Published in final edited form as:

Neuropharmacology. 2011 January ; 60(1): 66–81. doi:10.1016/j.neuropharm.2010.07.007.

Allosteric Modulation of Metabotropic Glutamate Receptors: Structural Insights and Therapeutic Potential

Karen J. Gregory^a, Elizabeth N. Dong^b, Jens Meiler^{a,b,c}, and P. Jeffrey Conn^a

Karen J. Gregory: karen.j.gregory@vanderbilt.edu; Elizabeth N. Dong: elizabeth.n.dong@vanderbilt.edu

^aDepartment of Pharmacology, Vanderbilt Program in Drug Discovery, Vanderbilt University Medical Center, Nashville, TN, USA

^bCenter for Structural Biology, Vanderbilt University Medical Center, Nashville, TN, USA

^cDepartment of Chemistry and the Institute for Chemical Biology, Vanderbilt University Medical Center, Nashville, TN, USA

Abstract

Allosteric modulation of G protein-coupled receptors (GPCRs) represents a novel approach to the development of probes and therapeutics that is expected to enable subtype-specific regulation of central nervous system target receptors. The metabotropic glutamate receptors (mGlu) are class C GPCRs that play important neuromodulatory roles throughout the brain, as such they are attractive targets for therapeutic intervention for a number of psychiatric and neurological disorders including anxiety, depression, Fragile X Syndrome, Parkinson's disease and schizophrenia. Over the last fifteen years, selective allosteric modulators have been identified for many members of the mGlu family. The vast majority of these allosteric modulators are thought to bind within the transmembrane-spanning domains of the receptors to enhance or inhibit functional responses. A combination of mutagenesis-based studies and pharmacological approaches are beginning to provide a better understanding of mGlu allosteric sites. Collectively, when mapped onto a homology model of the different mGlu subtypes based on the β_2 -adrenergic receptor, the previous mutagenesis studies suggest commonalities in the location of allosteric sites across different members of the mGlu family. In addition, there is evidence for multiple allosteric binding pockets within the transmembrane region that can interact to modulate one another. In the absence of a class C GPCR crystal structure, this approach has shown promise with respect to the interpretation of mutagenesis data and understanding structure-activity relationships of allosteric modulator pharmacophores.

Keywords

allosteric modulator; chimeric receptors; G protein-coupled receptors; neuromodulation; site-directed mutagenesis; homology model

© 2010 Elsevier Ltd. All rights reserved.

Co-corresponding Authors: Jens Meiler, jens.meiler@vanderbilt.edu, Center for Structural Biology, Vanderbilt University Medical Center, 5144B BIOSCI/MRBIII, 465 21st Avenue South, Nashville, TN, 37232-8725, Phone:+1-615-936-5662, Fax:+1-615-936-2211, P. Jeffrey Conn, jeff.conn@vanderbilt.edu, Department of Pharmacology, Vanderbilt University Medical Center, 1215 Light Hall, 2215-B Garland Ave, Nashville, TN, 37232-0697, Phone:+1-615-936-2478, Fax:+1-615-343-3088.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction¹

