo-controlled study as a single agent for treatment of anxiety compared with diazepam and was reported to have fewer adverse effects (Pecknold et al., 1982). Subsequently, fenobam was trialed in an open-label pilot study for treatment of fragile X syndrome behavioral deficits, where it was well tolerated but lacked efficacy (Berry-Kravis et al., 2009). Multiple phase II trials of mGlu5 receptor NAMs in patients with fragile X syndrome (both adolescents and adults) have now been completed, with bosinglurant (RO4917523) and mavoglurant (AFQ056) being well tolerated, improving behavioral symptoms but failing to meet primary outcomes (Jacquemont et al., 2011; Bailey et al., 2016; Berry-Kravis et al., 2016; Youssef et al., 2018). These failures may in part be attributable to the difficulties associated with study design for indications lacking rigorous criteria for assessment of behavioral symptoms, or a need to stratify patient populations. Basimglurant also failed to show efficacy in primary clinician assessed measures for major depressive disorder, although patient-reported outcomes suggested an antidepressive effect (Quiroz et al., 2016). Mavoglurant entered phase II trials for obsessive-compulsive disorder but was terminated early due to lack of efficacy and a higher incidence of adverse effects (Rutrick et al., 2017). Beyond psychiatric indications, mavoglurant lacked efficacy in treating patients with levodopa-induced dyskinesia (LID) in PD or chorea in patients with Huntington's disease (Reilman et al., 2015; Trenkwalder et al., 2016).

In contrast, dipraglurant (ADX48621) was also assessed in a phase IIa trial for PD-LID, showing promising indications of antidyskinetic efficacy (Tison et al., 2016). Raseglurant (ADX10059) showed antireflux efficacy for gastroesophageal reflux disease (Keywood et al., 2009; Zerbib et al., 2010, 2011), but further development was ultimately discontinued due to liver toxicity concerns.

With respect to modulators of other mGlu receptor subtypes, building on preclinical efficacy in addiction models for nicotine and methamphetamine (Caprioli et al., 2015; Justinova et al., 2015; Li et al., 2016), mavoglurant lacked efficacy as an antipsychotic or for negative symptoms in patients with schizophrenia (Litman et al., 2016). Another mGlu2 receptor PAM, ADX71149/JNJ-40411813, was also trialed for smoking cessation and found to improve attention and memory as well as reverse effects of ketamine (Salih et al., 2015). In a phase II trial of major depressive disorder patients with significant anxiety, JNJ-40411813 as an adjunct therapy to standard of care failed to relieve anxiety (Kent et al., 2016).

A group II receptor NAM, decoglurant, also commenced trials for major depressive disorder; results are yet to be posted, but development was discontinued. The efficacy and safety of an mGlu4 PAM, foliglurax, was recently evaluated in a phase II clinical trials in patients with PD treated with levodopa, experiencing end-of-dose wearing off and levodopa-induced dyskinesia (Charvin et al., 2017, 2018a), but the program was discontinued due to insufficient efficacy. The varying degrees of success and failures with clinical translation for mGlu receptor allosteric modulators are in keeping with high attrition rates for neuroscience targets. Moreover, these results speak to a need to better understand the pharmacological properties of allosteric modulators and harness novel modes of action and activity.

H. Secondary Allosteric Sites within the VFT and 7 TM Domains

The vast majority of allosteric modulators for mGlu receptors are believed to interact with a common allosteric site within the 7TM domain, analogous to the
biogenic amine orthosteric binding pocket of class A GPCRs. However, allosteric modulators interacting at alternate allosteric sites may offer the means to engender unique pharmacological profiles and increased subtype selectivity. Within the context of a full-length dimeric mGlu receptor, there are multiple possible sites to exploit for allosteric ligands. Multiple subtype-selective single domain antibodies, also referred to as nanobodies, have now been described that recognize epitopes within the VFT. For mGlu2, three nanobodies that recognize overlapping epitopes but have different pharmacological properties have been described (Scholler et al., 2017). DN1 recognizes both active and inactive mGlu2 receptors, whereas DN10 and DN13 require active homodimeric mGlu2 receptor states to bind and potentiate orthosteric agonist activity (Scholler et al., 2017). Similarly, a nanobody that recognizes a loop region with lobe 1 of the mGlu5 receptor VFT potentiates agonist binding and function but can also recognize both active and inactive receptor states (Koehl et al., 2019). To date, nanobodies have been used to facilitate structural studies or as biosensors for active receptor conformational changes. For mGlu2 receptors, a monoclonal antibody, MB1/28, binds to the dimeric VFT, inhibiting receptor activation but is able to induce receptor internalization (Ullmer et al., 2012). Beyond antibodies, the naturally sourced sweet protein monellin was recently revealed as an mGlu5 receptor allosteric agonist that is also thought to interact with the N terminus, and interacts allosterically with small molecule allosteric modulators (Chen et al., 2020).

