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Molecular Insights into Metabotropic Glutamate Receptor Allosteric Modulation[□]

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ABSTRACT

The metabotropic glutamate (mGlu) receptors are a group of eight family C G protein–coupled receptors that are expressed throughout the central nervous system (CNS) and periphery. Within the CNS the different subtypes are found in neurons, both pre- and/or postsynaptically, where they mediate modulatory roles and in glial cells. The mGlu receptor family provides attractive targets for numerous psychiatric and neurologic disorders, with the majority of discovery programs focused on targeting allosteric

sites, with allosteric ligands now available for all mGlu receptor subtypes. However, the development of allosteric ligands remains challenging. Biased modulation, probe dependence, and molecular switches all contribute to the complex molecular pharmacology exhibited by mGlu receptor allosteric ligands. In recent years we have made significant progress in our understanding of this molecular complexity coupled with an increased understanding of the structural basis of mGlu allosteric modulation.

Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS), eliciting its effects via two

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distinct receptor classes. The ionotropic glutamate receptors are ligand-gated ion channels that mediate fast synaptic responses. The metabotropic glutamate (mGlu) receptors are G protein–coupled receptors (GPCRs) that play a modulatory role in the CNS. Ubiquitously expressed throughout the CNS in glia and neurons, found both pre- and postsynaptically, the mGlu receptors are involved in a broad range of physiologic functions in the brain. Comprised of eight members, the mGlu receptor family is often subclassified into three groups based on homology, pharmacology, and G protein coupling. Group I includes mGlu₁ and mGlu₅ that preferentially couple to G_{q/11} and are typically found postsynaptically. Group II, mGlu₂ and

ABBREVIATIONS: AMN082, *N,N'*-dibenzhydrylethane-1,2-diamine dihydrochloride; BAY36-7620, (3*aS*,6*aS*)-hexahydro-5-methylene-6*a*-(2-naphthalenylmethyl)-1*H*-cyclopenta[*c*]furan-1-one; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; CNS, central nervous system; CPCCOEt, 7-(hydroxyimino)cyclopropa[*b*]chromen-1*a*-carboxylate ethyl ester; CPPHA, *N*-(4-chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phenyl)-2-hydroxybenzamide; DCG-IV, (2*S*,2',3'*R*)-2-(2',3'-dicarboxycyclopropyl)glycine; DFB, ((3-fluorophenyl)methylene)hydrazone-3-fluorobenzaldehyde; DHPG, 3,5-dihydroxyphenylglycine; FITM, 4-fluoro-*N*-(4-(6-(isopropylamino)pyrimidin-4-yl)thiazole-2-yl)-*N*-methylbenzamide; GPCR, G protein–coupled receptor; JNJ-40068782, 3-cyano-1-cyclo-propylmethyl-4-(4-phenyl-piperidin-1-yl)-pyridine-2(1*H*)-one; L-AP4, L-2-amino-4-phosphonobutyrate; LSP4-2022, (2*S*)-2-amino-4-[[4-(carboxymethoxy)phenyl](hydroxy)methyl](hydroxy)phosphoryl)butanoic acid; Lu AF21934, (1*S*,2*R*)-2-[(aminooxy)methyl]-*N*-(3,4-dichlorophenyl)cyclohexane-1-carboxamide; LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; LY379268, (–)-2-oxa-4-minobicyclo[3.1.0]hexane-4,6-dicarboxylate; LY541850, (1*S*,2*S*,4*R*,5*R*,6*S*)-2-amino-4-methylbicyclo[3.1.0]hexane-2,6-dicarboxylic acid; mGlu, metabotropic glutamate; M-5MPEP, 2-(2-(3-methoxyphenyl)ethynyl)-5-methylpyridine; MPEP, 2-methyl-6-(phenylethynyl)pyridine; mPEPy, 3-methoxy-5-(2-pyridinylethynyl)pyridine; NAM, negative allosteric modulator; NCFP, *N*-(4-chloro-2-[(1,3-dioxoisindolin-2-yl)methyl]phenyl)picolinamide; PAM, positive allosteric modulator; pERK1/2, phosphorylated extracellular-signal regulated kinases 1 and 2; PET, positron emission tomography; PHCCC, *N*-phenyl-7-(hydroxyimino)cyclopropa[*b*] chromen-1*a*-carboxamide; R214127, 1-(3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-2-phenylethanone; Ro67-4853, butyl (9*H*-xanthene-9-carbonyl)carbamate; SAR, structure-activity relationships; TM, transmembrane; VFT, venus flytrap domain; VU0092273, 3'-(8-methyl-4-oxo-7-trifluoromethyl-4,5-dihydro-3*H*-benzo[*b*][1,4]diazepin-2-yl)-biphenyl-3-sulfonic acid; VU0155041, (1*R*,2*S*)-2-[(3,5-dichlorophenyl)carbamoyl]cyclohexane-1-carboxylic acid; VU0155094, methyl 4-(3-(2-[(4-acetamidophenyl)thio]acetyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)benzoate; VU0357121, 4-butoxy-*N*-(2,4-difluorophenyl)benzamide; VU0366248, *N*-(3-chloro-2-fluorophenyl)-3-cyano-5-fluorobenzamide; VU0400100, 2-[2-chloro-*N*-methylsulfonyl-5-(trifluoromethyl)anilino]-*N*-(cyclopropylmethyl)acetamide; VU0409551, 5-[(4-fluorophenyl)carbonyl]-2-(phenoxymethyl)-4,5,6,7-tetrahydro[1,3]oxazolo[5,4-*c*]pyridine; VU0422288, *N*-(3-chloro-4-[(5-chloropyridin-2-yl)oxy]phenyl)picolinamide; VU29, *N*-(1,3-diphenyl-1*H*-pyrazolo-5-yl)-4-nitrobenzamide.

mGlu₃; and group III, mGlu₄, mGlu₆, mGlu₇, and mGlu₈, members couple to G_{i/o} and with the exception of mGlu₆, which is exclusively found in the retina, are predominantly located presynaptically. Multiple recent reviews have extensively discussed the roles of mGlu receptors in different neuronal circuits, modulating synaptic transmission, neuronal excitability, and other cellular functions within the CNS (Conn and Pin, 1997; Anwyl, 1999; Valenti et al., 2002; Coutinho and Knopfel, 2002; Bellone et al., 2008; Pinheiro and Mulle, 2008) as well as physiology in the periphery (Julio-Pieper et al., 2011). Based on this understanding, targeting activation or inhibition of specific mGlu receptor subtypes may provide a therapeutic benefit for a range of psychiatric and neurologic disorders. For neuropathic pain, inhibition of mGlu₁ is being pursued as a therapeutic intervention with success reported in preclinical models (Mabire et al., 2005; Bennett et al., 2012). Agonists of group II receptors have reached clinical trials for schizophrenia and anxiety with varying success (Schoepp et al., 2003; Patil et al., 2007; Dunayevich et al., 2008; Kinon et al., 2011). Group II mGlu receptors are also attractive targets for addiction and depression (Ma et al., 2007; Moussawi and Kalivas, 2010; Chaki et al., 2013). Inhibitors of mGlu₅ have demonstrated efficacy in preclinical studies for anxiety, depression, and Parkinson's disease Levodopa-induced dyskinesias (Porter et al., 2005; Li et al., 2006; Belozertseva et al., 2007; Rylander et al., 2010; Hughes et al., 2013). Promisingly, multiple mGlu₅ inhibitors have reached (Berry-Kravis et al., 2009; Keywood et al., 2009; Zerbib et al., 2010) or are currently in phase II clinical trials (www.clinicaltrials.gov) with varying successes and failures for fragile X syndrome, Parkinson's disease Levodopa-induced dyskinesias, gastroesophageal reflux disorder, and migraine. On the other hand, activators and/or potentiators of mGlu₅ have efficacy in preclinical models for schizophrenia and cognition (Moghaddam, 2004; Noetzel et al., 2012a). Activation or potentiation of group III mGlu receptors, in particular mGlu₄, has therapeutic potential for Parkinson's disease (Marino et al., 2003; Jones et al., 2012), with mGlu₄ also a potential target for pain (Vilar et al., 2013) and autism (Becker et al., 2014).

Therapeutic Targeting of mGlu with Allosteric Modulators

The prevailing strategy for GPCR-based drug discovery has been to target the endogenous ligand binding site, referred to as an orthosteric site, to competitively block or mimic the actions of the endogenous ligand. However, multiple receptor families and transporters recognize glutamate, and as such, glutamate binding pockets are highly conserved such that orthosteric ligands often lack sufficient selectivity. Based on this and difficulties in optimizing glutamate analogs as drug candidates, mGlu receptor-based drug discovery programs have turned their attention to alternative strategies to develop more selective agents. One such approach is to target allosteric binding sites that are topographically distinct from the orthosteric site, such that the receptor can be simultaneously bound by more than one ligand. These ligands are referred to as allosteric modulators and have the capacity to perturb the affinity and/or efficacy of an orthosteric ligand. Because allosteric sites are often located in regions of the

receptor that show greater diversity between subtypes, allosteric modulators offer the potential for greater subtype selectivity than their orthosteric counterparts. Allosteric modulators may potentiate or inhibit the affinity and/or efficacy of an orthosteric ligand, a property referred to as “cooperativity.” A potentiator that has positive cooperativity with the orthosteric ligand is a positive allosteric modulator (PAM), whereas an inhibitor is a negative allosteric modulator (NAM). Cooperativity is saturable, therefore allosteric modulators have the theoretical advantage of being safer in the case of overdose, as there is a limit to the modulatory effect. A third class of ligands that interact with allosteric sites but have neutral cooperativity with the orthosteric ligand are called neutral allosteric ligands and represent excellent pharmacological tools for dissecting allosteric modulator pharmacology (Christopoulos et al., 2014). A third advantage of allosteric modulators is the potential to maintain spatial and temporal aspects of receptor activation, i.e., modulation will only occur when and where the orthosteric agonist is present. However, it is important to note that allosteric ligands may possess intrinsic efficacy (either positive or inverse) in addition to, or exclusive of, cooperativity with orthosteric ligands (Annoura et al., 1996; Litschig et al., 1999; Niswender et al., 2008; Duvoisin et al., 2010; Gregory et al., 2012, 2013a; Noetzel et al., 2012b; Lavreysen et al., 2013; Rook et al., 2013). The first allosteric modulator discovered for the mGlu family was CPCCOEt [7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester], an mGlu₁ NAM of multiple orthosteric agonists, including glutamate (Annoura et al., 1996; Litschig et al., 1999). The past 15 years has seen the discovery of selective allosteric modulators for most of the mGlu family, and for many subtypes multiple chemotypes and different classes of allosteric ligands have been disclosed (Tables 1, 2, and 3).

Complexities of mGlu Receptor Allosteric Modulator Pharmacology

Stimulus-Bias. Despite definite success in the discovery of mGlu allosteric ligands, the pharmacology of allosteric modulators is inherently more complex than their orthosteric counterparts. The overall pharmacological effect of an allosteric ligand includes affinity and efficacy as well as the cooperativity between the allosteric and orthosteric ligands. With respect to ligand efficacy it has become increasingly accepted that occupancy and efficacy are not necessarily linearly linked. The concept that orthosteric ligands may differentially impact the G protein coupling and other functional responses mediated by activation of the receptor has come to the fore in recent years (Fig. 1). This phenomenon referred to as “stimulus-bias” (although it has had many other monikers including “agonist-directed trafficking of receptor stimulus,” “functional selectivity,” and “biased agonism”) has now been observed for many GPCRs, including mGlu₁, mGlu₄, mGlu₇, and mGlu₈ (Emery et al., 2012; Gregory et al., 2012; Jalan-Sakrikar et al., 2014). For example, stimulus-bias is evident when comparing orthosteric agonists L-AP4 [L-2-amino-4-phosphonobutyrate] and LSP4-2022 [(2S)-2-amino-4-([4-(carboxymethoxy)phenyl](hydroxy)methyl)(hydroxy)phosphoryl]butanoic acid] with glutamate between Ca²⁺ mobilization and G protein-coupled inwardly rectifying

TABLE 1

Molecular pharmacology of selected examples of allosteric ligands for group I metabotropic glutamate receptors

Ligand/Selectivity		Allosteric Pharmacology	References
CPCCOEt	1	NAM: glutamate, DHPG, quisqualate, and ACPD mediated inositol phosphate accumulation (IP ₁); glutamate mediated intracellular Ca ²⁺ mobilization (iCa ²⁺); quisqualate induced changes in cell impedance (xCelligence); extracellular Ca ²⁺ induced changes in iCa ²⁺ . NAL: [³ H]glutamate binding.	Annoura et al., 1996; Litschig et al., 1999; Scandroglio et al., 2010; Jiang et al., 2014
JNJ-16259685	1 > >5	NAM: glutamate mediated iCa ²⁺ and IP ₁ ; quisqualate induced changes in cell impedance (xCelligence). Inverse agonist: IP ₁ . NAL: [³ H]quisqualate binding.	Lavreysen et al., 2004; Scandroglio et al., 2010;
R214127	1/5	NAM: glutamate mediated iCa ²⁺ . Inverse agonist: IP ₁ .	Lavreysen et al., 2003
Ro 67-7476	1	PAM: glutamate efficacy for mediating iCa ²⁺ and cAMP accumulation. Agonist: pERK1/2.	Hemstapat et al., 2006; Sheffler et al., 2008
Ro0711401	1	Agonist: iCa ²⁺ . PAM: glutamate mediated K ⁺ currents via GIRK activation.	Vieira et al., 2009
YM-298198	1	NAM: glutamate induced IP ₁ ; quisqualate induced changes in cell impedance (xCelligence). NAL: orthosteric agonists and affinity of radiolabeled ligand.	Kohara et al., 2005; Scandroglio et al., 2010
5MPEP	5	NAL: glutamate efficacy for iCa ²⁺ ; glutamate and DHPG efficacy for IP ₁ ; quisqualate affinity.	Rodriguez et al., 2005; Chen et al., 2007, 2008; Ayala et al., 2009; Hammond et al., 2010; Bradley et al., 2011
5PAM523	5	PAM: glutamate efficacy for iCa ²⁺ . NAL: [³ H]quisqualate binding.	Parmentier-Batteur et al., 2014
ADX47273	5	PAM: quisqualate affinity; quisqualate efficacy for IP ₁ .	Bradley et al., 2011
CDPPB	5	PAM: glutamate efficacy for iCa ²⁺ ; quisqualate affinity; quisqualate efficacy for IP ₁ . PAM-agonist: glutamate efficacy for iCa ²⁺ , pERK1/2 and p-Akt.	Chen et al., 2007; Bradley et al., 2011; Gregory et al., 2012; Doria et al., 2013; Parmentier-Batteur et al., 2014
CPPHA	5 > 1 > 4/8	mGlu ₅ PAM: glutamate efficacy for iCa ²⁺ ; quisqualate efficacy for IP ₁ and iCa ²⁺ . mGlu ₅ NAL: quisqualate and glutamate affinity. mGlu ₅ PAM-agonist: glutamate efficacy for pERK1/2. NAM-agonist: DHPG efficacy for pERK1/2 (cortical astrocytes). mGlu ₄ and mGlu ₈ NAM: glutamate-mediated Ca ²⁺ signaling.	O'Brien et al., 2004; Zhang et al., 2005; Chen et al., 2008; Bradley et al., 2011; Gregory et al., 2012;
DPFE	5	PAM-agonist: glutamate efficacy for iCa ²⁺ and pERK1/2.	Gregory et al., 2013b
M-5MPEP	5	NAM (low cooperativity): glutamate efficacy for iCa ²⁺ ; quisqualate and DHPG efficacy for IP ₁ . NAM: glutamate, DHPG and quisqualate mediated Ca ²⁺ oscillations; glutamate efficacy for pERK1/2. NAL: quisqualate affinity.	Rodriguez et al., 2005; Bradley et al., 2011; Gregory et al., 2012
MPEP	5 > >4	mGlu ₅ NAM: glutamate efficacy for iCa ²⁺ and pERK1/2; quisqualate and DHPG stimulated IP ₁ ; glutamate, DHPG and quisqualate induced Ca ²⁺ oscillations. mGlu ₅ inverse agonist: IP ₁ . mGlu ₅ NAL: quisqualate and glutamate affinity. mGlu ₄ PAM-agonist: L-AP4 mediated iCa ²⁺ . mGlu ₄ PAM: L-AP4 induced changes in VFT.	Gasparini et al., 1999; Chen et al., 2007; Bradley et al., 2011; Gregory et al., 2012; Rovira et al., 2015
VU0357121	5	NAL: glutamate affinity. PAM: glutamate efficacy for iCa ²⁺ . PAM-agonist: glutamate efficacy for pERK1/2.	Hammond et al., 2010; Gregory et al., 2012
VU0360172	5	PAM: glutamate efficacy for iCa ²⁺ . NAL: glutamate affinity. PAM-agonist: glutamate efficacy for pERK1/2.	Gregory et al., 2012