In addition to eliciting fast excitatory synaptic responses, the neurotransmitter glutamate can modulate neuronal excitability, synaptic transmission, and other cell functions by activation of metabotropic glutamate receptors (mGlu). Due to the ubiquitous distribution of glutamatergic synapses and the broad range of functions of the mGlu, members of this receptor family participate in many different processes in the central nervous system (CNS). As such, mGlu are an attractive target for therapeutic intervention for a range of neurological and psychiatric disorders. mGlu are members of the G protein-coupled receptor (GPCR) superfamily, the largest class of cell-surface receptors. Despite their tractability as drug targets, the majority of GPCR-based drug discovery programs have failed to yield highly selective compounds. The traditional approach to drug discovery has been to target the endogenous ligand (orthosteric)-binding site, to either mimic or block the actions of the endogenous neurotransmitter or hormone in a competitive manner. However, this approach has suffered from a paucity of suitably subtype-selective ligands. This is not surprising given that orthosteric binding sites are often highly conserved between subtypes of a single GPCR subfamily. An alternative approach is to target allosteric sites that are topographically distinct from the orthosteric site, to either enhance or inhibit receptor activation. This approach has been highly successful for ligand-gated ion channels. For example benzodiazepines, positive allosteric modulators (PAMs) of GABA_A receptors, are an effective and safe treatment for anxiety and sleep disorders (Mohler et al. 2002). Discovery and characterization of allosteric modulators of GPCRs has gained significant momentum over the last few years, especially since the clinical validity of GPCR allosteric modulators was demonstrated with two allosteric modulators entering the market. In 2004, cinacalcet (an allosteric enhancer of the Calcium-sensing receptor (CaSR)) was approved for the treatment of hyperparathyroidism, a disease associated with CaSR deficiency (Lindberg et al., 2005). In 2007, maraviroc (an allosteric inhibitor of the chemokine receptor CCR5) was approved for the treatment of HIV infections. This drug stabilizes CCR5 receptor conformations that have a lower affinity for the HIV virus, blocking CCR5-dependent entry of HIV-1 into cells (Dorr et al., 2005). Thus, allosteric modulation represents an exciting novel means of targeting GPCRs particularly for CNS disorders, a therapeutic area with one of the highest rates of attrition in drug discovery (Kola & Landis, 2004).

¹Abbreviations: ADX47273, *S*-(4-fluoro-phenyl)-[3-[3-(4-fluoro-phenyl)-[1,2,4]oxadiazol-5-yl]-piperidin-1-yl]-methanone; AMN082, *N,N'*-Bis(diphenylmethyl)-1,2-ethanediamine; ATCM, allosteric ternary complex model; BINA, Biphenyl-indanone A; Br-5MPEPy, 2-(2-(5-bromopyridin-3-yl)ethynyl)-5-methylpyridine; CaSR, Calcium-sensing receptor; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; CFMMC, 3-cyclohexyl-5-fluoro-6-methyl-7-(2-morpholin-4-ylethoxy)-4*H*-chromen-4-one; 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; DFB, [(3-Fluorophenyl)methylene]hydrazone-3-fluorobenzaldehyde; EM-TBPC, 1-ethyl-2-methyl-6-oxo-4-(1,2,4,5-tetrahydro-benzo[*d*]azepin-3-yl)-1,6-dihydro-pyrimidine-5-carbonitrile; ERK1/2, extracellular signal-regulated kinases 1 and 2; FMRP, fragile X mental retardation protein; FTIDC, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide; FXS, Fragile X Syndrome; GABA, γ -aminobutyric acid; GPCR, G protein-coupled receptor; mGlu, metabotropic glutamate receptor; LY404039, (-)-(1*R*,4*S*,5*S*,6*S*)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid; LY456066, (2-[4-(indan-2-ylamino)-5,6,7,8-tetrahydro-quinazolin-2-ylsulfanyl]-ethanol hydrochloride); LY487379, 2,2,2-Trifluoro-*N*-[4-(2-methoxyphenoxy) phenyl]-*N*-(3-pyridinylmethyl)ethanesulfonamide; M-5MPEP, 2-(2-(3-methoxyphenyl)ethynyl)-5-methylpyridine; M-MPEP, 2-methyl-6-(3-methoxyphenyl)ethynyl-pyridine; MMPIP, 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo [4,5-*c*]pyridine-4(5*H*)-one hydrochloride; MPEP, 2-Methyl-6-(phenylethynyl)pyridine; MTEP, 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]pyridine; NAM, negative allosteric modulator; NMDA, *N*-methyl-D-aspartate; PAM, positive allosteric modulator; PCP, Phencyclidine; PD, Parkinson's Disease; 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-phenyl-1-ethanone; Ro 67-7476, (S)-2-(4-fluorophenyl)-1-(toluene-4-sulfonyl)pyrrolidine; S-4C3H-PG, (S)-4-carboxy-3-hydroxyphenylglycine; SAR, structure-activity relationship; SIB-1757, 6-Methyl-2-(phenylazo)-3-pyridinol; SIB-1893, 2-Methyl-6-(2-phenylethenyl)pyridine; TM, transmembrane; VFD, Venus-Flytrap domain; VU0155041, *cis*-2-[[[3,5-Dichlorophenyl]amino]carbonyl]cyclohexanecarboxylic acid; VU29, 4-nitro-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; VU48, 4-nitro-*N*-(1-(2-bromophenyl)-3-phenyl-1*H*-pyrazol-5-yl)benzamide; VU71, 4-nitro-*N*-(1,4-diphenyl-1*H*-pyrazol-5-yl)benzamide; YM298198, 6-amino-*N*-cyclohexyl-*N*,3-dimethylthiazolo[3,2-*a*]benzimidazole-2-carboxamide.