Multiple allosteric sites have been postulated for the mGlu5 receptor 7TM domain; however, the precise location of these secondary allosteric sites has proven elusive (Chen et al., 2008; Hammond et al., 2010; Noetzel et al., 2013). With the recent publication of a full-length cryo-electron microscopy structure of mGlu5 receptors, we now appreciate that the cysteine-rich domain is a stalk that holds the VFT above the 7TM domains and interacts with the second extracellular loop to transmit conformational changes (Koehl et al., 2019). It is tempting to speculate that the inability to identify these secondary pockets may have been due to a monomeric view of the 7TM domain. However, biochemical studies as well as the new structures demonstrate that the 7TM domains themselves dimerize when activated (El Moustaine et al., 2012; Doumazane et al., 2013; Xue et al., 2015; Koehl et al., 2019). Appreciation of the full-length dimeric structure offers the possibility of identifying new allosteric sites to exploit through targeting these newly appreciated interfaces.

VII. Evolving Concepts

A. Biased Modulators

Allosteric modulators elicit potentiation or inhibition through stabilizing different receptor conformations than can be achieved with an orthosteric ligand alone. Therefore, there is the potential that these conformations can give rise to biased pharmacology. Where intrinsic efficacy differs between pathways relative to a reference agonist, this is referred to as biased agonism and can be quantified as discussed previously for biased orthosteric agonists. For mGlu5 receptors, PAM agonists from diverse scaffolds are biased agonists relative to DHPG in both recombinant and native cells; however, the bias profile differs between scaffolds, with 5-[2-[2-(3-fluorophenyl)ethynyl]-N-[(4R)-2-hydroxy-1,2-dimethylpropyl]-2-pyridinecarboxamide, DPFE, and [6,7-dihydro-2-(phenoxy)methyl]oxazolo[5,4-c]pyridin-5(4H)-yl][fluorophenyl]methanone (VU0409551) each exhibiting different bias profiles for mGlu5 receptor signaling and receptor desensitization (Sengmany et al., 2017; Hellyer et al., 2019). However, biased modulation is also possible, where the direction or magnitude of modulation of the same agonist differs between pathways and may manifest either as differential apparent affinity or cooperativity (Sengmany et al., 2019). For structurally diverse mGlu5 receptor PAMs (VU0360172, DPFE, VU0409551), the magnitude of cooperativity with DHPG was lower when measured in IP1 accumulation compared with iCa2+ mobilization (Sengmany et al., 2017). Moreover, DPFE and CDPPB inhibit mGlu5 receptor orthosteric agonist stimulated ERK1/2 phosphorylation in primary cultures (Zhang et al., 2005; Sengmany et al., 2017). Importantly, biased allosteric agonism and potentiation of mGlu5 receptors, as well as probe dependence, are mediated via dynamic interactions within the common allosteric pocket (Hellyer et al., 2020; Sengmany et al., 2020). Allosteric interactions with distinct binding sites would be expected to offer further diversity in these biased pharmacological fingerprints. Furthermore, differential cooperativity has also been noted for mGlu5 receptor NAMs, where N-(3-chloro-2-fluorophenyl)-3-cyano-5-fluoro-benzamide inhibits iCa2+ mobilization but is an NAL with respect to IP1 accumulation (Sengmany et al., 2019). Recent studies indicate biased agonism and modulation of mGlu5 receptor NAMs and PAMs extends to receptor regulatory processes such as internalization and desensitization (Hellyer et al., 2019; Arsova et al., 2020). Another contributing factor to biased modulator pharmacology at mGlu5 receptors may be ligand binding kinetics as suggested by two recent studies on structurally diverse NAMs (Sengmany et al., 2019; Arsova et al., 2020).