ACPD, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid; ADX47273, (4-fluorophenyl)-[(3*S*)-3-[3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl]piperidin-1-yl]methanone; DPFE, 1-(4-(2,4-difluorophenyl)piperazin-1-yl)-2-((4-fluorobenzyl)oxy)ethanone; GIRK, G protein-coupled inwardly rectifying potassium channel; JNJ-16259685, 3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl-(4-methoxycyclohexyl)methanone; NAL, neutral allosteric ligand; 5PAM523, 5-fluoro-2-(3-[(3*S*,6*R*)-1-[(4-fluorophenyl)carbonyl]-6-methylpiperidin-3-yl]-1,2,4-oxadiazol-5-yl)pyridine; Ro0711401, *N*-[4-(trifluoromethyl)-1,3-oxazol-2-yl]-9*H*-xanthene-9-carboxamide; Ro 67-7476, (5*S*)-2-(4-fluorophenyl)-1-(toluene-4-sulfonyl)pyrrolidine; VU0360172, *N*-cyclobutyl-6-[2-(3-fluorophenyl)ethynyl]pyridine-3-carboxamide; YM-298198, 6-amino-*N*-cyclohexyl-*N*,3-dimethylthiazolo[3,2-*a*]benzimidazole-2-carboxamide.

potassium channel activation at a single group III subtype (Jalan-Sakrikar et al., 2014). Interestingly, when these orthosteric agonist bias profiles are compared between mGlu₇ and either mGlu₄ or mGlu₈, there is a complete reversal in the direction of bias for the same ligands across the same two pathways. This finding highlights the important concept that the endogenous ligand itself will have a “natural bias,” revealed in this study by differences between different group III mGlu subtypes. Stimulus-bias is thought to arise from ligands stabilizing different complements of receptor conformations that favor one suite of receptor activation outcomes over others. It is perhaps not surprising then that there is preliminary evidence for stimulus-bias when comparing allosteric agonist activity to that with glutamate. For example, VU29 [*N*-(1,3-diphenyl-1*H*-pyrazolo-5-yl)-4-nitrobenzamide] and CDPPB [3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide] are PAM-agonists of mGlu₅ for both intracellular Ca²⁺ mobilization and phosphorylated extracellular-signal regulated kinases 1

and 2 (pERK1/2) and notably have higher intrinsic efficacy for pERK1/2 relative to Ca²⁺ signaling (Gregory et al., 2012). Glutamate, however, shows the reverse, with higher efficacy for Ca²⁺ signaling over pERK1/2. Similar observations have been reported for mGlu₁ PAM-agonists relative to glutamate between Ca²⁺ mobilization and cAMP accumulation (Sheffler and Conn, 2008). Moreover, many of the mGlu₅ PAM-agonists for pERK1/2 are super-agonists, capable of achieving higher maximal responses than glutamate (Gregory et al., 2012). Although it should be noted that both ligand and system kinetics may contribute to these observations of biased agonism. Negative allosteric modulators are also often reported to be inverse agonists (Tables 1, 2, and 3); however, these observations are assay dependent, likely a result of receptor constitutive activity and assay resolution. Comprehensive investigation into stimulus-bias that factors in these contributing mechanisms and confounds is sorely needed.

TABLE 2

Molecular pharmacology of selected allosteric ligands for group II metabotropic glutamate receptors

Ligand/Selectivity		Allosteric Pharmacology	References
BINA	2	PAM-agonist: glutamate stimulated [³⁵ S]GTPγS binding at human mGlu ₂ . PAM: glutamate stimulated [³⁵ S]GTPγS binding at rat mGlu ₂ and iCa ²⁺ at human and rat mGlu ₂ ; [³ H]DCG-IV binding to human mGlu ₂ ; LY541850 mediated decreases in Ca ²⁺ oscillation frequency in primary neuronal cultures. NAL: [³ H]LY341495 binding to human mGlu ₂ , LY379268 mediated decreases in Ca ²⁺ oscillation frequency in primary neuronal cultures.	Galici et al., 2006; Hemstapat et al., 2007; Lavreysen et al., 2013; Sanger et al., 2013
CBiPES		PAM: glutamate mediated iCa ²⁺ ; LY379268 and LY541850 mediated decreases in Ca ²⁺ oscillation frequency in primary neuronal cultures.	Johnson et al., 2005; Sanger et al., 2013
JNJ-40068782	2	PAM-agonist: glutamate stimulated [³⁵ S]GTPγS binding and iCa ²⁺ at human mGlu ₂ . PAM: glutamate stimulated [³⁵ S]GTPγS binding at rat mGlu ₂ ; [³ H]DCG-IV binding to human mGlu ₂ ; glutamate affinity (displacement of [³ H]DCG-IV). NAL: [³ H]LY341495 binding.	Lavreysen et al., 2013
LY2607540 (THIC)	2	PAM-agonist: glutamate stimulation of iCa ²⁺ and [³⁵ S]GTPγS binding at human receptor. PAM: glutamate stimulation of iCa ²⁺ and [³⁵ S]GTPγS binding at rat receptor.	Fell et al., 2011; Lavreysen et al., 2013
LY487379	2	PAM-agonist: glutamate stimulation of [³⁵ S]GTPγS binding to rat cerebral cortical membranes. PAM: glutamate, DCG-IV, LCCG-I and LY379268 stimulated [³⁵ S]GTPγS binding and glutamate mediated iCa ²⁺ ; [³ H]DCG-IV binding to human mGlu ₂ . NAL: [³ H]LY341495 binding to human mGlu ₂ .	Schaffhauser et al., 2003; Hemstapat et al., 2007; Odagaki et al., 2011, 2013; Lavreysen et al., 2013
MNI-137	2/3	mGlu ₂ NAM: glutamate and DCG-IV mediated iCa ²⁺ and glutamate stimulated [³⁵ S]GTPγS binding. mGlu ₃ NAM: glutamate mediated iCa ²⁺ and [³⁵ S]GTPγS binding. mGlu ₂ NAL: [³ H]LY341495 binding and glutamate affinity.	Hemstapat et al., 2007; Yin et al., 2014;
RO4491533	2/3	mGlu ₂ NAM: [³ H]LY354740 affinity; ACPD inhibition of cAMP accumulation; glutamate stimulation of GIRK channels and [³⁵ S]GTPγS binding. mGlu ₂ NAL: [³ H]LY341495 binding to human mGlu ₂ . mGlu ₃ NAM: glutamate stimulation of GIRK channels.	Campo et al., 2011; Woltering et al., 2010
RO4988546	2/3	mGlu ₂ NAM: [³ H]LY354740 binding; LY354740 stimulation of [³⁵ S]GTPγS binding and iCa ²⁺ . mGlu ₂ NAL: [³ H]HYDIA binding.	Lundstrom et al., 2011
RO5488608	2/3	mGlu ₂ NAM: [³ H]LY354740 binding; LY354740 stimulation of [³⁵ S]GTPγS binding and iCa ²⁺ . mGlu ₂ NAL: [³ H]HYDIA binding.	Lundstrom et al., 2011

ACPD, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid; BINA, biphenyl-indanone A; CBiPES, *N*-(4'-cyano-biphenyl-3-yl)-*N*-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride; DCG-IV, (2*S*,2'*R*,3'*R*)-2-(2',3'-dicarboxycyclopropyl)glycine; GIRK, G protein-coupled inwardly rectifying potassium channel; HYDIA, (1*S*,2*R*,3*R*,5*R*,6*S*)-2-amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; L-CCG-I, (2*S*,1'*S*,2'*S*)-2-(carboxycyclopropyl)glycine; LY2607540, THIC, *N*-(4-(2-(trifluoromethyl)-3-hydroxy-4-(isobutyl)phenoxy)methyl)benzyl)-1-methyl-1*H*-imidazole-4-carboxamide; LY354740, (1*S*,2*R*,5*R*,6*S*)-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY487379, 2,2,2-trifluoro-*N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(pyridin-3-ylmethyl)ethanesulfonamide; MNI-137, 4-(8-bromo-5-oxo-3,4,5,6-tetrahydro-1,6-benzodiazocin-2-yl)pyridine-2-carbonitrile; NAL, neutral allosteric ligand; RO4491533, 4-[3-(2,6-dimethylpyridin-4-yl)phenyl]-7-methyl-8-(trifluoromethyl)-1,3-dihydro-1,5-benzodiazepin-2-one; RO4988546, 5-[7-trifluoromethyl-5-(4-trifluoromethyl-phenyl)-pyrazolo[1,5-*a*]pyrimidin-3-ylethynyl]-pyridine-3-sulphonic acid; RO5488608, 3'-(8-methyl-4-oxo-7-trifluoromethyl-4,5-dihydro-3*H*-benzo[*b*][1,4]diazepin-2-yl)-biphenyl-3-sulphonic acid.

An additional mechanism that may also contribute to observations of stimulus-bias relates to receptor compartmentalization. For example, a large pool of intracellular mGlu₅ is located on the nuclear membrane (Jong et al., 2005; Kumar et al., 2008). Activation of mGlu₅ at the cell surface versus the nuclear membrane results in the activation of different signaling cascades, as demonstrated through the use of permeable/transported and impermeable/nontransported orthosteric and allosteric ligands of mGlu₅ (Jong et al., 2009; Kumar et al., 2012). Furthermore, activation of cell surface mGlu₅ leads to long-term potentiation and long-term depression in the hippocampus, whereas activation of intracellular mGlu₅ only mediates long-term depression (Purgert et al., 2014). These findings highlight that stimulus-bias may also arise from a differential ability of different chemotypes to access distinct subcellular compartments where receptors may be located. Therefore the lipophilicity or transportability of an allosteric ligand may impact the overall functional outcome observed.

Biased Modulation. A related concept to biased agonism is the phenomenon of biased modulation (Fig. 1), if we consider that the conformations engendered by the simultaneous occupation of a receptor with an orthosteric and allosteric ligand that give rise to cooperativity differ from those engendered by binding of orthosteric ligand alone. Therefore this raises the possibility that the “natural bias” of an endogenous ligand may be influenced by an allosteric modulator. Conceptually, this may manifest as a change in the magnitude and/or direction of the cooperativity factors

that describe the allosteric interaction depending on the receptor activation outcome measured. Alternatively, allosteric modulators may also show differential apparent affinity estimates depending upon the receptor activation outcome measured. In recent years, there have been a growing number of reports of biased mGlu allosteric modulation. For example at mGlu₅, CPPHA [*N*-(4-chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isindol-2-yl)methyl]phenyl)-2-hydroxybenzamide], and DFB [(3-fluorophenyl)methylene]hydrazono-3-fluorobenzaldehyde are both PAMs for glutamate-mediated Ca²⁺ mobilization in rat cortical astrocytes; however, DFB potentiates, whereas CPPHA inhibits DHPG [3,5-dihydroxyphenylglycine]-stimulated pERK1/2 (Zhang et al., 2005). Furthermore, multiple mGlu₅ modulators have lower positive cooperativity for glutamate-mediated pERK1/2 versus intracellular Ca²⁺ mobilization (Gregory et al., 2012). Recent studies suggest that biased modulation has relevance in more physiologic systems, raising the possibility that biased modulation may have clinical implications. For example, a novel mGlu₅ selective modulator from the same chemotype as CPPHA, NCFP [*N*-(4-chloro-2-[(1,3-dioxoisindolin-2-yl)methyl]phenyl)picolinamide], potentiated DHPG-induced depolarization of subthalamic nucleus neurons but lacked the ability to potentiate induction of long-term potentiation or long-term depression in the hippocampus (Noetzel et al., 2013). Importantly, another mGlu₅ allosteric modulator, VU0092273 [3'-(8-methyl-4-oxo-7-trifluoromethyl-4,5-dihydro-3*H*-benzo[*b*][1,4]diazepin-2-yl)-biphenyl-3-sulfonic acid], that shared a similar pharmacological mGlu₅ PAM profile in recombinant cells, potentiated all three slice

TABLE 3

Molecular pharmacology of selected allosteric ligands for group III metabotropic glutamate receptors