2. Allosteric modulation of metabotropic glutamate receptors

2.1. Quantifying allosteric interactions

The binding of an allosteric ligand to its site will change the conformation of the receptor, meaning that the “geography” of the orthosteric site and any other potential receptor-ligand/protein interfaces, also have the potential to change. As a consequence, the binding affinity and/or signaling efficacy of the orthosteric ligand are likely to be modulated, either in a positive or negative manner. The simplest allosteric GPCR model assumes that the binding of an allosteric ligand to its site modulates only the affinity of the orthosteric ligand and vice versa; this model is referred to as the allosteric ternary complex model (ATCM; Figure 1A). Within the framework of an ATCM, the interaction is governed by the concentration of each ligand, the equilibrium dissociation constants of the orthosteric and allosteric ligands (K_A and K_B , respectively), and the “cooperativity factor” α , a measure of the magnitude and direction of the allosteric interaction between the two conformationally linked sites (Stockton et al., 1983; Ehlert, 1988). A value of $\alpha < 1$ (but greater than 0) indicates negative cooperativity, such that the binding of an allosteric ligand inhibits the binding of the orthosteric ligand. Values of $\alpha > 1$ indicate positive cooperativity, such that the allosteric modulator promotes the binding of orthosteric ligand, whereas values of $\alpha = 1$ indicate neutral cooperativity, i.e. no net change in binding affinity at equilibrium. Because the two sites are conformationally linked, the allosteric interaction is reciprocal, i.e., the orthosteric ligand will modulate the binding of the allosteric ligand in the same manner and to the same extent.

The simple ATCM describes the effect of the modulator only in terms of changes in orthosteric ligand affinity, and vice versa, thus the stimulus that is generated by the ARB ternary complex (a receptor (R) simultaneously occupied by both agonist (A) and modulator (B)) is assumed to be no different to that imparted by the binary AR complex. In general, many allosteric modulators studied to date, particularly those interacting with class A GPCRs, appear to behave in a manner consistent with this simple ATCM. However, there is no *a priori* reason why the conformational change engendered by an allosteric modulator in the GPCR does not perturb signaling efficacy in addition to, or independently of, any effects on orthosteric ligand binding affinity. Indeed, for mGluR the majority of allosteric modulators influence orthosteric ligand efficacy in the absence of effects on affinity. This is most likely a reflection of the fact that the orthosteric and allosteric binding sites are located in very distinct regions of the receptor i.e. the extracellular N-terminus and the transmembrane-spanning domains respectively (Conn et al., 2009; see later for discussion). It is also important to note that an allosteric modulator can have differential effects on affinity versus efficacy. A striking example of this is the cannabinoid CB₁ receptor allosteric modulator, Org27569, which is an allosteric enhancer of [³H]CP 55940 binding but an allosteric inhibitor of CP 55940 function (Price et al., 2005). This potential for differential effects on efficacy as well as affinity has necessitated the development of alternative models to describe allosteric interactions.