Biased pharmacology of mGlu5 receptor PAMs extends beyond second messenger signaling in cultures to intact circuitry. VU0409551 potentiates mGlu5-dependent long-term depression in the hippocampus (Rook et al., 2015b), prefrontal cortex (Ghosal et al., 2017), and nucleus accumbens (Turner et al., 2018) but unlike other PAMs is unable to potentiate DHPG stimulation of mGlu5 receptor-mediated modulation of NMDA receptor currents (Rook et al., 2015b). Differential potentiation of mGlu5 receptor-dependent synaptic
plasticity and modulation of NMDA receptor activity in the hippocampus has also been noted for mGlu5 receptor PAMs structurally unrelated to VU0409551. N-(1,3-Diphenyl-1H-pyrazolo-5-yl)-4-nitrobenzamide (VU29) potentiates hippocampal long-term potentiation but not NMDA receptor currents (Xiang et al., 2019). Within these brain slice electrophysiology experiments, mGlu5 receptor PAM effects are mediated via enhancement of endogenous glutamate tone, or exogenously applied DHPG, suggesting that similar to VU0409551, VU29 has biased cooperativity between these two measures of mGlu5 activity. Another structurally unrelated mGlu5 receptor PAM, N-[4-chloro-2-[(4-fluoro-1,3-dioxoisindol-2-yl)methyl]phenyl]pyridine-2-carboxamide, failed to potentiate DHPG-mGlu5 receptor long-term potentiation and long-term depression in brain slice electrophysiology experiments (Noetzel et al., 2013). For mGlu5 receptor allosteric ligands, biased pharmacology may offer the means to selectively modulate therapeutically beneficial effects while avoiding those linked to adverse effects. To realize this potential, there remains a need to better understand how different mGlu5 receptor signaling and cellular responses are linked to behavioral effects in the whole animal and the translation of these effects to the clinic. By example, it was recently demonstrated for a series of mGlu5 receptor PAMs closely related to VU0409551 that cooperativity with glutamate (for iCa^{2+} mobilization) rather than ligand affinity was predictive of relative efficacy in rats for reversing amphetamine-induced hyperlocomotion (Gregory et al., 2019). However, whether mGlu5 receptor PAM affinity, cooperativity, bias, or agonism proves to be the best predictor for therapeutic efficacy and safety across multiple in vivo measures for different scaffolds remains to be rigorously tested.

Beyond mGlu5 receptors, the mGlu7 receptor-targeting monoclonal antibody, MB1/28, is an NAM for orthosteric agonist inhibition of cAMP accumulation but has intrinsic efficacy for inducing receptor internalization (Ullmer et al., 2012). At mGlu2 receptors, the PAM agonist BINA was found to be a biased agonist relative to LY354740, favoring ERK1/2 phosphorylation over coupling to Gα15 (Hellyer et al., 2020). Furthermore, biased pharmacology can contribute to apparent selectivity of allosteric ligands. Reportedly, selective allosteric ligands across the class C GPCR family were recently shown to either have neutral cooperativity for mGlu5 receptors or have biased pharmacology (Hellyer et al., 2018). Collectively, the potential for biased allosteric ligand pharmacology highlights the importance of considering the assay/system/approach used to define mechanism of action, classify pharmacological effects, and selectivity.

B. Location- and Context-Dependent Pharmacology

Related to the phenomenon of biased allosteric agonism and modulation is the concept of context-dependent pharmacology, including the contribution of “location bias.” Quite simply put, the observed pharmacological effect is influenced by the cellular context within which it is studied. The first report for context-dependent pharmacology was for the mGlu2 receptor NAM, MMPIp, which shows different magnitudes of inhibition of mGlu2 receptor activity for the same agonist between different recombinant cell lines (Niswender et al., 2010). For mGlu5 receptor modulators, both PAMs and NAMs have context-dependent pharmacology, manifested as distinct biased agonism profiles (Sengmany et al., 2017; Hellyer et al., 2019) or differential apparent affinities (Sengmany et al., 2019) or potencies (Jong et al., 2019) of NAMs between recombinant and native cells from different brain regions. Quantitative pharmacological differences between cell types may be a consequence of different stimulus-response coupling efficiencies, the presence or absence of receptor interacting proteins (other GPCRs, transducers, or scaffolding partners), or differences in receptor subcellular compartmentalization and relative accessibility by different ligands.