Ligand/Selectivity		Allosteric Pharmacology	References
ADX71743	7	NAM: L-AP4 efficacy for iCa^{2+} and inhibition of cAMP accumulation; glutamate in mediating inhibition of cAMP accumulation.	Kalinichev et al., 2013
AMN082	7 [#]	NAL-agonist: inhibition of cAMP accumulation, stimulates pERK1/2 and receptor internalization. NAL: MSOP (does not block AMN082 internalization).	Mitsukawa et al., 2005; Pelkey et al., 2007; Suzuki et al., 2007; Iacovelli et al., 2014
AZ12216052	8	PAM-agonist: glutamate stimulation of [^{35}S]GTP γ S binding.	Duvoisin et al., 2010
MMPIP	7	NAM: L-AP4 efficacy for iCa^{2+} and changes in cell mass distribution; L-CCG-I and CPPG efficacy for iCa^{2+} ; glutamate stimulation of GIRK channels. Inverse/agonist: cAMP accumulation and Epic label-free assay. NAL: [3H]LY341495 binding.	Suzuki et al., 2007; Niswender et al., 2010
PHCCC	4/6	PAM: glutamate for iCa^{2+} and GIRK channel activation; L-AP4 stimulated iCa^{2+} and changes in VFT. PAM-agonist: L-AP4 and glutamate stimulated [^{35}S]GTP γ S binding. mGlu ₁ NAM: glutamate mediated iCa^{2+} . mGlu ₆ agonist: glutamate mediated calcium current inhibition.	Maj et al., 2003; Marino et al., 2003; Beqollari and Kammermeier, 2008; Niswender et al., 2008; Yin et al., 2014; Rovira et al., 2015
VU0155041	4	PAM-agonist: glutamate mediated iCa^{2+} and GIRK channel activation. PAM: L-AP4 mediated iCa^{2+} and changes in VFT.	Niswender et al., 2008; Yin et al., 2013; Rovira et al., 2015
VU0155094	4-8	PAM: glutamate apparent affinity (mGlu _{4, 7, 8}); glutamate efficacy for stimulation of GIRK channels (mGlu ₈) and iCa^{2+} (mGlu _{4, 7}); L-AP4 apparent affinity (mGlu _{7, 8}); L-AP4 efficacy for iCa^{2+} (mGlu ₄); LSP4-2022 apparent affinity (mGlu _{4, 7, 8}); LSP4-2022 efficacy for iCa^{2+} (mGlu ₄). NAL/NAM: L-AP4 apparent affinity (mGlu ₄); L-AP4 and LSP4-2022 efficacy for iCa^{2+} (mGlu ₇). NAL: glutamate, L-AP4, LSP4-2022 efficacy for iCa^{2+} (mGlu ₈).	Jalan-Sakrikar et al., 2014
VU0422288	4-8	PAM: glutamate, L-AP4 and LSP4-2022 efficacy for iCa^{2+} and GIRK channel activation (mGlu _{4, 7, 8}); glutamate, L-AP4, LSP4-2022 apparent affinity (mGlu _{7, 8}). NAL: glutamate apparent affinity (mGlu ₄). NAM: L-AP4 and LSP4-2022 apparent affinity (mGlu ₄).	Jalan-Sakrikar et al., 2014

ADX71743, 6-(2,4-dimethylphenyl)-2-ethyl-4,5,6,7-tetrahydro-1,3-benzoxazol-4-one; AZ12216052, 2-[[[4-(bromophenyl)methyl]sulfanyl]-N-[4-(butan-2-yl)phenyl]acetamide; CPPG, 2-amino-2-cyclopropyl-2-(4-phosphonophenyl)acetic acid; GIRK, G protein-coupled inwardly rectifying potassium channel; L-CCG-I, (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine; MMPIP, 6-(4-methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo [4,5-c]pyridine-4(5H)-one hydrochloride; MSOP, 2-amino-2-methyl-3-phosphonoxypropanoic acid; NAL, neutral allosteric ligand.

[#]Compound and/or its metabolite hits other non-mGlu targets (Ayala et al., 2008; Sukoff Rizzo et al., 2011).

electrophysiological responses (Noetzel et al., 2013), suggesting that NCFP is showing true biased modulation in a native environment. Another novel mGlu₅ allosteric modulator, VU0409551 [5-[(4-fluorophenyl)carbonyl]-2-(phenoxyethyl)-4,5,6,7-tetrahydro[1,3]oxazolo[5,4-c]pyridine], was recently disclosed that potentiates multiple glutamate-induced mGlu₅ signaling responses in cell-based assays, but has no efficacy in modulating hippocampal long-term potentiation or *N*-methyl-D-aspartate receptor currents (Rook et al., 2015). However, VU0409551 has efficacy in preclinical rodent models of psychosis and cognition (Rook et al., 2015), suggesting that biased allosteric modulators of mGlu₅ can possess preclinical efficacy.

Biased modulation is not limited to mGlu₅ PAMs, both positive and negative modulators of group III receptors have been suggested to induce biased receptor signaling (Niswender et al., 2010; Jalan-Sakrikar et al., 2014). The extent and prevalence of biased modulators is likely to be highly underappreciated. This is due to the fact that the vast majority of mGlu allosteric modulators are characterized in a single cell-based assay, generally Ca^{2+} mobilization, to classify and rank compounds on the basis of potency. Furthermore, the therapeutic relevance of biased pharmacology has yet to be realized; however, it is tempting to speculate that unappreciated bias may contribute to differential efficacy of allosteric modulators in behavioral models. For example, 1-(4-(2,4-difluorophenyl)piperazin-1-yl)-2-((4-fluorobenzyl)oxy)ethanone, an mGlu₅ PAM of glutamate, requires far lower doses for efficacy in cognitive models compared with reversing amphetamine-induced hyperlocomotion, a model for antipsychotic efficacy (Gregory et al., 2013a). Moreover, there is the potential that biased modulation may be exploited to develop therapeutics that are not only subtype selective but also

pathway selective, modulating receptor responses that yield therapeutic effects and avoiding those that give rise to on-target adverse effects.

Probe Dependence. A related phenomenon to biased modulation is that of probe dependence, where allosteric interactions are dependent upon the chemical nature of the two ligands under investigation. The result being that the direction and/or magnitude of cooperativity may be

Fig. 1. Schematic depicting the concepts of biased agonism and modulation. (A) The endogenous ligand (blue triangle) activates the receptor, resulting in intracellular signal transduction. However, not all pathways will be activated to the same extent; this is the natural bias of the receptor/system. (B) A surrogate orthosteric agonist (red triangle) may engender a different suite of active receptor conformations, resulting in biased signaling compared with the endogenous ligand; in this case the strength of signaling to response 1 versus 2 is reversed. (C) The binding of an allosteric modulator (orange star) potentiates the response to the endogenous agonist for all signal pathways to the same extent. (D) In contrast, a biased allosteric modulator (purple star) may have different magnitude or direction on cooperativity depending on the response measured; in this case, response 1 is potentiated whereas response 2 is inhibited.

significantly different. There are multiple examples of probe dependence at the group II mGlu receptors. Biphenylindanone A potentiates Ca^{2+} oscillations in cortical neurons induced by the mGlu₂ selective orthosteric agonist LY541850 [(1*S*,2*S*,4*R*,5*R*,6*S*)-2-amino-4-methylbicyclo[3.1.0]hexane-2,6-dicarboxylic acid] but not the mixed group II orthosteric agonist LY379268 [(−)-2-oxa-4-minobicyclo[3.1.0]hexane-4,6-dicarboxylate] (Sanger et al., 2013). JNJ-40068782 [3-cyano-1-cyclopropylmethyl-4-(4-phenyl-piperidin-1-yl)-pyridine-2(1*H*)-one], a PAM-agonist of glutamate-stimulation of [³⁵S]GTPγS binding and Ca^{2+} mobilization at mGlu₂, has neutral cooperativity with the orthosteric antagonist radioligand [³H]LY341495 [(2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid] and positive cooperativity with the orthosteric agonist radioligand [³H]DCG-IV [(2*S*,2'*R*,3'*R*)-2-(2',3'-dicarboxycyclopropyl)glycine] (Lavreysen et al., 2013). For group I and III mGlu receptors, there are also select examples of probe dependence. Affinity modulation occurs between the orthosteric agonist quisqualate and mGlu₅ PAMs (Bradley et al., 2011); however, a separate study found no evidence for affinity modulation (i.e., neutral cooperativity) between glutamate and mGlu₅ PAMs of glutamate in functional assays (Gregory et al., 2012). At mGlu₄ and mGlu₇, VU0155094 [methyl 4-(3-(2-((4-acetamidophenyl)thio)acetyl)-2,5-dimethyl-1*H*-pyrrol-1-yl) benzoate] and VU0422288 [*N*-(3-chloro-4-((5-chloropyridin-2-yl)oxy)phenyl)picolinamide] have different degrees of cooperativity depending upon both the orthosteric agonist and the assay endpoint used (Jalan-Sakrikar et al., 2014). For example, at mGlu₇, VU0155094 is neutral with respect to the apparent affinity of LSP4-2022 and L-AP4 but a positive modulator of glutamate apparent affinity (Jalan-Sakrikar et al., 2014). Clearly, probe dependence is evident for the mGlu family and should be taken into consideration when designing screening paradigms and characterizing mGlu allosteric modulators. The choice of assay and/or probe can result in a very different pharmacological profile. This is of particular importance for mGlu₇ where L-AP4 is most commonly used as the orthosteric agonist given the low affinity of this subtype for glutamate (in the millimolar range). Moreover, extracellular Ca^{2+} ions can activate mGlu₁, influencing the responses to orthosteric agonists, indicating that two endogenous orthosteric agonists need to be considered with respect to probe dependence but also that different endogenous ligands can allosterically interact at mGlu₁ (Jiang et al., 2014). Importantly, extracellular Ca^{2+} activation of mGlu₁ can be modulated by small molecule positive and negative allosteric modulators of glutamate (Ro67-4853 [butyl (9*H*-xanthene-9-carbonyl)carbamate] and CPCCOEt) (Jiang et al., 2014). Probe dependence can also be an important consideration when assessing allosteric modulators in native systems (brain slice electrophysiology or primary neuronal cultures) where surrogate agonists are typically used to selectively activate a particular mGlu subtype and avoid the confounds of glutamate activating ionotropic glutamate receptors and being subject to endogenous transporters.

Protein-Protein Interactions. Another important consideration in assessing allosteric modulator pharmacology in native systems is the fact that the mGlu family are obligate dimers (El-Moustaine et al., 2012) that also have the capacity to form heteromers with other subtypes (Doumazane et al., 2011; Kammermeier, 2012; Sevastyanova and Kammermeier, 2014; Yin et al., 2014) as well as with other unrelated GPCRs

(Gama et al., 2001; Gonzalez-Maeso et al., 2008; Cabello et al., 2009). Unsurprisingly, such complexity in receptor configurations can influence the pharmacology of small molecules. Heterodimerization of mGlu₄ and mGlu₂ has differential effects on the pharmacology of different allosteric ligands (Kammermeier, 2012; Yin et al., 2014). For example, at the mGlu_{2/4} heteromer PHCCC [*N*-phenyl-7-(hydroxyimino)cyclopropa[*b*] chromen-1*α*-carboxamide] has lower cooperativity with L-AP4, whereas Lu AF21934 [(1*S*,2*R*)-2-[(aminooxy)methyl]-*N*-(3,4-dichlorophenyl)cyclohexane-1-carboxamide] and VU0155041 [(1*R*,2*S*)-2-[(3,5-dichlorophenyl)carbamoyl]cyclohexane-1-carboxylic acid] have decreased affinity but higher cooperativity compared with mGlu₄ homodimers (Yin et al., 2014). This perturbation in the pharmacology of allosteric ligands has consequences in vivo at synapses where mGlu₂ and mGlu₄ are coexpressed. VU0155041 and Lu AF21934 are both effective at potentiating the actions of L-AP4 at corticostriatal synapses (Gubellini et al., 2001; Yin et al., 2014). However, PHCCC does not potentiate the actions of L-AP4 at corticostriatal synapses (Yin et al., 2014), despite being an efficacious mGlu₄ PAM of L-AP4-induced responses at other synapses (Marino et al., 2003; Valenti et al., 2005; Jones et al., 2008). Direct protein-protein interactions are not necessarily required for a coexpressed receptor to impact the pharmacological effect of an mGlu ligand. Coexpression and coactivation of G_q-coupled receptors enhances mGlu₄ stimulus-response coupling to orthosteric and allosteric agonists in intracellular Ca^{2+} mobilization but not cAMP accumulation assays (Yin et al., 2013). It is apparent that cellular context, measure of receptor function and coexpression of other GPCRs can impact the pharmacology of small molecule allosteric ligands, leading to unanticipated consequences in native systems. However, this complexity extends further to the structure-activity relationships of allosteric modulators themselves.

Molecular Switches. Given the complexities of allosteric modulation surrounding stimulus-bias and probe dependence it is perhaps not surprising that structure-activity relationships (SAR) for mGlu receptor allosteric modulators are notoriously challenging. SAR is often reported to be “steep,” with minimal changes resulting in a complete loss of activity (Conn et al., 2014; Lindsley, 2014). In addition, mGlu allosteric chemotypes often show “molecular switches” where the same scaffold can give rise to modulators with negative, positive, and neutral cooperativity with the same orthosteric agonist (Wood et al., 2011). The phenomenon was first observed during the development of DFB, an mGlu₅ PAM for glutamate-mediated intracellular Ca^{2+} mobilization (O'Brien et al., 2003). Recently, this propensity for molecular switching was exploited with the development of a photo-switchable allosteric modulator for mGlu₅, where negative cooperativity with glutamate could be controlled by exposure to different light wavelengths (Pittolo et al., 2014). However, unexpected changes in cooperativity continue to be a challenge for medicinal chemistry programs and interpretation of SAR (Hammer et al., 1980; Sharma et al., 2009; Rodriguez et al., 2010; Schann et al., 2010; Zhou et al., 2010; Lamb et al., 2011; Sams et al., 2011; Sheffler et al., 2012). These difficulties in SAR interpretation can in part be attributed to a paucity in our understanding of the structural basis of allostereism. The majority of discovery programs rely on modulator potency curves in the presence of a single concentration of agonist to

classify allosteric ligands as active/inactive and ascribe either positive or negative cooperativity. However, potency represents a composite measure of efficacy, affinity, and cooperativity (Gregory et al., 2012). As such, modifications to a chemotype may influence any one of these three parameters. Therefore, more quantitative analysis of allosteric chemotypes is required to truly understand how SAR relates to efficacy, affinity, and cooperativity. Going forward a key challenge will be coupling SAR interpretation as we improve our understanding of the structural basis of allostereism.