To account for such allosteric effects on efficacy, the ATCM has been extended into an allosteric “two-state” model (ATSM) (Hall, 2000). An alternate extension of the ATCM has been proposed by Parmentier and colleagues to model the functioning of class C GPCRs (Parmentier et al., 2002). This model accounts for the fact that class C GPCRs have very distinct ligand binding and effector coupling domains and proposes an allosteric interaction between these two domains. While these models provide a framework to describe the vast array of allosteric modulator effects on receptor binding and functional properties, they are not amenable to fitting to experimental biological data due to the large number of model parameters. Recently an “operational model of allosterism” was reported that more simply describes both allosteric modulation of affinity and efficacy and incorporates allosteric agonism (Leach et al., 2007; May et al., 2007b). The operational model of allosterism (Fig. 1B) combines the simple ATCM with an operational model of agonism (Black & Leff,

1983). According to the model, the pharmacological response, initiated by the stimulus (S), can be the result of three different receptor species: the agonist bound (AR), the modulator bound (BR) and the ternary complex (ARB). As for the simple ATCM, allosteric modulation of binding affinity is governed by the cooperativity factor α , whilst allosteric modulation of efficacy is incorporated into the model by the introduction of another parameter, β . The parameters τ_A and τ_B relate to the ability of the orthosteric and allosteric ligands, respectively, to engender receptor activation. Both τ_A and τ_B incorporate the intrinsic efficacy of each ligand, the total density of receptors and the stimulus-response coupling efficiency of the system under investigation. The parameters E_m and n denote the maximal possible system response and the slope factor of the transducer function that links occupancy to response, respectively.

In this operational model of allosterism, allosteric modulation is governed by two cooperativity parameters, α and β , which can vary for each and every set of interacting ligands at a GPCR. However, these should not change for a given set of ligands and GPCR between different assays of GPCR function. An important caveat to this is the potential for pathway specific modulation, in which case the β values will change. It should also be noted that the τ values are determined not only by the individual ligands but also by the biological assay system under investigation, and thus can change between different systems. Analyzing an effector pathway that has low stimulus-response coupling efficiency or alternatively a very low level of receptor expression can yield a low τ value for an agonist and, as such, its efficacy may not be discernible; if the compound is allosteric the interaction will manifest primarily as a change in the potency or maximal response to orthosteric ligand, with no effect on basal signaling. As the τ_B value approaches 0, the species RB becomes equivalent to R and the operational model of allosteric modulation is essentially the ATCM, incorporating efficacy modulation with respect to the species ARB. In the case of receptor over-expression or high stimulus-response coupling efficiency and subsequently high τ_B values, the allosteric ligand efficacy will substantially increase the basal responsiveness of the assay system, and may also shift the orthosteric agonist potency. However, enhancement of the maximal response may not be evident, as a GPCR that has high coupling efficiency may already be approaching the maximal possible response of the entire cellular system (E_m). These are important considerations in terms of designing screening strategies for allosteric ligand-based drug discovery programs, interpreting the pharmacology of putative allosteric modulators and also translating research from recombinant systems to tissues and beyond.

2.2 Structural features of metabotropic glutamate receptors

Arguably, one of the most well studied GPCR families with respect to allosteric modulation are the mGlu. Indeed, the full spectrum of allosteric ligands has been described for these receptors. There are eight mGlu subtypes that are classified into three major groups based on sequence homology, pharmacological properties, and coupling to different second-messenger pathways. Group I includes mGlu₁ and mGlu₅; group II, mGlu₂ and mGlu₃; and group III, mGlu₄, mGlu₆, mGlu₇ and mGlu₈. mGlus of the same group show ~70% sequence identity whereas between groups this percentage falls to ~45% (Conn & Pin, 1997). Group I mGlus preferentially couple to activation of the G_{q/11} family of G proteins activating phosphoinositide hydrolysis as the major signaling mechanism. In contrast, group II and group III mGlus preferentially couple to G_{i/o} and inhibition of adenylyl cyclases. Members of each group have a unique pharmacological profile and can be selectively activated by specific agonists or allosteric modulators that have no effects on members of the other groups.