Group I mGlu receptors associate with different lipid microdomains (Burgueño et al., 2003; Francesconi et al., 2009b), the balance of which can be altered by receptor activation or membrane cholesterol content, which in turn can modulate signaling to ERK1/2 phosphorylation (Kumar et al., 2008, 2013). Furthermore, mGlu5 is also found on intracellular membranes (e.g., nucleus; Jong et al., 2005), with signaling arising from these intracellular sites differing from that elicited by plasma membrane receptors (Jong et al., 2009; Kumar et al., 2012; Purger et al., 2014). Subcellular compartmentalization of mGlu receptors can shape the physiologic responses to orthosteric agonists, particularly for surrogate agonists that cannot access intracellular receptors. Such effects may contribute to observations of probe dependence by allosteric ligands. To date, the influence of allosteric ligands on GPCR subcellular localization (and vice versa) has been relatively unexplored. However, Ca-sensing receptor allosteric ligands can act as “pharmacochaperones” to increase Ca-sensing receptor cell surface expression (White et al., 2009), suggesting that mGlu receptor allosteric ligands may also have the potential to alter receptor location.

In addition to physiologic context differences, the disease state can also impact mGlu receptor signaling and subsequently ligand pharmacology. In the setting of melanoma, mGlu1 loses the capacity to signal via classic G protein pathways; however, glutamate retains the ability to stimulate mGlu1 internalization, which promotes melanoma cell survival (Gelb et al., 2015b). In a preclinical model of Huntington’s disease, the balance of mGlu5 receptor signaling pathways are perturbed, where IP1 accumulation is reduced, but iCa^{2+} mobilization, Akt, and ERK1/2 phosphorylation are increased (Ribeiro et al., 2010). Brain region-specific changes in group I mGlu receptor signaling have also been noted.
after chronic cocaine administration, such that the mGlu5 receptor NAM MPEP becomes an agonist for inducing cAMP response element-binding protein phosphorylation in the nucleus accumbens but not striatum (Hoffmann et al., 2017). Changes in the balance of intracellular signaling responses specific to the disease setting could be exploited by the development of biased ligands; however, they also offer another layer of complexity with respect to designing appropriate discovery pipelines for the translation of biased ligands. Indeed, VU049551, which does not potentiate mGlu5 receptor modulation of NMDA receptor currents in wild-type animals, does potentiate these responses in a genetic model of schizophrenia (Balu et al., 2016). These data highlight the paucity in our understanding of how different diseases and pathologic processes reshape the intracellular responses to mGlu receptors. Parsing out these differences will offer the prospect for rational discovery efforts to tailor therapeutic efficacy to the pathway level to restore neurotransmission to the healthy setting.

C. Heteromerization of mGlu Receptors

Historically, mGlu receptors were believed to form strict homodimers, unlike other class C GPCRs such as the obligatory heterodimeric GABA<sub>B</sub> or sweet or umami taste receptors. The first evidence of mGlu receptor heteromers came from the demonstration that group I receptors can assemble and function together when cotransfected in HEK293 cells but cannot assemble with either group II or group III receptors (Doumazane et al., 2011). This same study demonstrated that heteromers are also formed among group II and group III receptors, prompting investigations into the existence and function of native mGlu receptors heteromers in vivo.

One anticipates that the formation of heterocomplexes by mGlu receptors, where natively expressed in the same cells, should result in specific pharmacological signatures that differ from homomeric receptors. Intriguing pharmacological responses aroused suspicion on the existence of mGlu2-mGlu4 receptor heteromers in rat dorsal striatum where the well established mGlu4 receptor PAM PHCCC failed to potentiate mGlu4 receptor activity at corticostriatal synapses in rat dorsal striatum, whereas VU0155041 retained its expected mGlu4 receptor PAM activity (Yin et al., 2014). In vitro studies confirmed that mGlu4-mGlu7 receptor heteromers are differentially potentiated by mGlu4 receptor PAMs from different scaffolds (Kammermeier, 2012; Yin et al., 2014; Niswender et al., 2016). At the mechanistic level, these pharmacological differences arise from complex asymmetric functioning of mGlu2-mGlu4 receptor heteromers. Indeed, after orthosteric agonist activation, the signaling of mGlu2-mGlu4 receptor heteromer only occurs through the transmembrane domain of mGlu4 receptor (Liu et al., 2017); however, the mGlu2 receptor subunit can signal if potentiated by an mGlu2 receptor PAM. This is reminiscent of previous studies showing that only one subunit is active at a time in an mGlu receptor homodimer (Goudet et al., 2005; Hlavackova et al., 2005). Evidence of the presence of mGlu2-mGlu4 receptor heteromers at this corticostriatal synapses were reinforced by immunoprecipitation studies showing the presence of protein complexes containing mGlu2 and mGlu4 receptors in striatum (Yin et al., 2014). Pharmacological evidence of mGlu2-mGlu4 receptor heteromers has also been detected in lateral perforant path terminals in rat hippocampus (Moreno Delgado et al., 2017).