Structural Basis of Allosteric Modulation

The mGluRs are family C GPCRs typified by their large extracellular N-terminal Venus flytrap domain (VFT), linked via a cysteine-rich domain to the seven transmembrane (TM) spanning α -helical domains that are the hallmark of the GPCR superfamily. The orthosteric site is located in the N-terminal domain and to date all small molecule allosteric modulators are thought to bind within the 7TMs. Historically, receptor chimeras proved valuable to (1) definitively demonstrate an allosteric mechanism of action for small molecule ligands and (2) to localize allosteric binding sites to the 7TM domains. Chimeric constructs swapping the VFT of one subtype onto another or even exchanging with non-mGluR family C GPCRs have been used. Chimeras between mGlu₁ and mGlu₅ or mGlu₄ and the calcium-sensing receptor were used to validate the allosteric mechanism of action of mGlu₁ selective allosteric modulator CPCCOEt (Brauner-Osborne et al., 1999; Litschig et al., 1999; Gasparini et al., 2001). Subsequent studies used mGlu_{1/5} chimeras to demonstrate MPEP (2-methyl-6-(phenylethynyl)pyridine; an mGlu₅ selective NAM), and multiple mGlu₁ PAMs recognized an overlapping binding site with that of CPCCOEt (Pagano et al., 2000; Knoflach et al., 2001). Chimeric constructs were also used to validate an allosteric mechanism of action for PHCCC, AMN082 [*N,N'*-dibenzhydrylethane-1,2-diamine dihydrochloride], and BAY36-7620 [(3*aS*,6*aS*)-hexahydro-5-methylene-6*a*-(2-naphthalenylmethyl)-1*H*-cyclopenta[*c*]furan-1-one] at mGlu₄, mGlu₇, and mGlu₁, respectively (Carroll et al., 2001; Maj et al., 2003; Mitsukawa et al., 2005). Another approach to achieve gross localization of allosteric binding sites is through the generation of N-terminal truncated receptors. The extracellular VFT can be removed to generate a “headless” mGlu, yielding a receptor construct that does not respond to glutamate but can couple to intracellular signaling pathways (Goudet et al., 2004). Headless mGlu₁ and mGlu₅ are constitutively active such that negative allosteric modulators behave as inverse agonists (Goudet et al., 2004; Chen et al., 2007; Suzuki et al., 2007). Furthermore, headless mGlu₅ can be stimulated by small molecule positive allosteric modulators that do not necessarily have intrinsic efficacy at the full-length receptor (Chen et al., 2007; Goudet et al., 2004; Noetzel et al., 2013). Interestingly, NCFP had little or no efficacy for stimulating headless mGlu₅, despite having a similar magnitude of positive cooperativity as VU0092273, which can activate headless mGlu₅ (Noetzel et al., 2013). The headless mGlu 7TM is thought to behave as a monomer (Goudet et al., 2004), so perhaps this discrepancy relates to receptor dimerization in the full-length receptor being important either for NCFP binding or cooperativity.

Two recent X-ray crystal structures were solved for the 7TMs of group I mGluR with allosteric modulators bound [PDB IDs 4O09 and 4OR2 (Dore et al., 2014; Wu et al., 2014)]. Before this, researchers relied on family A 7TM templates to perform homology modeling and interpret site-directed mutagenesis data. Despite low sequence homology (<20%), these approaches proved fruitful, localizing allosteric binding pockets of mGlu₁, mGlu₂, mGlu₄, and mGlu₅ to a region analogous to that of the biogenic amine binding sites in family A GPCRs (Ott et al., 2000; Pagano et al., 2000; Malherbe et al., 2003a,b; Lundstrom et al., 2011; Molck et al., 2012; Gregory et al., 2013b, 2014; Rovira et al., 2015). In light of the new crystal structures, although alignments and secondary structure predictions of TMs 5–7 deviated from that observed in the mGlu₁ and mGlu₅ structures, the general localization of the pocket was consistent with earlier reports. Many residues that had previously been identified in mutagenesis-based studies were recapitulated in the crystal structures (discussed in detail below). Ongoing research continues to refine our understanding of binding pocket(s) within the 7TM bundle in particular in relation to determinants that govern selectivity, affinity, cooperativity, and efficacy of allosteric modulators.

The Common mGlu Allosteric Site

The concept for a common allosteric site between mGlu subtypes arises from three lines of evidence: (1) allosteric modulators that lack subtype selectivity; (2) competitive interactions between diverse chemotypes and allosteric radioligands; (3) overlapping binding pockets based on mutagenesis studies and observed in X-ray crystal structures. Although attaining selectivity with allosteric modulators is considerably more effective than for orthosteric ligands, there are a number of examples of allosteric modulators that have efficacy at multiple subtypes. MPEP is both a negative allosteric modulator of glutamate at mGlu₅ and a positive allosteric modulator of L-AP4 at mGlu₄ (Mathiesen et al., 2003). Conversely, DFB and CPPHA, which potentiate glutamate signaling at mGlu₅ are weak negative allosteric modulators of glutamate at mGlu₄ and mGlu₈ (O'Brien et al., 2003, 2004). In addition, PHCCC, an mGlu₄ PAM for glutamate and L-AP4, negatively modulates glutamate at mGlu₁ (Annoura et al., 1996; Maj et al., 2003). The recent report of a molecular switch to mGlu₃ NAM from an mGlu₅ PAM scaffold (Sheffler et al., 2012) reveals that the subtleties of interactions within this common pocket that dictate subtype selectivity are not limited to crossover between group I and group III mGlu receptors alone. This lack of selectivity within the common binding pocket is also associated with differential cooperativity between subtypes, highlighting that the small molecule interactions within the common allosteric site can elicit either positive or negative cooperativity.

Identification of allosteric ligands that interact with this common mGlu allosteric site has been facilitated by the development of radiolabeled mGlu allosteric modulators. Multiple selective tritiated and positron emission tomography (PET) allosteric ligands have been developed for mGlu₁, mGlu₂, and mGlu₅ (Anderson et al., 2002, 2003; Gasparini et al., 2002; Cosford et al., 2003b; Lavreysen et al., 2003; Kohara et al., 2005; Malherbe et al., 2006; Ametamey et al., 2007; Treyer et al., 2007; Baumann et al., 2010). Through the use of inhibition binding assays with these allosteric ligands

a direct assessment of competitive versus noncompetitive binding modes can be performed (Lavreysen et al., 2003, 2004; Kinney et al., 2005; Malherbe et al., 2006; Hemstapat et al., 2006; Chen et al., 2007; Lundstrom et al., 2011). Such experiments are often the first step toward identifying allosteric modulators that bind to alternative allosteric sites [(Chen et al., 2008; Hammond et al., 2010) discussed in further detail later]. However, competition binding experiments may also be used to identify neutral allosteric ligands that interact with the common allosteric site but have neutral cooperativity with agonist(s) in functional screening assays (Rodriguez et al., 2005; Sams et al., 2011).

Early mutational studies investigating group I mGlu receptor allosteric binding pockets exploited nonconserved residues within the 7TMs. Substitution of mGlu₁ nonconserved amino acids onto the equivalent positions in mGlu₅, and vice versa, resulted in gain-of-function of selective allosteric modulators (Litschig et al., 1999; Pagano et al., 2000; Knoflach et al., 2001; Goudet et al., 2005; Surin et al., 2007). These mutagenesis studies pointed to a common location for allosteric binding pockets for group I mGlu receptors. Additional studies have shown that residues at positions 3.40 and 7.38 are important for allosteric modulation of both mGlu₁ and mGlu₅ [Ballesteros-Weinstein numbering (Ballesteros and Weinstein, 1995) adopted from mGlu₁ structural alignment to family A GPCRs; Malherbe et al., 2003a,b, 2006; Muhlemann et al., 2006; Suzuki et al., 2007; Fukuda et al., 2009; Gregory et al., 2012, 2013b, 2014; Turlington et al., 2014; Wu et al., 2014]. Subsequently, key residues for the potency and/or binding of mGlu₁, mGlu₂, mGlu₄, and mGlu₅ allosteric modulators from diverse chemical

scaffolds have been mapped to TMs 3–7. Across the four subtypes multiple residues have repeatedly been reported as contributing to allosteric modulation. For all four subtypes, residues in positions 3.36, 5.43, and 6.48 are crucial for both positive and negative allosteric modulators (Pagano et al., 2000; Malherbe et al., 2003a, 2006; Muhlemann et al., 2006; Fukuda et al., 2009; Lundstrom et al., 2011; Gregory et al., 2012, 2013b, 2014; Molck et al., 2012; Turlington et al., 2014; Wu et al., 2014; Rovira et al., 2015). Position 7.45 has also been implicated in mGlu₄ and mGlu₅ allosteric modulation (Molck et al., 2012; Gregory et al., 2013b, 2014; Turlington et al., 2014; Rovira et al., 2015); there are no reports investigating the influence of this amino acid on selective allosteric modulators of mGlu₁ or mGlu₂. Between group I mGlu receptors and mGlu₂, three residues are common: 5.44, 5.47, and 6.55 (Knoflach et al., 2001; Malherbe et al., 2003b, 2006; Schaffhauser et al., 2003; Hemstapat et al., 2006; Muhlemann et al., 2006; Rowe et al., 2008; Fukuda et al., 2009; Lundstrom et al., 2011; Gregory et al., 2012, 2013b, 2014; Molck et al., 2012). Group 1 receptors and mGlu₄ share a common determinant in F6.51 (Malherbe et al., 2003a,b, 2006; Muhlemann et al., 2006; Suzuki et al., 2007; Fukuda et al., 2009; Gregory et al., 2014; Rovira et al., 2015). Two residues at the top of TM3 (3.28 and 3.29) are important for allosteric modulation of both mGlu₂ and mGlu₄ (Lundstrom et al., 2011; Rovira et al., 2015), with position 3.29 also implicated in the activity of some mGlu₅ allosteric modulators (Malherbe et al., 2003b, 2006; Molck et al., 2012). Therefore these mutagenesis data reinforce the notion of overlapping binding pockets across the mGlu family.

Comparison of the recently solved mGlu₁ and mGlu₅ crystal structures reveals a similar architecture for the backbone of

Fig. 2. Comparison of allosteric pockets within the 7TM domains of mGlu₁ and mGlu₅. (A) X-ray crystal structures of 7TMs of mGlu₅ (gray, PDB ID 4009) and mGlu₁ (teal; PDB ID 4OR2) were aligned using MacPyMOL (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC, New York, NY). (B) Inside surface rendering of the allosteric binding pockets of mavoglurant (black) in mGlu₅ (gray) and FITM (blue) in mGlu₁ (teal). (C) Zoomed-in image of the bottom of the allosteric pockets with mavoglurant shown. Two amino acids differ between mGlu₁ and mGlu₅ in this region, the differences in the side chains (depicted as sticks) create more space in mGlu₅, allowing mavoglurant to bind deeper within the 7TM bundle. All images created using MacPyMOL.

the transmembrane-spanning helices (Fig. 2A). Overlay of the 7TM binding pockets for FITM [4-fluoro-*N*-(4-(6-isopropylamino)pyrimidin-4-yl)thiazole-2-yl]-*N*-methylbenzamide] and mavoglurant in mGlu₁ and mGlu₅, respectively, confirmed that these subtypes have overlapping allosteric binding pockets within the 7TMs (Dore et al., 2014; Wu et al., 2014). Examination of binding pockets for mavoglurant and FITM reveals that mavoglurant binds deeper within the 7TM bundle (Fig. 2B). Side chain conformations of two key nonconserved residues in TM3 of mGlu₅: P655^{3,36} and S658^{3,39} (mGlu₁ equivalents are S668 and C671, respectively) create greater space within the pocket that allows mavoglurant to bind deeper within the 7TM bundle (Fig. 2C). These crystal structures of closely related mGlu receptors have yielded structural insights with respect to how selectivity can be achieved in the common mGlu allosteric site.

Delineating Affinity versus Cooperativity Determinants

The vast majority of research on allosteric modulator SAR and structure-function has relied on measures of allosteric modulator potency; however, potency values represent a composite of affinity and cooperativity. In recent years the application of rigorous analytical pharmacological methods has facilitated delineation of the impact of mutations on allosteric modulator affinity, cooperativity, and efficacy based on functional interaction data alone. By combining this research with earlier studies using radiolabeled allosteric modulators where affinity was determined or a loss of binding was observed we can start to map affinity determinants in the binding pocket. Residues known to be crucial affinity determinants are summarized in Table 4 (also see Supplemental Table 1) where the most well characterized representatives from individual allosteric modulator scaffolds were included. These affinity determinants are also mapped onto

the mGlu₁ and mGlu₅ structures (Fig. 3, A and B). Significant overlap is observed between mGlu₁ and mGlu₅, although there is limited affinity data for mGlu₁. For mGlu₅, six residues have been implicated in the binding of most PAMs and NAMs: P655^{3,36}, Y659^{3,40}, T781^{6,44}, W785^{6,48}, S809^{7,45}, and A810^{7,46} within the common allosteric site. Mapping the known affinity determinants for MPEP, the most well studied mGlu₅ allosteric ligand with respect to structure-function, onto the mGlu₅ crystal structure yields additional insights (Fig. 4; Pagano et al., 2000; Malherbe et al., 2003b; Gregory et al., 2012, 2013b, 2014). Coloring residues on the basis of the impact of mutations reveals that substitutions that cause a moderate (<10-fold) reduction in affinity are located on the fringes of the mavoglurant binding pocket or are immediately adjacent to key residues (Fig. 4; Supplemental Table 1).

In addition to effects on affinity, molecular determinants for a large set of allosteric modulators spanning multiple scaffolds with both positive and negative cooperativity have been explored at mGlu₅. Some residues that are important for modulator affinity also contribute to cooperativity; however, there are select amino acids that only influence cooperativity (Fig. 3). Negative cooperativity with glutamate of multiple allosteric modulators at mGlu₅ is decreased at P655^{3,36} mutants (Gregory et al., 2013b, 2014). Additionally mutations in TMs 3–7 [Y659^{3,40}, N747^{5,47}, T781^{6,44}, W785^{6,48}, S809^{7,45} (Molck et al., 2012; Gregory et al., 2014)] reduced cooperativity for negative modulators of glutamate at mGlu₅. Notably, in mGlu₁ mutations of T794^{6,44} and S822^{7,45} had no effect on the interaction between glutamate and FITM (Wu et al., 2014), suggesting that the structural determinants that govern cooperativity can be subtype selective even for conserved amino acids.

Negative cooperativity with glutamate at mGlu₅ was increased for M-5MPEP [2-(2-(3-methoxyphenyl)ethynyl)-5-methylpyridine] and/or VU0366248 [*N*-(3-chloro-2-fluorophenyl)-3-cyano-5-fluorobenzamide] when mutations were introduced to S658^{3,39},

TABLE 4

Known affinity determinants for mGlu allosteric modulators interacting with the common allosteric site.

Most well characterized representatives from individual allosteric modulator scaffolds were selected for inclusion. Alignment of amino acids and assignment of Ballesteros-Weinstein numbering was adopted from the structural alignment to class A GPCRs in Wu et al., 2014. A colored version of this table (Supplemental Table 1) is included in the online supplemental materials.