GPCRs are predicted to share a common topology consisting of seven transmembrane-spanning α -helical domains, an extracellular N terminus and intracellular C terminus. The mGlus are sub-classified into class C GPCRs along with Calcium-sensing, GABA_B, pheromone and taste receptors. Most class C GPCRs are distinguished by their large

extracellular N-terminal domain, termed the Venus Flytrap domain (VFD), that contains the endogenous ligand-binding site (Pin et al., 2003). The crystal structures of the N-terminal domains of mGlu₁, mGlu₃ and mGlu₇ suggest that the VFD of the mGlu is made up of two lobes (Kunishima et al., 2000; Tsuchiya et al., 2002; Muto et al., 2007). This forms a clam shell-shaped structure, with the glutamate binding site residing between the two lobes. Evidence suggests that the mGlu dimerize via interactions between their VFDs. When glutamate binds, the globular domains close into a stable conformation with glutamate inside (Bessis et al., 2000; 2002; Kunishima et al., 2000; Tsuchiya et al., 2002). The conformation changes induced by glutamate binding at the VFD are transmitted via a cysteine-rich domain. The cysteine-rich domain, unique to class C GPCRs (with the exception of the GABA_B receptor which does not have one), links the VFD to the transmembrane-spanning α -helices by a conserved disulfide bridge, subsequently promoting coupling to intracellular G proteins and activation of second messenger pathways (Liu et al., 2004; Rondard et al., 2006; Muto et al., 2007).

While the extracellular N-terminal domain of several mGlu has been crystallized, the structure of the hepta-helical transmembrane domain of the receptor has yet to be determined. In the absence of a crystal structure, homology modeling with class A GPCR templates has been shown to provide substantial insight into the transmembrane region of mGlu. Despite the low sequence identity (less than 20%) between the different classes of GPCRs, confirmation of a common hepta-helical architecture in the mGlu transmembrane region provides support for the use of class A templates as a starting point for homology modeling (Bhave et al., 2003). Differences between GPCR classes prevent homology models from providing structural information at atomic resolution, therefore modeling best occurs synergistically alongside experimental studies of allosteric modulators (Ballesteros and Palczewski, 2001). Early models of mGlu₁ and mGlu₅ (Ott et al., 2000; Pagano et al., 2000) were based on an alpha-carbon template constructed from a sequence analysis of the transmembrane helices in the rhodopsin family of GPCRs (Baldwin et al., 1997). After the 2.8 angstrom resolution structure of bovine rhodopsin was crystallized (Palczewski et al., 2000), homology models of class C GPCRs revealed possible binding modes of known allosteric modulators within the transmembrane domain of the receptor, which will be discussed in detail below (Ott et al., 2000; Malherbe 2003a, 2003b; Miedlich et al., 2004; Vanejevs et al., 2008). Since the bovine rhodopsin crystal structure, two additional mammalian GPCR crystal structures have become available: the human β_2 -adrenergic receptor (Cherezov et al., 2007; Rasmussen et al., 2007; Rosenbaum et al., 2007) and the human A_{2A} adenosine receptor (Jaakola et al., 2008). The growing number of available templates has sparked development of high-throughput homology modeling of GPCRs (Yarnitzky et al., 2010) and will enrich the understanding of the transmembrane region of these receptors.

2.3 Verifying allosteric modulation through metabotropic glutamate receptor constructs

One way of determining that a putative allosteric modulator is not binding the orthosteric site of a mGlu is through the use of chimeric receptors. Chimeric receptors are often constructed by exchanging the VFD of the receptor for which a modulator is selective with that of a different subtype that the ligand does not affect. If the ligand remains active at the chimeric construct, its activity must then be localized to the transmembrane region or C-terminal region, i.e. an allosteric site. Chimeric receptors were first used to determine agonist selectivity of orthosteric ligands (Takahashi et al., 1993; Tones et al., 1995) and have now become increasingly useful for determining an allosteric mode of action. Interestingly, CPCCOEt was characterized as an allosteric modulator of mGlu₁ using a chimeric CaSR and mGlu₁ construct (Brauner-Osborne et al., 1999) as well as using chimeras with other mGlu (Litschig et al., 1999; Gasparini et al., 2001). This strategy has proved effective for characterization of numerous negative allosteric modulators (NAMs) and PAMs, with chimeric receptor constructs often used as the first step

in the validation of an allosteric mechanism (Pagano et al., 2000; Carroll et al., 2001; Knoflach et al., 2001; Maj et al., 2003; Mitsukawa et al., 2005).