Recently, mGlu2-mGlu7 receptor heteromers were reported in the hippocampus and mGlu3-mGlu7 receptor heteromers in the cortex (Habrian et al., 2019). Interestingly, further in vitro investigations using a single molecular Förster resonance energy transfer approach revealed that the glutamate affinity and efficacy at mGlu7 receptors are greatly enhanced when associated to an mGlu2 receptor subunit, as compared with the mGlu7 receptor homodimer. Also, association with mGlu2 receptors confers to the mGlu7 receptor subunit the ability to be fully activated by the selective group III agonist LSP4-2022. Of note, previous neuroanatomical study revealed that mGlu2 and mGlu5 receptors may be expressed in the same boutons in the hippocampus (Ferraguti et al., 2005), raising the possibility the mGlu7-mGlu16 receptor heteromers may also be of relevance in the hippocampus. Heterodimerization of mGlu2 receptors with other mGlu receptor subtypes may provide a means to enhance the range of glutamate concentrations sensed by the mGlu7 receptor, which is otherwise insensitive to low glutamate levels.

There is also evidence of mGlu1 and mGlu5 receptors forming complexes in mouse hippocampus and cortex as shown by a knockout-controlled interaction proteomics strategy and further confirmed by immunoprecipitation and superresolution microscopy imaging of hippocampal primary neurons revealing mGlu1-mGlu5 receptor coexpression at the synaptic level (Pandya et al., 2016). Indeed, these data are in keeping with evidence that blockade of both group I receptor subtypes is required to ablate DHPG-induced long-term depression in the hippocampus (Volk et al., 2006). More recently, a single-cell RNA sequencing study revealed the coexpression of different mGlu subtypes within the same cell in the adult mouse cortex (Lee et al., 2020). Notably, most pyramidal cells contained at least four receptor subtypes. Probing the propensity of different mGlu receptors to coassemble by fluorescent-based complementation assays, the authors concluded that mGlu2 and mGlu3 receptors are particularly prone to form heteromers when coexpressed in heterologous cells (Lee et al., 2020). Other prominent mGlu receptor pairs included mGlu2/4, mGlu1/5, mGlu4/7 and mGlu3/7. The coexpression of native mGlu2 and mGlu3 receptors in mouse frontal cortex was confirmed by in situ
hybridization and coimmunoprecipitation (Lee et al., 2020). The prevalence of heteromerization between mGlu receptors adds considerable complexity to understanding and interpreting molecular pharmacological properties of ligands and particularly the notion of selectivity.

Beyond heteromerization with other mGlu receptor subtypes, increasing evidence suggests mGlu receptors form heteromers and larger-order complexes with class A GPCRs, including mGlu2-5-HT2A receptors (González-Maes et al., 2008; Fribourg et al., 2011; Delille et al., 2013; Moreno et al., 2013; Moreno et al., 2016; Felsing et al., 2018), group I receptors with multiple adenosine receptor subtypes (Ciruela et al., 2001; Ferré et al., 2002; Nishi et al., 2003; Domenici et al., 2004; Rodrigues et al., 2005), mGlu5-dopamine D1 receptors (Sebastianutto et al., 2020), mGlu5-dopamine D2 receptors (Ferré et al., 1999; Popoli et al., 2001), and mGlu5-dopamine D2-adenosine A2A receptors (Díaz-Cabiale et al., 2002; Cabello et al., 2009). For each pairing with a class A GPCR, the functional responses arising when receptors are coactivated or coincidentally inhibited changes the pharmacological profile to when mGlu receptor is activated in isolation. By example, heteromerization with dopamine D1 receptors enhances the proportion of mGlu5 receptors in active states, elevating basal Gq coupling and signaling toward iCa2+ mobilization over cAMP pathways (Sebastianutto et al., 2020). Heteromerization is often observed in a cell type– or brain region–specific fashion. In this respect, selectively targeting mGlu receptor heteromers offers the intriguing prospect of achieving tissue-level selectivity of drug action. The study of mGlu receptors heteromers is still in its infancy, and it is clear that further investigations will be needed to better understand its functional consequences in brain function and therapeutic potential.