Ligand/Subtype	Amino Acid Residue Position																
	3.29	3.36	3.39	3.40	5.43	5.44	5.47	5.48	6.44	6.48	6.51	6.52	6.55	6.56	7.38	7.45	7.46
EM-TBPC	1			Y		V/L				W+	F	–	Y		T		
FITM	1	s			P				–						T	–	
MPEP	5	R	P	S	Y	P	L	–	G	T	W	F	V	Y	f+	S	A
Fenobam	5	R	P	S	Y					T	W	F		Y			A
VU0366248	5		P	S	Y		N	–	t	W	f [#]	–	Y	–		S	A
VU0285683	5		p [#]		Y	–	N	–	T	W	F	–	Y			S	A
VU0366058	5		p [#]		Y		–	g+		W	F+	v+	Y			s	A
VU0409106	5		p [#]		Y		N	–	T	W	F	–	Y	f		S	A
DPFE	5	–	P		Y	P	L+	–	T	W	–	V+	y+	f+		s	A
VU29	5	–	p [#]		–	p	l	G	T	w+	f	v	y	f+		–	A
VU0403602	5	–	P	s	Y [#]	p	–	–	T	W		–				S	A

DPFE, 1-(4-(2,4-difluorophenyl)piperazin-1-yl)-2-((4-fluorobenzyl)oxy)ethanone; EM-TBPC, 1-ethyl-2-methyl-6-oxo-4-(2,3,4,5-tetrahydro-1*H*-3-benzazepin-3-yl)-1,6-dihydropyrimidine-5-carbonitrile; VU0285683, 3-fluoro-5-(3-pyridin-2-yl-1,2,4-oxadiazol-5-yl)benzonitrile; VU0366058, 2-[(1,3-benzoxazol-2-yl)amino]-4-(4-fluorophenyl)pyrimidine-5-carbonitrile; VU0403602, *N*-cyclobutyl-5-((3-fluorophenyl)ethynyl)picolinamide.

Lower case, mutations caused a change (2- to 10-fold) in affinity estimates that did not reach significance (determined from either radioligand binding or from functional interactions with orthosteric agonists); upper case, mutations caused a significant change in affinity (determined from either radioligand binding or from functional interactions with orthosteric agonists; not included: T6.43 for MPEP, C7.52 for VU0403602). Grayscale: white, 3- to 10-fold change; light gray, 10- to 30-fold change; mid gray, 30- to 100-fold decrease; dark gray, >100-fold decrease or complete loss of radiolabeled allosteric modulator binding (data summarized from: Pagano et al., 2000; Malherbe et al., 2003a,b, 2006; Gregory et al., 2012, 2013b, 2014; Turlington et al., 2014; Wu et al., 2014).

[#]Mutation caused a loss in allosteric modulation of orthosteric agonist functional response; impact on affinity inferred from effects across other scaffolds.

–Mutation(s) had no effect on modulator affinity (determined from either radioligand binding or from functional interactions with orthosteric agonists).

+Mutation increases modulator affinity, in all other cases mutations cause a decrease (determined from either radioligand binding or from functional interactions with orthosteric agonists).

Fig. 3. Affinity and cooperativity determinants for allosteric modulation of metabotropic glutamate receptors. (A) X-ray crystal structure of mGlu₁ 7TMs with FITM (rendered in blue sticks) bound (PDB ID 4OR2) with known mGlu₁ allosteric ligand affinity determinants highlighted in dark blue. (B) X-ray crystal structure of mGlu₅ 7TMs with mavoglurant (rendered in black sticks) bound (PDB ID 4O09), affinity determinants for mGlu₅ allosteric ligands are highlighted in red. (C) X-ray crystal structure of mGlu₅ and mGlu₁ 7TM domains were aligned; only the mGlu₅ structure is represented in cartoon. The binding poses of FITM and mavoglurant show the overlapping allosteric pockets of mGlu₁ and mGlu₅. Highlighted are residues that when mutated change allosteric ligand cooperativity: dark red, reduce mGlu₅ NAM cooperativity; light red, increase NAM cooperativity; orange, differential effects on NAM cooperativity; dark blue, increase PAM cooperativity; light blue, increase cooperativity of NAMs and PAMs; purple, differential effects on cooperativity of NAMs versus PAMs. (D) Structures of mGlu₁ and mGlu₅ were aligned as per (C); highlighted are residues that when mutated switch allosteric modulator cooperativity: blue, PAM to either NAM or neutral switch; red, NAM to PAM switch; purple, PAM to NAM and vice versa for NAMs. All images created using MacPyMOL.

P743^{5.43}, G748^{5.48}, and V789^{6.52} (Gregory et al., 2014). Both M-5MPEP and VU0366248 have limited negative cooperativity at the wild-type receptor, with saturating concentrations unable to completely abolish the glutamate response (Rodriguez et al., 2005; Felts et al., 2010). The cooperativity of other mGlu₅-negative allosteric modulators tested at these mutations was not discernibly different to wild type (Gregory et al., 2014). However, given that these all have strong negative cooperativity at wild type, fully abolishing the maximal glutamate response, further increases in negative cooperativity were likely beyond the limits of detection. For P743^{5.43} this increase is opposite to that observed for the equivalent mGlu₁ mutation (Wu et al., 2014), again highlighting the subtype selective nature of cooperativity. Interestingly, two point mutations (N747^{5.47} and S809^{7.45}) had differential effects on allosteric ligands, actually increasing negative cooperativity of M-5MPEP and VU0366248, respectively (Fig. 3C; Gregory et al., 2014). It is clear that the amino acids within the common allosteric site can differentially contribute to affinity and the manifestation of negative cooperativity in a manner that is very much ligand dependent.

With respect to positive allosteric modulators, no mutations have yet been demonstrated to unequivocally reduce cooperativity. However, there are multiple examples of mutations that increase the magnitude of positive cooperativity with glutamate (Fig. 3C). Shared with the low cooperativity negative modulators, mutation of P743^{5.43} and V789^{6.52} also

increased positive cooperativity of diverse allosteric modulators with glutamate at mGlu₅ (Gregory et al., 2013b, 2014). Four residues (L744^{5.44}, Y792^{6.55}, A810^{7.46}, and C816^{7.52}) are implicated in positive but not negative cooperativity (Gregory et al., 2013b, 2014). Interestingly, compared with negative allosteric modulators, mutation of T781^{6.44} and W785^{6.48} had differential effects, increasing positive cooperativity with glutamate at mGlu₅ (Gregory et al., 2013b, 2014). The complicated nature of cooperativity determinants is further highlighted through evidence of mutations that engender “molecular switches” in allosteric modulator cooperativity (Fig. 3D). Substitution of Y6.51 at mGlu₅ switches DFB from a PAM of glutamate to a NAM, whereas the reverse is true for YM298198 at mGlu₁ (Muhlemann et al., 2006; Fukuda et al., 2009). Multiple additional point mutations in TMs 3, 6, and 7 can also switch acetylenic PAMs to have either neutral or negative cooperativity with glutamate at mGlu₅ (Gregory et al., 2013b; Turlington et al., 2014). Mutation of conserved W785^{6.48} in mGlu₅ can switch the cooperativity of select NAMs of glutamate to positive (Gregory et al., 2014). Collectively, these data highlight the subtleties and complexities of interactions within the common allosteric site that dictate whether the binding of a ligand will result in enhancement or inhibition of mGlu receptor function. Furthermore, developing a better understanding of the dynamic ligand-receptor interactions that give rise to cooperativity will

Fig. 4. Relative contribution of 7TM residues to the binding affinity of prototypical mGlu₅ allosteric modulator, MPEP. Known affinity determinants for MPEP are mapped onto the mGlu₅-7TM X-ray crystal structure with mavoglurant bound (PDB ID 4OO9). The relative contribution of pocket residues are color-coded with side chains rendered as sticks based on mutational data as indicated in the key. All images created using MacPyMOL

likely improve interpretation of allosteric modulator SAR and perhaps inform as to why “molecular switches” are prevalent for some chemical scaffolds.

Additional mGlu Allosteric Sites

As mentioned earlier, the development of radiolabeled allosteric ligands has identified allosteric ligands that are noncompetitive. The first such ligand identified was CPPHA, a group I mGlu selective positive allosteric modulator of glutamate. CPPHA is unable to fully displace the mGlu₅ radioligands [³H]mPEPy [3-methoxy-5-(2-pyridinylethynyl)pyridine] and [³H]MPEP or compete with [³H]R214127 [1-(3,4-dihydro-2H-pyran[2,3-b]quinolin-7-yl)-2-phenylethanone] for binding to mGlu₁ (O'Brien et al., 2004; Chen et al., 2008; Bradley et al., 2011; Noetzel et al., 2013). Another structurally related selective mGlu₅ PAM of glutamate, NCFP, is also thought to bind to this alternate allosteric site (Noetzel et al., 2013). Potentiation of glutamate by both NCFP and CPPHA is noncompetitively inhibited by the neutral allosteric ligand 5-methyl-2-(2-phenylethynyl)pyridine (Chen et al., 2008; Noetzel et al., 2013). This indicates that the second allosteric site is conformationally linked to the common allosteric site such that ligands binding to these sites can allosterically influence one another. Isoleucine substitution of F1.42 reduces potentiation by CPPHA (both mGlu₁ and mGlu₅) and NCFP (mGlu₅ only) (Chen et al., 2008; Noetzel et al., 2013). Interestingly, NCFP also loses either affinity or the capacity to allosterically modulate [³H]mPEPy binding and has very little efficacy as an agonist at an N-terminal truncated mGlu₅ (Noetzel et al., 2013). This suggests that the intact full-length receptor is

required for this alternate allosteric site and/or receptor dimerization is also necessary. Also for mGlu₅, VU0357121 [4-butoxy-*N*-(2,4-difluorophenyl)benzamide] and VU0400100 [2-[2-chloro-*N*-methylsulfonyl-5-(trifluoromethyl)anilino]-*N*-(cyclopropylmethyl)acetamide] do not completely displace [³H]mPEPy binding (Hammond et al., 2010; Gregory et al., 2012; Rodriguez et al., 2012). Multiple mGlu₁ PAM scaffolds have also been flagged as binding to alternate allosteric site(s) based on no or incomplete displacement of the allosteric radioligand [³H]R214127 (Hemstapat et al., 2006). Overall, these data strongly support the hypothesis that multiple allosteric sites are present on the group I mGlu receptors.

In the absence of an allosteric radioligand, multiple allosteric sites have also been proposed for mGlu₄. The concentration-response curve for VU0155041 as an allosteric agonist at mGlu₄ was unaffected by coapplication of PHCCC, an mGlu₄ PAM of glutamate and L-AP4 (Niswender et al., 2008). Recent mutational analysis of mGlu₄ allosteric modulators suggests that these two allosteric ligands interact with the common allosteric pocket made up of TMs 3–7 but in a nonoverlapping manner (Rovira et al., 2015). Based on homology modeling and mutational data, PHCCC is proposed to bind deep within the allosteric site, analogous to the binding pose of mavoglurant in the mGlu₅ structure (Rovira et al., 2015). On the other hand, VU0155041 binds higher in the pocket, interacting with residues at the very top of the TM domains as well as extracellular loops 1 and 2 (Rovira et al., 2015). This suggests that two allosteric ligands can be accommodated simultaneously by mGlu₄; however, at least for PHCCC and VU0155041, there is neutral cooperativity between the binding of these ligands. Interestingly, VU0415374 appears to be bitopic, with homology modeling proposing it can span both binding sites. It is tempting to speculate that the reason VU0415374 has 10-fold (or more) higher apparent affinity than other mGlu₄ positive allosteric modulators is due to its ability to engage more residues within the pocket. Furthermore, it is interesting to consider that CPPHA has weak activity at mGlu₄ (Chen et al., 2008), as such there is the possibility that allosteric ligands may interact with mGlu₄ in three distinct binding modes. The extensive literature surrounding orthosteric and allosteric binding pockets of class A GPCRs may offer some insights into the location of alternative allosteric sites (Christopoulos, 2014). As confirmed in the recent cocrystal structure of the M2 muscarinic acetylcholine receptor, class A GPCR allosteric modulators recognize a binding pocket defined by the top of the TMs and the second and third extracellular loops (Kruse et al., 2013). Given the parallels between the common allosteric site of class C GPCRs and the orthosteric site in class A GPCRs, there is the possibility that alternate allosteric sites in class C GPCRs may also involve the extracellular surfaces of the receptor. Allosteric modulators that interact with different binding pockets are likely to engender a different complement of receptor conformational states than those that interact with the common allosteric site. Therefore the functional consequences of allosteric modulation through different binding pockets are likely to be divergent. Indeed, CPPHA and related compounds have biased pharmacology at mGlu₅ when compared with common site modulators (Zhang et al., 2005; Noetzel et al., 2013). We are just beginning to scratch the surface with respect to our understanding of what drives efficacy, cooperativity, and

affinity through the different allosteric sites across the entire mGlu family.

Concluding Remarks

Since the discovery of the first mGlu receptor allosteric modulator in 1996, the mGlu allosteric modulator pharmacological toolbox has continued to expand with selective allosteric ligands now available for the majority of mGlu family members. This toolbox includes chemically diverse allosteric ligands that may possess positive, negative, or neutral cooperativity and/or positive or inverse agonism. At least two allosteric sites have been proposed, one of which is shared across mGlu receptor subtypes, but can still be selectively targeted. Which allosteric site is best targeted for therapeutic efficacy remains unknown. Given the complexity introduced by the phenomenon of biased modulation, the optimal allosteric ligand pharmacological profile and the relative contribution of affinity, efficacy, and cooperativity will likely be both pathophysiology and mGlu subtype dependent. With two X-ray crystal structures of the 7TMs for group I mGlu receptors now available, the field is now entering an exciting new era in our understanding of the dynamic interactions that govern allosteric ligand affinity, cooperativity, and efficacy.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Gregory, Conn.