Another method of confirming allosteric binding is to eliminate the extracellular VFD from the receptor altogether through the construction of a 'headless' mGlu. The headless receptor lacks the N-terminal extracellular VFD but retains an intact transmembrane region and a functional C terminus (Goudet et al., 2004). Headless mGlu behave like wild type receptors in terms of G protein coupling and can be positively or negatively regulated by ligands, like any other class A GPCR (Goudet et al., 2004), however, they no longer respond to orthosteric ligands. Allosteric modulators retain activity in cells expressing the headless receptor, PAMs are agonists and NAMs become inverse agonists, as such these constructs are useful tools to identify allosteric ligands (Chen et al., 2007). The headless construct of mGlu₅ has been used to localize the binding site of the mGlu₅ allosteric modulators MPEP, VU29 and CPPHA to the transmembrane domain (Chen et al., 2007, 2008). FTIDC, an mGlu₁ NAM, was also shown to bind to an allosteric site through the use of headless mGlu₁ (Suzuki et al., 2007). Chimeric and headless receptors constructs are useful for delineating the location of allosteric binding sites and investigating allosteric interactions, however, they do not provide detailed structural information.

Localization of an allosteric modulator's activity can be narrowed down further to functionally important residues and binding determinants using site-directed mutagenesis (see later for further discussion). Such efforts have been greatly facilitated by the development of radioligands for mGlu allosteric sites. The selective mGlu₅ radioligands [³H]-M-MPEP (Gasparini et al., 2002), [³H]methoxy-PEPy (Cosford et al., 2003) and [³H]-methoxymethyl-MTEP (Cosford et al., 2003), provide the opportunity for the characterization of the MPEP binding site on mGlu₅. Two radioligands selective for mGlu₁ have also been developed, including [³H]R214127 (Lavreysen et al., 2003) and [³H]EM-TBPC (Malherbe et al., 2003a). While second messenger assays are useful for probing the functional effect of a mutation on the interaction between an orthosteric agonist and allosteric modulator, radioligand binding based studies can be used to quantify the influence of a mutation on the affinity of an allosteric modulator. In addition, inhibition binding experiments can be used to determine if a novel allosteric modulator is competitive for known allosteric sites.

2.4. Advantages of allosteric modulation

Allosteric modulators theoretically offer a number of advantages over competitive (orthosteric) agonists and antagonists. Allosteric modulators that have no agonist activity in their own right are quiescent in the absence of the endogenous agonist and will only modulate the receptor once the endogenous agonist is present, thereby retaining spatial and temporal aspects of endogenous receptor signaling. Such 'fine-tuning' of the physiological response is likely to have a better therapeutic outcome than the sustained blockade or activation achieved by orthosteric agonists. A second advantage is the potential for greater subtype selectivity due to either interaction with sites that show greater divergence between subtypes compared to the orthosteric site, or due to selective cooperativity at a particular subtype at the exclusion of others (Lazareno et al., 2004). Another means of generating selectivity is to combine orthosteric and allosteric moieties with the same molecule yielding a bitopic (also referred to as dualsteric) ligand (Valant et al., 2008; Antony et al., 2009). Furthermore, modulators with limited cooperativity, such as the mGlu₅ NAMs M-5MPEP and Br-5MPEPy (Rodriguez et al., 2005), will have an in-built "ceiling" level to their effect, suggesting that they may be potentially safer than orthosteric ligands if administered in very large doses. For negative allosteric modulators with limited cooperativity this also introduces the capability to "dial down" receptor activity, maintaining a residual level of receptor activation, which may in fact be a more desirable therapeutic endpoint than complete blockade. Clearly, allosteric

modulators offer a number of advantages over their orthosteric counterparts, although both PAMs and NAMs rely upon the presence of the endogenous ligand. It is also worth noting that drug discovery programs centered on small molecules, be it orthosteric or allosteric, share common problems concerning solubility and formulation, generation of active metabolites, clearance and lack of brain penetrance.