D. Optical Tools to Probe and Control mGlu Receptors

Irreversible ligands or photoaffinity probes have been widely used to study ligand-receptor interactions and aid structural determinations across diverse protein targets. Such tools have not been available for mGlu receptors; however, recent efforts exploiting selective allosteric chemotypes have proven successful. The first in class were bifunctional mGlu5 receptor NAMs that included a photoactivatable moiety to irreversibly bind receptors and a click chemistry handle to allow secondary attachment of clickable reporter (e.g., fluorophore) for identification (Gregory et al., 2016). Installation of a covalent or photoreactive moiety has been successfully achieved within three distinct mGlu2 receptor PAM scaffolds (Doornbos et al., 2019; Hellyer et al., 2020). The development of covalent or photoactivatable ligands is not without its challenges. Covalent ligands require proximity to an appropriate amino acid for reactivity. Furthermore, the bifunctional clickable photoprobes for mGlu2 and mGlu5 receptors revealed substantial nonspecific interactions, which may limit how these tools can be applied. There has been considerable interest in alternative approaches to optically control mGlu receptor function. Two main strategies exist to control mGlu receptors by light: an optogenetic pharmacology approach based on attached photoswitchable ligands (Fig. 9) and a photopharmacology approach based on freely diffusible light-operated ligands (Fig. 10) (Goudet et al., 2018). The aim of both strategies is to use light to achieve precise spatiotemporal control over receptor activity.

Fig. 9. Optogenetic pharmacology of mGlu receptors. Optogenetic pharmacology consists in covalently attaching a photoswitchable tethered ligand to a genetically modified protein, enabling the photoactivation or photoantagonism of the receptor. Several strategies have been applied to mGlu receptors. (A) Photoswitchable tethered ligands. (B) PORTL. (C) Membrane-anchored PORTL. (D) Antibody-based photocontrol. ST, SNAP-tag; GFP, green fluorescent protein.
Optogenetic Pharmacology

Optogenetic pharmacology consists of covalently attaching a photoswitchable tethered ligand to a genetically modified protein (Kramer et al., 2013), which will then enable the photoactivation or photoantagonism of the receptor. In most cases, the receptor itself is modified to allow anchoring of the photoswitchable ligand, but several variants of this technique have been developed, using 1) a transmembrane protein at the proximity of the receptor or 2) an antibody targeting the receptor (Fig. 9). Optogenetic pharmacology consists of covalently attaching a photoswitchable tethered ligand to a genetically modified protein (Kramer et al., 2013), which will then enable the photoactivation or photoantagonism of the receptor.

Optogenetic pharmacological approaches allows for greater selectivity for studying the functional roles of a target receptor. The attached photoswitchable ligand can rapidly oscillate between activating and inactivating a receptor, thus being a useful optogenetic tool to understand mGlu receptor activation mechanisms (Levitz et al., 2016) or to study synaptic activity of neural circuits with high spatiotemporal resolution and pharmacological specificity. The drawback is the requirement for genetic manipulation, which can limit in vivo application, but this can be circumvented by using a viral infection approach (Acosta-Ruiz et al., 2020).

The first generation of light-controlled mGlu receptors was based on photoswitchable tethered ligands, which contain glutamate linked via a photoisomerizable azobenzene linker to a maleimide that reacts with a free cysteine within the receptor. These molecules, called MAGs, bind covalently to genetically engineered mGlu receptors that possess geometrically appropriate cysteine attachment points (Fig. 9A). Light-controlled mGlu2, mGlu3, and mGlu6 receptors were designed using this strategy. Precise optical control can be achieved in cells, in mouse brain slices, and in living zebrafish (Levitz et al., 2013). An improved spatiotemporal resolution can also be achieved via two-photon activation of light-controlled mGlu receptors (Carroll et al., 2015).