References

- Ametamey SM, Treyer V, Streffer J, Wyss MT, Schmidt M, Blagoev M, Hintermann S, Auberson Y, Gasparini F, and Fischer UC et al. (2007) Human PET studies of metabotropic glutamate receptor subtype 5 with 11C-ABP688. *J Nucl Med* **48**: 247–252.
- Anderson JJ, Bradbury MJ, Giracello DR, Chapman DF, Holtz G, Roppe J, King C, Cosford ND, and Varney MA (2003) In vivo receptor occupancy of mGlu5 receptor antagonists using the novel radioligand [3H]-methoxy-5-(pyridin-2-ylethynyl)pyridine. *Eur J Pharmacol* **473**:35–40.
- Anderson JJ, Rao SP, Rowe B, Giracello DR, Chapman DF, Tehrani L, Bradbury MJ, Cosford ND, and Varney MA (2002) [3H]Methoxymethyl-3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine binding to metabotropic glutamate receptor subtype 5 in rodent brain: in vitro and in vivo characterization. *J Pharmacol Exp Ther* **303**:1044–1051.
- Annoura H, Fukunaga A, Uesugi M, Tatsuoka T, and Horikawa Y (1996) A novel class of antagonists for metabotropic glutamate receptors, 7-(hydroxymino)cyclopropa[b]chromen-1a-carboxylates. *Bioorg Med Chem Lett* **6**:763–766.
- Anwyl R (1999) Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. *Brain Res Brain Res Rev* **29**:83–120.
- Ayala JE, Chen Y, Banko JL, Sheffler DJ, Williams R, Telk AN, Watson NL, Xiang Z, Zhang Y, and Jones PJ et al. (2009) mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. *Neuropsychopharmacology* **34**:2057–2071.
- Ayala JE, Niswender CM, Luo Q, Banko JL, and Conn PJ (2008) Group III mGluR regulation of synaptic transmission at the SC-CA1 synapse is developmentally regulated. *Neuropharmacology* **54**:804–814.
- Ballesteros J and Weinstein H (1995) Integrated methods for the construction of three dimensional models and computational probing of structure-function relations in G-protein coupled receptors. *Methods Neurosci* **25**:366–428.
- Baumann CA, Mu L, Werli N, Krämer SD, Honer M, Schubiger PA, and Ametamey SM (2010) Syntheses and pharmacological characterization of novel thiazole derivatives as potential mGluR5 PET ligands. *Bioorg Med Chem* **18**:6044–6054.
- Becker JA, Clesse D, Spiegelhalter C, Schwab Y, Le Merrer J, and Kieffer BL (2014) Autistic-like syndrome in mu opioid receptor null mice is relieved by facilitated mGluR4 activity. *Neuropsychopharmacology* **39**:2049–2060.
- Bellone C, Lüscher C, and Mamel M (2008) Mechanisms of synaptic depression triggered by metabotropic glutamate receptors. *Cell Mol Life Sci* **65**:2913–2923.
- Belozertseva IV, Kos T, Popik P, Danysh W, and Bespalov AY (2007) Antidepressant-like effects of mGluR1 and mGluR5 antagonists in the rat forced swim and the mouse tail suspension tests. *Eur Neuropsychopharmacol* **17**:172–179.
- Bennett CE, Burnett DA, Greenlee WJ, Knutson CE, Korakas P, Li C, Tulshian D, Wu WL, Bertorelli R, and Fredduzzi S et al. (2012) Fused tricyclic mGluR1 antagonists for the treatment of neuropathic pain. *Bioorg Med Chem Lett* **22**: 1575–1578.
- Beqollari D and Kammermeier PJ (2008) The mGlu(4) receptor allosteric modulator N-phenyl-7-(hydroxymino)cyclopropa[b]chromen-1a-carboxamide acts as a direct agonist at mGlu(6) receptors. *Eur J Pharmacol* **589**:49–52.
- Berry-Kravis E, Hessel D, Coffey S, Hervey C, Schneider A, Yuhus J, Hutchison J, Snape M, Tranfaglia M, and Nguyen DV et al. (2009) A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J Med Genet* **46**:266–271.
- Bradley SJ, Langmead CJ, Watson JM, and Challiss RA (2011) Quantitative analysis reveals multiple mechanisms of allosteric modulation of the mGlu5 receptor in rat astroglia. *Mol Pharmacol* **79**:874–885.
- Bräuner-Osborne H, Jensen AA, and Krosgaard-Larsen P (1999) Interaction of CPCCOEt with a chimeric mGlu1b and calcium sensing receptor. *Neuroreport* **10**:3923–3925.
- Cabello N, Gandía J, Bertarelli DC, Watanabe M, Lluís C, Franco R, Ferré S, Luján R, and Ciruela F (2009) Metabotropic glutamate type 5, dopamine D2 and adenosine A2a receptors form higher-order oligomers in living cells. *J Neurochem* **109**: 1497–1507.
- Campo B, Kalinichev M, Lambeng N, El Yacoubi M, Royer-Urios I, Schneider M, Legrand C, Parron D, Girard F, and Bessif A et al. (2011) Characterization of an mGluR2/3 negative allosteric modulator in rodent models of depression. *J Neurogenet* **25**:152–166.
- Carroll FY, Stolle A, Beart PM, Voerste A, Brabet I, Mauler F, Joly C, Antonicek H, Bockaert J, and Müller T et al. (2001) BAY36-7620: a potent non-competitive mGlu1 receptor antagonist with inverse agonist activity. *Mol Pharmacol* **59**: 965–973.
- Chaki S, Ago Y, Palucha-Paniewiera A, Matrisciano F, and Pilc A (2013) mGlu2/3 and mGlu5 receptors: potential targets for novel antidepressants. *Neuropharmacology* **66**:40–52.
- Chen Y, Goudet C, Pin JP, and Conn PJ (2008) N-4-Chloro-2-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]phenyl-2-hydroxybenzamide (CPPHA) acts through a novel site as a positive allosteric modulator of group 1 metabotropic glutamate receptors. *Mol Pharmacol* **73**:909–918.
- Chen Y, Nong Y, Goudet C, Hemstapat K, de Paulis T, Pin JP, and Conn PJ (2007) Interaction of novel positive allosteric modulators of metabotropic glutamate receptor 5 with the negative allosteric antagonist site is required for potentiation of receptor responses. *Mol Pharmacol* **71**:1389–1398.
- Christopoulos A (2014) Advances in G protein-coupled receptor allostery: from function to structure. *Mol Pharmacol* **86**:463–478.
- Christopoulos A, Changeux JP, Catterall WA, Fabbro D, Burris TP, Cidlowski JA, Olsen RW, Peters JA, Neubig RR, and Pin JP et al. (2014) International union of basic and clinical pharmacology. XC. multisite pharmacology: recommendations for the nomenclature of receptor allostery and allosteric ligands. *Pharmacol Rev* **66**: 918–947.
- Conn PJ, Lindsley CW, Meiler J, and Niswender CM (2014) Opportunities and challenges in the discovery of allosteric modulators of GPCRs for treating CNS disorders. *Nat Rev Drug Discov* **13**:692–708.
- Conn PJ and Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol* **37**:205–237.
- Cosford ND, Roppe J, Tehrani L, Schweiger EJ, Seiders TJ, Chaudary A, Rao S, and Varney MA (2003b) [3H]-methoxymethyl-MTEP and [3H]-methoxy-PEPy: potent and selective radioligands for the metabotropic glutamate subtype 5 (mGlu5) receptor. *Bioorg Med Chem Lett* **13**:351–354.
- Coutinho V and Knöpfel T (2002) Metabotropic glutamate receptors: electrical and chemical signaling properties. *Neuroscientist* **8**:551–561.
- Doré AS, Okrasa K, Patel JC, Serrano-Vega M, Bennett K, Cooke RM, Errey JC, Jazayeri A, Khan S, and Tehan B et al. (2014) Structure of class C GPCR metabotropic glutamate receptor 5 transmembrane domain. *Nature* **511**:557–562.
- Doria JG, Silva FR, de Souza JM, Vieira LB, Carvalho TG, Reis HJ, Pereira GS, Dobransky T, and Ribeiro FM (2013) Metabotropic glutamate receptor 5 positive allosteric modulators are neuroprotective in a mouse model of Huntington's disease. *Br J Pharmacol* **169**:909–921.
- Doumazane E, Scholler P, Zwier JM, Trinquet E, Rondard P, and Pin JP (2011) A new approach to analyze cell surface protein complexes reveals specific heterodimeric metabotropic glutamate receptors. *FASEB J* **25**:66–77.
- Dunayevich E, Erickson J, Levine L, Landbloom R, Schoepp DD, and Tollefson GD (2008) Efficacy and tolerability of an mGlu2/3 agonist in the treatment of generalized anxiety disorder. *Neuropsychopharmacology* **33**:1603–1610.
- Duvoisin RM, Pfankuch T, Wilson JM, Grabell J, Chhajlani V, Brown DG, Johnson E, and Raber J (2010) Acute pharmacological modulation of mGluR8 reduces measures of anxiety. *Behav Brain Res* **212**:168–173.
- El Moustaine D, Granier S, Doumazane E, Scholler P, Rahmeh R, Bron P, Mouillac B, Banères JL, Rondard P, and Pin JP (2012) Distinct roles of metabotropic glutamate receptor dimerization in agonist activation and G-protein coupling. *Proc Natl Acad Sci USA* **109**:16342–16347.
- Emery AC, Diraddo JO, Miller E, Hathaway HA, Pshenichkin S, Takoudjou GR, Grajkowska E, Yasuda RP, Wolfe BB, and Wroblewski J (2012) Ligand Bias at Metabotropic Glutamate 1a Receptor: Molecular Determinants that Distinguish beta-arrestin from G Protein Mediated Signaling. *Mol Pharmacol* **82**:95–105.
- Fell MJ, Witkin JM, Falcone JF, Katner JS, Perry KW, Hart J, Rorick-Kehn L, Overshiner CD, Rasmussen K, and Chaney SF et al. (2011) N-4-((2-(trifluoromethyl)-3-hydroxy-4-(isobutyl)phenoxy)methyl)benzyl)-1-methyl-1H-imidazole-4-carboxamide (THIIC), a novel metabotropic glutamate 2 potentiator with potential anxiolytic/antidepressant properties: in vivo profiling suggests a link between behavioral and central nervous system neurochemical changes. *J Pharmacol Exp Ther* **336**:165–177.
- Felts AS, Lindsley SR, Lamb JP, Rodriguez AL, Menon UN, Jadhav S, Jones CK, Conn PJ, Lindsley CW, and Emmitte KA (2010) 3-Cyano-5-fluoro-N-arylbenzamides as negative allosteric modulators of mGlu(5): Identification of easily prepared tool compounds with CNS exposure in rats. *Bioorg Med Chem Lett* **20**:4390–4394.
- Fukuda J, Suzuki K, Kimura T, Nagatomi Y, Ito S, Kawamoto H, Ozaki S, and Ohta H (2009) Identification of a novel transmembrane domain involved in the negative modulation of mGluR1 using a newly discovered allosteric mGluR1 antagonist, 3-cyclohexyl-5-fluoro-6-methyl-7-(2-morpholin-4-ylethoxy)-4H-chromen-1-one. *Neuropharmacology* **57**:438–445.