In recent years it has become increasingly evident that the consequences of receptor activation are not limited to G protein activation and subsequent downstream second messengers. Thus, depending upon the measure of receptor activation being employed, ligand pharmacology described can differ. This phenomenon has been given many names including ‘stimulus trafficking’, ‘biased agonism’ and ‘functional selectivity’ (Urban et al., 2007; Kenakin, 2007; Galandrin et al., 2007). Given that allosteric modulators engender unique receptor conformations it is perhaps not surprising that there is the potential for the pharmacology of allosteric modulators to differ dramatically depending upon the assay of receptor activity. For example, MMPiP a negative allosteric modulator of mGlu₇, shows differential effects on the receptor activation depending upon the measure of receptor activation and cellular background (Niswender et al., 2010).

It is also becoming increasingly evident that allosteric modulators for a variety of GPCRs can not only modulate orthosteric ligand signaling, but also act as agonists in their own right (Spalding et al., 2002, 2006; Sachpatzidis et al., 2003; Mitsukawa et al., 2005; Zhang et al., 2005; Langmead et al., 2006; Nawaratne et al., 2007; Tu et al., 2007; Pelkey et al., 2007; Lee et al., 2007; May et al., 2007a; Jones et al., 2008; Niswender et al., 2008; Holst et al., 2009; Lebois et al., 2010). These so-called allosteric agonists add an additional layer of complexity and even more scope for treatment options. It should also be noted that there is no reason why a modulator could not express more than one of these properties concomitantly, e.g., agonism (positive or inverse) together with enhancement or inhibition of orthosteric ligand binding/function (Schwartz & Holst, 2007; May et al., 2007b). If a particular pathway can be associated with the pathophysiology of a disease or therapeutic improvement, then selective activation or inhibition of particular pathways by allosteric ligands may also represent a novel means of altering receptor activation. Currently, it remains to be determined whether a single phenotype (modulator only) or a combination of both modulator and agonist properties is the optimal approach to treating GPCR-based diseases with allosteric ligands. Most likely, different therapies will benefit differently from one phenotype relative to another.

3. Physiological roles of metabotropic glutamate receptors

With the exception of mGlu₆ which is localized to the retina, mGlu_s are ubiquitously expressed throughout the CNS in both neurons and glia, although each subtype is differentially localized in different brain regions (specific brain localizations for each of the mGlu subtypes is reviewed in detail in Ferraguti & Shigemoto, 2006). In recent years, all mGlu subtypes have been genetically deleted in mice; studies using these animals have yielded further insights into the biological functions of mGlu as well as potential diseases where mGlu_s may be a viable target for therapeutic intervention (see Niswender & Conn, 2010 for review). Detailed discussion of the physiological roles of mGlu_s has been presented in multiple reviews (Coutinho & Knopfel, 2002; Conn & Pin, 1997; Anwyl, 1999; Valenti et al., 2002; Bellone et al., 2008; Pinheiro & Mulle, 2008). Elucidation of these roles for mGlu_s suggests that selective activators and inhibitors of specific mGlu subtypes could subtly alter transmission in glutamatergic circuits in a therapeutically beneficial manner without eliciting the side effects commonly associated with drugs that interact with members of the ionotropic glutamate receptor family.

While it is clear that the specific mGlu subtype involved in mediating a given effect varies in different brain regions, some generalizations can be made regarding the common functions of

different groups of mGlu. Group I mGlu are generally found postsynaptically, whilst group II and III mGlu are often localized on presynaptic terminals or preterminal axons. Activation of postsynaptic group I mGlu often leads to cell depolarization and increases in neuronal excitability via modulation of a variety of ion channels. This modulation can range from robust excitation to more subtle changes in the pattern and frequency of cell firing and responses to excitatory inputs (Coutinho & Knopfel, 2002; Anwyl, 1999; Valenti et al., 2002). Presynaptic group II and III mGlu inhibit neurotransmitter release on a variety of excitatory (glutamatergic), inhibitory (GABAergic) and neuromodulatory (monoamines, ACh, peptides) synapses.