A second generation of light-controlled mGlu receptors has been developed based on photoswitchable orthogonal remotely tethered ligands (PORTL) and the SNAP tag technology (Keppeler et al., 2003) (Fig. 9B). The photoswitchable ligands are composed of a glutamate moiety, followed by a long flexible linker
The PORTL strategy has been applied to mGlu2 receptor with near-complete efficiency (Acosta-Ruiz et al., 2020). Since SNAP tag and CLIP tag possess orthogonal substrate specificities, SNAP- and CLIP-tagged proteins can be labeled simultaneously and specifically with different molecular probes in living cells. This has proven to be a very useful approach to analyze cell surface protein complexes and notably led to the discovery of specific heterodimeric mGlu receptors (Doumazane et al., 2011). By combining SNAP- and CLIP-tagged receptors and specific PORTL, Levitz and colleagues have created a family of light-gated group II/III mGlu receptors (Levitz et al., 2017), allowing multiplexed orthogonal optical control within homo or heterodimers. Optimized branched photoswitchable ligands have recently been developed permitting photoaognism of mGlu receptors with near-complete efficiency (Acosta-Ruiz et al., 2020). The PORTL strategy has been applied to mGlu2 receptor permitting light control of mGlu2 receptor-induced excitability in heterologous cells or transfected neurons (Broichhagen et al., 2015) and, more recently, to control working memory in mice (Acosta-Ruiz et al., 2020).

Alternative optogenetic pharmacology approaches have been developed. For example, tethered ligands have been designed to target a genetically modified plasma membrane protein bearing a SNAP tag, rather than directly targeting the receptor. This new approach is named membrane-anchored PORTL (Donthamsetti et al., 2019). These ligands anchor to the SNAP-tagged protein at the plasma membrane and come into close proximity to their target receptor via lateral diffusion to enable interaction (Fig. 9C). An alternative strategy consists in using ligands tethered to a SNAP-tagged antibody or nanobody targeting the receptor of interest (Fig. 9D). Proof of concept has been established using a nanobody recognizing a green fluorescent protein isomerized to its receptor (Fig. 10A). Therefore, these ligands are inactive photocaged ligands that are turned ON by light, enabling a precise spatiotemporal control of the onset of drug activity. Caged glutamate was developed in the 1990s and was most notably used for mapping neuronal circuits (Callaway and Katz, 1993) or for studying mGlu receptor function (Crawford et al., 1997). However, the use of caged glutamate is somehow limited due to the lack of subtype selectivity, leading to the development of ionotropic and metabotropic glutamate receptor-selective compounds. The first mGlu subtype-selective caged compound is (7-(diethylamino)-2-oxo-2H-chromen-4-yl)methyl (2-((3-fluorophenylethynyl)-4,6-dimethylpyridin-3-yl)carbamate, an inactive photocaged derivative of the mGlu5 receptor NAM raseglurant (Font et al., 2017) (Fig. 10B). The release of raseglurant is induced by a violet illumination, effectively blocking mGlu5 receptor activity in cells or in living mice. Interestingly, the caged compound can be injected systemically in preclinical murine models of chronic pain and uncaged locally by illumination, revealing the analgesic potential of mGlu5 blockade in peripheral tissues or in the thalamus (Font et al., 2017).

Photoswitchable ligands are designed to be rapidly and reversibly switched ON and OFF (Fig. 10C). Typical ligand design comprises incorporating a photoswitchable core into an active moiety that is selectively recognized by the target receptor. The photoswitchable core reversibly photosomerizes at specific wavelengths, modifying the overall structure of the ligand and thus its ability to interact with the target, resulting in the reversible control of a receptor in timed manner driven by light. The most common photoswitchable core used is azobenzene. Azobenzene changes geometry during photosomerization. In the dark or under white light, the azobenzene moiety is in a trans configuration converting to a cis configuration upon illumination with an appropriate wavelength (usually in the UV range). Relaxation to the thermodynamically more stable trans-isomer can be induced by irradiation or by thermal relaxation.

The first allosteric photoswitchable ligand targeting a GPCR is Alloswitch-1, an mGlu5 receptor NAM (Pittolo et al., 2014). An azobenzene was inserted in the core of N-(4-(2-chlorobenzamido)-3-methoxyphenyl) picolinamide, an mGlu4 receptor allosteric ligand having high chemical and structural homology with the pharmacological response kinetics can be slower. As with classic drugs, selectivity, pharmacokinetic, and absorption, distribution, metabolism, and excretion properties are also key considerations (Berizzi and Goudet, 2020). Another limitation is the local delivery of drug and light in vivo; however, this can be achieved using optic fibers coupled to a light source, as recently exemplified (Font et al., 2017; Zussy et al., 2018).