- Galici R, Jones CK, Hemstapat K, Nong Y, Echেমেন্দিয়া NG, Williams LC, de Paulis T, and Conn PJ (2006) Biphenyl-indanone A, a positive allosteric modulator of the metabotropic glutamate receptor subtype 2, has antipsychotic- and anxiolytic-like effects in mice. *J Pharmacol Exp Ther* **318**:173–185.
- Gama L, Wilt SG, and Breitwieser GE (2001) Heterodimerization of calcium sensing receptors with metabotropic glutamate receptors in neurons. *J Biol Chem* **276**:39053–39059.
- Gasparini F, Andres H, Flor PJ, Heinrich M, Inderbitzin W, Lingenhöhl K, Müller H, Munk VC, Omilusk K, and Stierlin C et al. (2002) [3H]-M-MPEP, a potent, subtype-selective radioligand for the metabotropic glutamate receptor subtype 5. *Bioorg Med Chem Lett* **12**:407–409.
- Gasparini F, Floersheim P, Flor PJ, Heinrich M, Inderbitzin W, Ott D, Pagano A, Stierlin C, Stoehr N, and Vranesic I et al. (2001) Discovery and characterization of non-competitive antagonists of group I metabotropic glutamate receptors. *Farmacologia* **56**:95–99.
- Gasparini F, Lingenhohl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I, Biollaz M, Allgeier H, Heckendorn R, Urwyler S et al. (1999) 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology* **38**:1493–503.
- González-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, López-Giménez JF, Zhou M, Okawa Y, Callado LF, and Milligan G et al. (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* **452**:93–97.
- Goudet C, Gaven F, Kniazeff J, Vol C, Liu J, Cohen-Gonsaud M, Acher F, Prézeau L, and Pin JP (2004) Heptahelical domain of metabotropic glutamate receptor 5 behaves like rhodopsin-like receptors. *Proc Natl Acad Sci USA* **101**:378–383.
- Goudet C, Kniazeff J, Hlavackova V, Malhaire F, Maurel D, Acher F, Blahos J, Prézeau L, and Pin JP (2005) Asymmetric functioning of dimeric metabotropic glutamate receptors disclosed by positive allosteric modulators. *J Biol Chem* **280**:24380–24385.
- Gregory KJ, Herman EJ, Ramsey AJ, Hammond AS, Byun NE, Stauffer SR, Manka JT, Jadhav S, Bridges TM, and Weaver CD et al. (2013a) N-aryl piperazine metabotropic glutamate receptor 5 positive allosteric modulators possess efficacy in preclinical models of NMDA hypofunction and cognitive enhancement. *J Pharmacol Exp Ther* **347**:438–457.
- Gregory KJ, Nguyen ED, Malosh C, Mendenhall JL, Zic JZ, Bates BS, Noetzel MJ, Squire EF, Turner EM, and Rook JM et al. (2014) Identification of specific ligand-receptor interactions that govern binding and cooperativity of diverse modulators to a common metabotropic glutamate receptor 5 allosteric site. *ACS Chem Neurosci* **5**:282–295.
- Gregory KJ, Nguyen ED, Reiff SD, Squire EF, Stauffer SR, Lindsley CW, Meiler J, and Conn PJ (2013b) Probing the metabotropic glutamate receptor 5 (mGlu5) positive allosteric modulator (PAM) binding pocket: discovery of point mutations that engender a “molecular switch” in PAM pharmacology. *Mol Pharmacol* **83**:991–1006.
- Gregory KJ, Noetzel MJ, Rook JM, Vinson PN, Stauffer SR, Rodriguez AL, Emmette KA, Zhou Y, Chun AC, and Felts AS et al. (2012) Investigating metabotropic glutamate receptor 5 allosteric modulator cooperativity, affinity, and agonism: enriching structure-function studies and structure-activity relationships. *Mol Pharmacol* **82**:860–875.
- Gubellini P, Saulle E, Centonze D, Bonsi P, Pisani A, Bernardi G, Conquet F, and Calabresi P (2001) Selective involvement of mGlu1 receptors in corticostriatal LTD. *Neuropharmacology* **40**:839–846.
- Hammer R, Berrie CP, Birdsall NJ, Burgen AS, and Hulme EC (1980) Pirenzepine distinguishes between different subtypes of muscarinic receptors. *Nature* **283**:90–92.
- Hammond AS, Rodriguez AL, Townsend SD, Niswender CM, Gregory KJ, Lindsley CW, and Conn PJ (2010) Discovery of a Novel Chemical Class of mGlu(5) Allosteric Ligands with Distinct Modes of Pharmacology. *ACS Chem Neurosci* **1**:702–716.
- Hemstapat K, Da Costa H, Nong Y, Brady AE, Luo Q, Niswender CM, Tamagnan GD, and Conn PJ (2007) A novel family of potent negative allosteric modulators of group II metabotropic glutamate receptors. *J Pharmacol Exp Ther* **322**:254–264.
- Hemstapat K, de Paulis T, Chen Y, Brady AE, Grover VK, Alagille D, Tamagnan GD, and Conn PJ (2006) A novel class of positive allosteric modulators of metabotropic glutamate receptor subtype 1 interact with a site distinct from that of negative allosteric modulators. *Mol Pharmacol* **70**:616–626.
- Hughes ZA, Neal SJ, Smith DL, Sukoff Rizzo SJ, Pulicchio CM, Lotarski S, Lu S, Dwyer JM, Brennan J, and Olsen M et al. (2013) Negative allosteric modulation of metabotropic glutamate receptor 5 results in broad spectrum activity relevant to treatment resistant depression. *Neuropharmacology* **66**:202–214.
- Iacovelli L, Felicioni M, Nisticò R, Nicoletti F, and De Blasi A (2014) Selective regulation of recombinantly expressed mGlu7 metabotropic glutamate receptors by G protein-coupled receptor kinases and arrestins. *Neuropharmacology* **77**:303–312.
- Jalan-Sakrinar N, Field JR, Klar R, Mattmann ME, Gregory KJ, Zamorano R, Engers DW, Bollinger SR, Weaver CD, and Days EL et al. (2014) Identification of positive allosteric modulators VU0155094 (ML397) and VU0422288 (ML396) reveals new insights into the biology of metabotropic glutamate receptor 7. *ACS Chem Neurosci* **5**:1221–1237.
- Jiang JY, Nagaraju M, Meyer RC, Zhang L, Hamelberg D, Hall RA, Brown EM, Conn PJ, and Yang JJ (2014) Extracellular calcium modulates actions of orthosteric and allosteric ligands on metabotropic glutamate receptor 1a. *J Biol Chem* **289**:1649–1661.
- Johnson MP, Barda D, Britton TC, Emkey R, Hornback WJ, Jagdmann GE, McKinzie DL, Nisenbaum ES, Tizzano JP, and Schoepp DD (2005) Metabotropic glutamate 2 receptor potentiators: receptor modulation, frequency-dependent synaptic activity, and efficacy in preclinical anxiety and psychosis model(s). *Psychopharmacology (Berl)* **179**:271–283.
- Jones CK, Bubser M, Thompson AD, Dickerson JW, Turle-Lorenzo N, Amalric M, Blobaum AL, Bridges TM, Morrison RD, and Jadhav S et al. (2012) The metabotropic glutamate receptor 4-positive allosteric modulator VU0364770 produces efficacy alone and in combination with L-DOPA or an adenosine 2A antagonist in preclinical rodent models of Parkinson's disease. *J Pharmacol Exp Ther* **340**:404–421.
- Jones PJ, Xiang Z, and Conn PJ (2008) Metabotropic glutamate receptors mGluR4 and mGluR8 regulate transmission in the lateral olfactory tract-piriform cortex synapse. *Neuropharmacology* **55**:440–446.
- Jong YJ, Kumar V, Kingston AE, Romano C, and O'Malley KL (2005) Functional metabotropic glutamate receptors on nuclei from brain and primary cultured striatal neurons. Role of transporters in delivering ligand. *J Biol Chem* **280**:30469–30480.
- Jong YJ, Kumar V, and O'Malley KL (2009) Intracellular metabotropic glutamate receptor 5 (mGluR5) activates signaling cascades distinct from cell surface counterparts. *J Biol Chem* **284**:35827–35838.
- Julio-Pieper M, Flor PJ, Dinan TG, and Cryan JF (2011) Exciting times beyond the brain: metabotropic glutamate receptors in peripheral and non-neural tissues. *Pharmacol Rev* **63**:35–58.
- Kalinichev M, Rouillier M, Girard F, Royer-Urios I, Bournique B, Finn T, Charvin D, Campo B, Le Poul E, and Mutel V et al. (2013) ADX71743, a potent and selective negative allosteric modulator of metabotropic glutamate receptor 7: in vitro and in vivo characterization. *J Pharmacol Exp Ther* **344**:624–636.
- Kammermeier PJ (2012) Functional and pharmacological characteristics of metabotropic glutamate receptors 2/4 heterodimers. *Mol Pharmacol* **82**:438–447.
- Keywood C, Wakefield M, and Tack J (2009) A proof-of-concept study evaluating the effect of ADX10059, a metabotropic glutamate receptor-5 negative allosteric modulator, on acid exposure and symptoms in gastro-oesophageal reflux disease. *Gut* **58**:1192–1199.
- Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, Chen TB, Wisnoski DD, Lindsley CW, and Tiller PR et al. (2005) A novel selective positive allosteric modulator of metabotropic glutamate receptor subtype 5 has in vivo activity and antipsychotic-like effects in rat behavioral models. *J Pharmacol Exp Ther* **313**:199–206.
- Kinon BJ, Zhang L, Millen BA, Osuntokun OO, Williams JE, Kollack-Walker S, Jackson K, Kryzhanovskaya L, and Jarkova N; HBBI Study Group (2011) A multicenter, inpatient, phase 2, double-blind, placebo-controlled dose-ranging study of LY2140023 monohydrate in patients with DSM-IV schizophrenia. *J Clin Psychopharmacol* **31**:349–355.
- Knöflach F, Mutel V, Jolidon S, Kew JN, Malherbe P, Vieira E, Wichmann J, and Kemp JA (2001) Positive allosteric modulators of metabotropic glutamate 1 receptor: characterization, mechanism of action, and binding site. *Proc Natl Acad Sci USA* **98**:13402–13407.
- Kohara A, Toya T, Tamura S, Watabiki T, Nagakura Y, Shitaka Y, Hayashibe S, Kawabata S, and Okada M (2005) Radioligand binding properties and pharmacological characterization of 6-amino-N-cyclohexyl-N,3-dimethylthiazolo[3,2-a]benzimidazole-2-carboxamide (YM-298198), a high-affinity, selective, and non-competitive antagonist of metabotropic glutamate receptor type 1. *J Pharmacol Exp Ther* **315**:163–169.
- Kruse AC, Ring AM, Manglik A, Hu J, Hu K, Eitel K, Hübner H, Pardon E, Valant C, and Sexton PM et al. (2013) Activation and allosteric modulation of a muscarinic acetylcholine receptor. *Nature* **504**:101–106.
- Kumar V, Fahey PG, Jong YJ, Ramanan N, and O'Malley KL (2012) Activation of intracellular metabotropic glutamate receptor 5 in striatal neurons leads to up-regulation of genes associated with sustained synaptic transmission including Arc/Arg3.1 protein. *J Biol Chem* **287**:5412–5425.
- Kumar V, Jong YJ, and O'Malley KL (2008) Activated nuclear metabotropic glutamate receptor mGlu5 couples to nuclear Gq/11 proteins to generate inositol 1,4,5-trisphosphate-mediated nuclear Ca²⁺ release. *J Biol Chem* **283**:14072–14083.
- Lamb JP, Engers DW, Niswender CM, Rodriguez AL, Venable DF, Conn PJ, and Lindsley CW (2011) Discovery of molecular switches within the ADX-47273 mGlu5 PAM scaffold that modulate modes of pharmacology to afford potent mGlu5 NAMs, PAMs and partial antagonists. *Bioorg Med Chem Lett* **21**:2711–2714.
- Lavreysen H, Janssen C, Bischoff F, Langlois X, Leyeys JE, and Lesage AS (2003) [3H]R214127: a novel high-affinity radioligand for the mGlu1 receptor reveals a common binding site shared by multiple allosteric antagonists. *Mol Pharmacol* **63**:1082–1093.
- Lavreysen H, Langlois X, Ahnaou A, Drinkenburg W, te Riele P, Biesmans I, Van der Linden I, Peeters L, Megens A, and Wintmolders C et al. (2013) Pharmacological characterization of JNJ-40068782, a new potent, selective, and systemically active positive allosteric modulator of the mGlu2 receptor and its radioligand [3H]JNJ-40068782. *J Pharmacol Exp Ther* **346**:514–527.
- Lavreysen H, Wouters R, Bischoff F, Nóbrega Pereira S, Langlois X, Blokland S, Somers M, Dillen L, and Lesage AS (2004) JNJ16259685, a highly potent, selective and systemically active mGlu1 receptor antagonist. *Neuropharmacology* **47**:961–972.
- Li X, Need AB, Baez M, and Witkin JM (2006) Metabotropic glutamate 5 receptor antagonism is associated with antidepressant-like effects in mice. *J Pharmacol Exp Ther* **319**:254–259.
- Lindsley CW (2014) 2013 Philip S. Portoghesi Medicinal Chemistry Lectureship: drug discovery targeting allosteric sites. *J Med Chem* **57**:7485–7498.
- Litschik G, Gasparini F, Rueegg D, Stoehr N, Flor PJ, Vranesic I, Prézeau L, Pin JP, Thomsen C, and Kuhn R (1999) CPCCOEt, a noncompetitive metabotropic glutamate receptor 1 antagonist, inhibits receptor signaling without affecting glutamate binding. *Mol Pharmacol* **55**:453–461.
- Lundström L, Bissant C, Beck J, Wettstein JG, Wolterling TJ, Wichmann J, and Gatti S (2011) Structural determinants of allosteric antagonism at metabotropic glutamate receptor 2: mechanistic studies with new potent negative allosteric modulators. *Br J Pharmacol* **164** (2b):521–537.
- Ma AWS, Redda DS, Pisterzi LF, Angers S, and Wells JW (2007) Recovery of oligomers and cooperativity when monomers of the M2 muscarinic cholinergic receptor are reconstituted into phospholipid vesicles. *Biochemistry* **46**:7907–7927.
- Mabire D, Coupa S, Adelinet C, Poncelet A, Simonnet Y, Venet M, Wouters R, Lesage AS, Van Beijsterveldt L, and Bischoff F (2005) Synthesis, structure-activity