Photocaged ligands, also named photoactivatable ligands, possess a protecting group that can be removed after illumination, enabling the uncaged ligand to bind to its receptor (Fig. 10A). Therefore, these ligands are inactive photocaged ligands that are turned ON by light, enabling a precise spatiotemporal control of the onset of drug activity. Caged glutamate was developed in the 1990s and was most notably used for mapping neuronal circuits (Callaway and Katz, 1993) or for studying mGlu receptor function (Crawford et al., 1997). However, the use of caged glutamate is somehow limited due to the lack of subtype selectivity, leading to the development of ionotropic and metabotropic glutamate receptor-selective compounds. The first mGlu subtype-selective caged compound is (7-(diethylamino)-2-oxo-2H-chromen-4-yl)methyl (2-((3-fluorophenylethynyl)-4,6-dimethylpyridin-3-yl)carbamate, an inactive photocaged derivative of the mGlu5 receptor NAM raseglurant (Font et al., 2017) (Fig. 10B). The release of raseglurant is induced by a violet illumination, effectively blocking mGlu5 receptor activity in cells or in living mice. Interestingly, the caged compound can be injected systemically in preclinical murine models of chronic pain and uncaged locally by illumination, revealing the analgesic potential of mGlu5 blockade in peripheral tissues or in the thalamus (Font et al., 2017).

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scaffold present in azobenzene (Engers et al., 2011), to minimally modify the steric occupancy, binding determinants, and physicochemical properties of the parent compounds. Illumination by green or violet light stabilizes either the trans or the cis configuration of the ligand that corresponds to high and low pharmacological activity, respectively, on heterologous or native cells expressing the mGlu₅ receptor. In vivo, Alloswitch-1 allows light-dependent control of the motility of Xenopus laevis tadpoles (Pittolo et al., 2014). More recently, a series of photoswitchable mGlu₅ receptor NAMs based on the phenylazopyridine scaffold was generated (Gómez-Santacana et al., 2017). Most of the trans-isomers of this series are active both in vitro, inhibiting mGlu₅ receptor function in heterologous cells, and in vivo, photocontrolling zebrafish motility. Optogluram is a derivative of Alloswitch-1, which acts as a photoswitchable mGlu₅ receptor PAM, enabling for selective, reversible, and repeated optical manipulation of mGlu₅ receptor activity (Zussy et al., 2018) (Fig. 10D). Optogluram allows the photocontrol of endogenous mGlu₅ receptor activity in specific brain of freely behaving mice, revealing the dynamic control of pain-related sensory and anxiodepressive symptoms by amygdala mGlu₅ receptors (Zussy et al., 2018). Since UV light could be potentially damaging to irradiated tissues, designing red-shifted photoswitchable ligands has been of considerable interest. Recently, OptoGlurAM4.1, a blue light-sensitive mGlu₅ receptor photoswitchable NAM, was described that is active both in vitro and in vivo, photocontrolling zebrafish larvae mobility or blocking the analgesic activity of an mGlu₅ receptor agonist in a mouse model of chronic pain (Rovira et al., 2016). Manipulating mGlu receptor with high spatial and temporal precision holds great promise for exploring physiologic and pathologic functions. As the field is rapidly evolving, the number of optical tools available will likely increase and provide new means to probe the biologic function of mGlu receptors.

VIII. Conclusion

From the initial identification of mGlu receptors in the early 1990s, the past 30 years have seen rapid progress, with the discovery of novel pharmacological agents and application of chemical, genetic, and optical biology tools to dissect the molecular properties of the eight subtypes. Each of the individual subtypes offers promise as a potential therapeutic target. Excitingly, the intensive drug discovery efforts have led to multiple candidates reaching clinical trials with varied mechanisms of action. Both orthosteric and allosteric ligands offer considerable complexity in their biologic effects, with biased agonism/modulation, context, and probe dependence, coupled with additional complexity presented by mGlu receptor heteromers. Although it poses a challenge for discovery, harnessing this pharmacological and biologic complexity presents new opportunities to precisely tailor the activity of mGlu receptors to maximize therapeutic efficacy and avoid adverse effect liability.

Authorship Contributions

Wrote the manuscript and prepared figures: Gregory, Goudet.
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Metabotropic Glutamate Receptors

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is anesthetic and has improved in vivo selectivity compared with the prototypical antagonist 2-methyl-6-phénylthienylpyrindine.

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