- relationship, and receptor pharmacology of a new series of quinoline derivatives acting as selective, noncompetitive mGlu1 antagonists. *J Med Chem* **48**:2134–2153.
- Maj M, Bruno V, Dragic Z, Yamamoto R, Battaglia G, Inderbitzin W, Stoehr N, Stein T, Gasparini F, and Vranesic I et al. (2003) (-)-PHCCC, a positive allosteric modulator of mGluR4: characterization, mechanism of action, and neuroprotection. *Neuropharmacology* **45**:895–906.
- Malherbe P, Kratochwil N, Knoflach F, Zenner MT, Kew JN, Kratzeisen C, Maerki HP, Adam G, and Mutel V (2003a) Mutational analysis and molecular modeling of the allosteric binding site of a novel, selective, noncompetitive antagonist of the metabotropic glutamate 1 receptor. *J Biol Chem* **278**:8340–8347.
- Malherbe P, Kratochwil N, Mühlemann A, Zenner M-T, Fischer C, Stahl M, Gerber PR, Jaeschke G, and Porter RHP (2006) Comparison of the binding pockets of two chemically unrelated allosteric antagonists of the mGlu5 receptor and identification of crucial residues involved in the inverse agonism of MPEP. *J Neurochem* **98**: 601–615.
- Malherbe P, Kratochwil N, Zenner MT, Piussi J, Diener C, Kratzeisen C, Fischer C, and Porter RH (2003b) Mutational analysis and molecular modeling of the binding pocket of the metabotropic glutamate 5 receptor negative modulator 2-methyl-6-(phenylethynyl)-pyridine. *Mol Pharmacol* **64**:823–832.
- Marino M, Williams DL, Jr, O'Brien JA, Valenti O, McDonald TP, Clements MK, Wang R, DiLella AG, Hess JF, and Kinney GG et al. (2003) Allosteric modulation of group III metabotropic glutamate receptor 4: a potential approach to Parkinson's disease treatment. *Proc Natl Acad Sci USA* **100**:13668–13673.
- Mathiesen JM, Svendsen N, Bräuner-Osborne H, Thomsen C, and Ramirez MT (2003) Positive allosteric modulation of the human metabotropic glutamate receptor 4 (hmGluR4) by SIB-1893 and MPEP. *Br J Pharmacol* **138**:1026–1030.
- Mitsukawa K, Yamamoto R, Ofner S, Nozulak J, Pescott O, Lukic S, Stoehr N, Mombereau C, Kuhn R, and McAllister KH et al. (2005) A selective metabotropic glutamate receptor 7 agonist: activation of receptor signaling via an allosteric site modulates stress parameters in vivo. *Proc Natl Acad Sci USA* **102**:18712–18717.
- Moghaddam B (2004) Targeting metabotropic glutamate receptors for treatment of the cognitive symptoms of schizophrenia. *Psychopharmacology (Berl)* **174**:39–44.
- Mølleck C, Harpoe K, Gloriam DE, Clausen RP, Madsen U, Pedersen LO, Jimenez HN, Nielsen SM, Mathiesen JM, and Bräuner-Osborne H (2012) Pharmacological characterization and modeling of the binding sites of novel 1,3-bis(pyrindylethynyl)benzenes as metabotropic glutamate receptor 5-selective negative allosteric modulators. *Mol Pharmacol* **82**:929–937.
- Moussawi K and Kalivas PW (2010) Group II metabotropic glutamate receptors (mGlu2/3) in drug addiction. *Eur J Pharmacol* **639**:115–122.
- Mühlemann A, Ward NA, Kratochwil N, Diener C, Fischer C, Stucki A, Jaeschke G, Malherbe P, and Porter RH (2006) Determination of key amino acids implicated in the actions of allosteric modulation by 3,3'-difluorobenzaldazine on rat mGlu5 receptors. *Eur J Pharmacol* **529**:95–104.
- Niswender CM, Johnson KA, Miller NR, Ayala JE, Luo Q, Williams R, Saleh S, Orton D, Weaver CD, and Conn PJ (2010) Context-dependent pharmacology exhibited by negative allosteric modulators of metabotropic glutamate receptor 7. *Mol Pharmacol* **77**:459–468.
- Niswender CM, Johnson KA, Weaver CD, Jones CK, Xiang Z, Luo Q, Rodriguez AL, Marlo JE, de Paulis T, and Thompson AD et al. (2008) Discovery, characterization, and antiparkinsonian effect of novel positive allosteric modulators of metabotropic glutamate receptor 4. *Mol Pharmacol* **74**:1345–1358.
- Noetzel MJ, Gregory KJ, Vinson PN, Manka JT, Stauffer SR, Lindsley CW, Niswender CM, Xiang Z, and Conn PJ (2013) A novel metabotropic glutamate receptor 5 positive allosteric modulator acts at a unique site and confers stimulus bias to mGlu5 signaling. *Mol Pharmacol* **83**:835–847.
- Noetzel MJ, Jones CK, and Conn PJ (2012a) Emerging approaches for treatment of schizophrenia: modulation of glutamatergic signaling. *Discov Med* **14**:335–343.
- Noetzel MJ, Rook JM, Vinson PN, Cho HP, Days E, Zhou Y, Rodriguez AL, Lavreysen H, Stauffer SR, and Niswender CM et al. (2012b) Functional impact of allosteric agonist activity of selective positive allosteric modulators of metabotropic glutamate receptor subtype 5 in regulating central nervous system function. *Mol Pharmacol* **81**:120–133.
- O'Brien JA, Lemaire W, Chen TB, Chang RS, Jacobson MA, Ha SN, Lindsley CW, Schaffhauser HJ, Sur C, and Pettibone DJ et al. (2003) A family of highly selective allosteric modulators of the metabotropic glutamate receptor subtype 5. *Mol Pharmacol* **64**:731–740.
- O'Brien JA, Lemaire W, Wittmann M, Jacobson MA, Ha SN, Wisnoski DD, Lindsley CW, Schaffhauser HJ, Rowe B, and Sur C et al. (2004) A novel selective allosteric modulator potentiates the activity of native metabotropic glutamate receptor subtype 5 in rat forebrain. *J Pharmacol Exp Ther* **309**:568–577.
- Odagaki Y, Kinoshita M, and Toyoshima R (2011) Functional coupling between metabotropic glutamate receptors and G-proteins in rat cerebral cortex assessed by guanosine 5'-O-(3-[(35S)]thio)triphosphate binding assay. *Basic Clin Pharmacol Toxicol* **109**:175–185.
- Odagaki Y, Kinoshita M, and Toyoshima R (2013) Group II metabotropic glutamate receptor-mediated activation of G-proteins in rat hippocampal and striatal membranes. *Neurosci Lett* **539**:22–26.
- Ott D, Floersheim P, Inderbitzin W, Stoehr N, Francotte E, Lecis G, Richert P, Rihs G, Flor PJ, and Kuhn R et al. (2000) Chiral resolution, pharmacological characterization, and receptor docking of the noncompetitive mGlu1 receptor antagonist (+/-)-2-hydroxyimino-1a, 2-dihydro-1H-7-oxacyclopropa[b]naphthalene-7a-carboxylic acid ethyl ester. *J Med Chem* **43**:4428–4436.
- Pagano A, Ruegg D, Litschig S, Stoehr N, Stierlin C, Heinrich M, Floersheim P, Prézéau L, Carroll F, and Pin JP et al. (2000) The non-competitive antagonists 2-methyl-6-(phenylethynyl)pyridine and 7-hydroxyimino-2-cyclopropylchromen-1a-carboxylic acid ethyl ester interact with overlapping binding pockets in the transmembrane region of group I metabotropic glutamate receptors. *J Biol Chem* **275**:33750–33758.
- Parmentier-Batteur S, Hutson PH, Menzel K, Uslander JM, Mattson BA, O'Brien JA, Magliaro BC, Forest T, Stump CA, and Tynebor RM et al. (2014) Mechanism based neurotoxicity of mGlu5 positive allosteric modulators—development challenges for a promising novel antipsychotic target. *Neuropharmacology* **82**:161–173.
- Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, Avedisova AS, Bardenstein LM, Gurovich IY, and Morozova MA et al. (2007) Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. *Nat Med* **13**:1102–1107.
- Pelkey KA, Yuan X, Lavezzari G, Roche KW, and McBain CJ (2007) mGluR7 undergoes rapid internalization in response to activation by the allosteric agonist AMN082. *Neuropharmacology* **52**:108–117.
- Pinheiro PS and Mulle C (2008) Presynaptic glutamate receptors: physiological functions and mechanisms of action. *Nat Rev Neurosci* **9**:423–436.
- Pittolo S, Gómez-Santacana X, Eckelt K, Rovira X, Dalton J, Goudet C, Pin JP, Llobet A, Giraldo J, and Llebaria A et al. (2014) An allosteric modulator to control endogenous G protein-coupled receptors with light. *Nat Chem Biol* **10**:813–815.
- Porter RH, Jaeschke G, Spooren W, Ballard TM, Büttelmann B, Kolczewski S, Peters JU, Prinssen E, Wichmann J, and Vieira E et al. (2005) Fenobam: a clinically validated nonbenzodiazepine anxiolytic is a potent, selective, and noncompetitive mGlu5 receptor antagonist with inverse agonist activity. *J Pharmacol Exp Ther* **315**:711–721.
- Purgert CA, Izumi Y, Jong YJ, Kumar V, Zorunski CF, and O'Malley KL (2014) Intracellular mGluR5 can mediate synaptic plasticity in the hippocampus. *J Neurosci* **34**:4589–4598.
- Rodriguez AL, Grier MD, Jones CK, Herman EJ, Kane AS, Smith RL, Williams R, Zhou Y, Marlo JE, and Days EL et al. (2010) Discovery of novel allosteric modulators of metabotropic glutamate receptor subtype 5 reveals chemical and functional diversity and in vivo activity in rat behavioral models of anxiolytic and antipsychotic activity. *Mol Pharmacol* **78**:1105–1123.
- Rodriguez AL, Nong Y, Sekaran NK, Alagille D, Tamagnan GD, and Conn PJ (2005) A close structural analog of 2-methyl-6-(phenylethynyl)-pyridine acts as a neutral allosteric site ligand on metabotropic glutamate receptor subtype 5 and blocks the effects of multiple allosteric modulators. *Mol Pharmacol* **68**:1793–1802.
- Rodriguez AL, Zhou Y, Williams R, Weaver CD, Vinson PN, Dawson ES, Steckler T, Lavreysen H, Mackie C, and Bartolomé JM et al. (2012) Discovery and SAR of a novel series of non-MPEP site mGlu5 PAMs based on an aryl glycine sulfonamide scaffold. *Bioorg Med Chem Lett* **22**:7388–7392.
- Rook JM, Noetzel MJ, Pouliot WA, Bridges TM, Vinson PN, Cho HP, Zhou Y, Gogliotti RD, Manka JT, and Gregory KJ et al. (2013) Unique signaling profiles of positive allosteric modulators of metabotropic glutamate receptor subtype 5 determine differences in in vivo activity. *Biol Psychiatry* **73**:501–509.
- Rook JM, Xiang Z, Lv X, Ghoshal A, Dickerson JW, Bridges TM, Johnson KA, Foster DJ, Gregory KJ, and Vinson PN et al. (2015) Biased mGlu5 positive allosteric modulators provide in vivo efficacy without potentiating mGlu5 modulation of NMDAR currents. *Neuron*, in press.
- Rovira X, Malhaire F, Scholler R, Rodrigo J, Gonzalez-Bulnes P, Llebaria A, Pin JP, Giraldo J, and Goudet C (2015) Overlapping binding sites drive allosteric agonism and positive cooperativity in type 4 metabotropic glutamate receptors. *FASEB J* **29**:116–130.
- Rowe BA, Schaffhauser H, Morales S, Lubbers LS, Bonnefous C, Kamenicka TM, McQuiston J, and Daggett LP (2008) Transposition of three amino acids transforms the human metabotropic glutamate receptor (mGluR)-3-positive allosteric modulation site to mGluR2, and additional characterization of the mGluR2-positive allosteric modulation site. *J Pharmacol Exp Ther* **326**:240–251.
- Rylander D, Iderberg H, Li Q, Dekundy A, Zhang J, Li H, Baishen R, Danysz W, Bezard E, and Cenci MA (2010) A mGluR5 antagonist under clinical development improves L-DOPA-induced dyskinesia in parkinsonian rats and monkeys. *Neurobiol Dis* **39**:352–361.
- Sams AG, Mikkelsen GK, Brodbeck RM, Pu X, and Ritzén A (2011) Efficacy switching SAR of mGluR5 allosteric modulators: highly potent positive and negative modulators from one chemotype. *Bioorg Med Chem Lett* **21**:3407–3410.
- Sanger H, Hanna L, Colvin EM, Grubisha O, Ursu D, Heinz BA, Findlay JD, Vivier RG, Sher E, and Lodge D et al. (2013) Pharmacological profiling of native group II metabotropic glutamate receptors in primary cortical neuronal cultures using a FLIPR. *Neuropharmacology* **66**:264–273.
- Scandroglio P, Brusa R, Lozza G, Mancini I, Petró R, Reggiani A, and Beltramo M (2010) Evaluation of cannabinoid receptor 2 and metabotropic glutamate receptor 1 functional responses using a cell impedance-based technology. *J Biomol Screen* **15**: 1238–1247.
- Schaffhauser H, Rowe BA, Morales S, Chavez-Noriega LE, Yin R, Jachec C, Rao SP, Bain G, Pinkerton AB, and Vernier JM et al. (2003) Pharmacological characterization and identification of amino acids involved in the positive modulation of metabotropic glutamate receptor subtype 2. *Mol Pharmacol* **64**:798–810.
- Schann S, Mayer S, Franchet C, Frauli M, Steinberg E, Thomas M, Baron L, and Neuville P (2010) Chemical switch of a metabotropic glutamate receptor 2 silent allosteric modulator into dual metabotropic glutamate receptor 2/3 negative/positive allosteric modulators. *J Med Chem* **53**:8775–8779.
- Schoepp DD, Wright RA, Levine LR, Gaydos B, and Potter WZ (2003) LY354740, an mGlu2/3 receptor agonist as a novel approach to treat anxiety/stress. *Stress* **6**: 189–197.
- Sevastyanova TN and Kammermeier PJ (2014) Cooperative signaling between homodimers of metabotropic glutamate receptors 1 and 5. *Mol Pharmacol* **86**:492–504.
- Sharma S, Kedrowski J, Rook JM, Smith RL, Jones CK, Rodriguez AL, Conn PJ, and Lindsley CW (2009) Discovery of molecular switches that modulate modes of metabotropic glutamate receptor subtype 5 (mGlu5) pharmacology in vitro and in vivo within a series of functionalized, regioisomeric 2- and 5-(phenylethynyl)pyrimidines. *J Med Chem* **52**:4103–4106.
- Sheffler DJ and Conn PJ (2008) Allosteric potentiators of metabotropic glutamate receptor subtype 1a differentially modulate independent signaling pathways in baby hamster kidney cells. *Neuropharmacology* **55**:419–427.
- Sheffler DJ, Wenthur CJ, Bruner JA, Carrington SJS, Vinson PN, Gogi KK, Blobaum AL, Morrison RD, Vamos M, and Cosford NDP et al. (2012) Development of a novel,

- CNS-penetrant, metabotropic glutamate receptor 3 (mGlu3) NAM probe (ML289) derived from a closely related mGlu5 PAM. *Bioorg Med Chem Lett* **22**:3921–3925.
- Sukoff Rizzo SJ, Leonard SK, Gilbert A, Dollings P, Smith DL, Zhang MY, Di L, Platt BJ, Neal S, and Dwyer JM et al. (2011) The metabotropic glutamate receptor 7 allosteric modulator AMN082: a monoaminergic agent in disguise? *J Pharmacol Exp Ther* **338**:345–352.
- Surin A, Pshenichkin S, Grajkowska E, Surina E, and Wroblewski JT (2007) Cyclothiazide selectively inhibits mGluR1 receptors interacting with a common allosteric site for non-competitive antagonists. *Neuropharmacology* **52**:744–754.
- Suzuki G, Kimura T, Satow A, Kaneko N, Fukuda J, Hikichi H, Sakai N, Maehara S, Kawagoe-Takaki H, and Hata M et al. (2007) Pharmacological characterization of a new, orally active and potent allosteric metabotropic glutamate receptor 1 antagonist, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2H)-carboxamide (FTIDC). *J Pharmacol Exp Ther* **321**:1144–1153.
- Treyer V, Streffer J, Wyss MT, Bettio A, Ametamey SM, Fischer U, Schmidt M, Gasparini F, Hock C, and Buck A (2007) Evaluation of the metabotropic glutamate receptor subtype 5 using PET and 11C-ABP688: assessment of methods. *J Nucl Med* **48**:1207–1215.
- Turlington M, Noetzel MJ, Bridges TM, Vinson PN, Steckler T, Lavreysen H, Mackie C, Bartolomé-Nebreda JM, Conde-Ceide S, and Tong HM et al. (2014) Discovery and SAR of a novel series of metabotropic glutamate receptor 5 positive allosteric modulators with high ligand efficiency. *Bioorg Med Chem Lett* **24**:3641–3646.
- Valenti O, Conn PJ, and Marino MJ (2002) Distinct physiological roles of the Gq-coupled metabotropic glutamate receptors Co-expressed in the same neuronal populations. *J Cell Physiol* **191**:125–137.
- Valenti O, Mannaioni G, Seabrook GR, Conn PJ, and Marino MJ (2005) Group III metabotropic glutamate-receptor-mediated modulation of excitatory transmission in rodent substantia nigra pars compacta dopamine neurons. *J Pharmacol Exp Ther* **313**:1296–1304.
- Vieira E, Huwyler J, Jolidon S, Knoflach F, Mutel V, and Wichmann J (2009) Fluorinated 9H-xanthene-9-carboxylic acid oxazol-2-yl-amides as potent, orally available mGlu1 receptor enhancers. *Bioorg Med Chem Lett* **19**:1666–1669.
- Vilar B, Busserolles J, Ling B, Laffray S, Ulmann L, Malhaire F, Chapuy E, Aissouni Y, Etienne M, and Bourinet E et al. (2013) Alleviating pain hypersensitivity through activation of type 4 metabotropic glutamate receptor. *J Neurosci* **33**:18951–18965.
- Woltering TJ, Wichmann J, Goetschi E, Knoflach F, Ballard TM, Huwyler J, and Gatti S (2010) Synthesis and characterization of 1,3-dihydro-benzo[b][1,4]diazepin-2-one derivatives: Part 4. In vivo active potent and selective non-competitive metabotropic glutamate receptor 2/3 antagonists. *Bioorg Med Chem Lett* **20**:6969–6974.
- Wood MR, Hopkins CR, Brogan JT, Conn PJ, and Lindsley CW (2011) “Molecular switches” on mGluR allosteric ligands that modulate modes of pharmacology. *Biochemistry* **50**:2403–2410.
- Wu H, Wang C, Gregory KJ, Han GW, Cho HP, Xia Y, Niswender CM, Katritch V, Meiler J, and Cherezov V et al. (2014) Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. *Science* **344**:58–64.
- Yin S, Noetzel MJ, Johnson KA, Zamorano R, Jalan-Sakrikar N, Gregory KJ, Conn PJ, and Niswender CM (2014) Selective actions of novel allosteric modulators reveal functional heteromers of metabotropic glutamate receptors in the CNS. *J Neurosci* **34**:79–94.
- Yin S, Zamorano R, Conn PJ, and Niswender CM (2013) Functional selectivity induced by mGlu(4) receptor positive allosteric modulation and concomitant activation of G(q) coupled receptors. *Neuropharmacology* **66**:122–132.
- Zerbib F, Keywood C, and Strabach G (2010) Efficacy, tolerability and pharmacokinetics of a modified release formulation of ADX10059, a negative allosteric modulator of metabotropic glutamate receptor 5: an esophageal pH-impedance study in healthy subjects. *Neurogastroenterol Motil* **22**: 859–865.
- Zhang Y, Rodriguez AL, and Conn PJ (2005) Allosteric potentiators of metabotropic glutamate receptor subtype 5 have differential effects on different signaling pathways in cortical astrocytes. *J Pharmacol Exp Ther* **315**:1212–1219.
- Zhou Y, Manka JT, Rodriguez AL, Weaver CD, Days EL, Vinson PN, Jadhav S, Hermann EJ, Jones CK, and Conn PJ et al. (2010) Discovery of N-Aryl Piperazines as Selective mGlu(5) Potentiators with Efficacy in a Rodent Model Predictive of Anti-Psychotic Activity. *ACS Med Chem Lett* **1**:433–438.

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