4. Therapeutic indications for allosteric modulators of metabotropic glutamate receptors

The first allosteric modulator of an mGlu identified was CPCCOEt, a NAM of mGlu₁ (Annoura et al., 1996). Selective promising allosteric modulators have been identified for many mGlu subtypes (see Figure 2 and Table 1) and are exciting potential therapeutics for a variety of CNS-related disorders including Alzheimer's disease (Lee et al., 2004), anxiety disorders (Spooren & Gasparini, 2004; Swanson et al., 2005), depression (Palucha & Pilc, 2007), epilepsy (Alexander & Godwin, 2006; Ure et al., 2006), and Parkinson's disease (Conn et al., 2005; Johnson et al., 2009) among others. Comprehensive reviews encompassing each of these therapeutic areas are available, however, several areas warrant mentioning as they signify important advances in the field.

4.1 Negative allosteric modulation of metabotropic glutamate receptor 5 for anxiety and Fragile X Syndrome

In brain regions implicated in the pathology of anxiety disorders, such as the amygdala, mGlu₅ is localized at postsynaptic sites where it increases the excitability of the N-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptors (Romano et al., 1995; Valenti et al., 2002). Based on this physiology, it has been hypothesized that antagonists of mGlu₅ might reduce the activity of glutamatergic synapses that are thought to contribute to the underlying mechanisms of anxiety disorders (Marino & Conn, 2006). Indeed, a role for group I mGlu in anxiety was indicated by the finding that intrahippocampal administration of the group I antagonist (S)-4-carboxy-3-hydroxyphenylglycine (S-4C3H-PG) had anxiolytic properties using the conflict drinking Vogel test in rats (Chojnacka-Wójcik et al., 1997). However, S-4C3H-PG also possesses partial agonism at group II mGlu (Hayashi et al., 1994). mGlu₁ selective antagonists, such as JNJ16259685, also showed efficacy in rodent models of anxiety, however, these compounds were associated with memory impairments that prohibited further development (Steckler et al., 2005a; 2005b; Gravius et al., 2005; Pietraszek et al., 2005). Fortunately, mGlu₅ selective antagonists whilst being anxiolytic, did not cause the same degree of impairments in memory (Steckler et al., 2005a; Gravius et al., 2005; Pietraszek et al., 2005). The first identified selective NAMs of mGlu₅ were SIB-1757 and SIB-1893 and subsequent structural analogues MPEP and MTEP have been developed that possess improved potency, selectivity and brain penetrance (Varney et al., 1999; Gasparini et al., 1999; Cosford et al., 2003a). There are now a large number of highly selective mGlu₅ NAMs, including radioligands and positron emission tomography (PET) ligands (Gasparini et al., 2002; Anderson et al., 2002, 2003; Cosford et al., 2003b; Ametamey et al., 2007; Treyer et al., 2008; Yu et al., 2007; Baumann et al., 2010). The availability of radioligands and PET ligands may prove useful for dose-finding studies for NAMs in clinical development as they provide the necessary tools to assess receptor occupancy.

The availability of selective and systemically active mGlu₅ NAMs has allowed for the validation of mGlu₅ inhibition as a viable therapeutic strategy for anxiety disorders. In multiple

rodent models of anxiety, MPEP and related compounds have been shown to be anxiolytic (Spooren et al., 2000; Schulz et al., 2001; Tatarczynska et al., 2001; Rodrigues et al., 2002; Spooren et al., 2002). Recently, fenobam, a non-benzodiazepine anxiolytic, was found to be a selective mGlu₅ NAM (Porter et al., 2005). Fenobam is efficacious in preclinical rodent models of anxiety (Patel et al., 1982; Goldberg et al., 1983; Porter et al., 2005) and in clinical trials (Pecknold et al., 1982). Collectively, these data support the hypothesis that negative allosteric modulation of mGlu₅ is an attractive avenue for the development of novel anxiolytics.

Fragile X Syndrome (FXS) is the leading cause of autism and the most common form of inherited mental retardation (Crawford et al., 2001; Garber et al., 2008). FXS is caused by a mutation in the gene encoding fragile X mental retardation protein (FMRP), which represses the translation of specific mRNAs regulating protein translation in neuronal dendrites. In mice, a loss of FMRP results in increased group I mGlu-dependent long-term depression in the hippocampus (Huber et al., 2002). Administration of MPEP to FXS mice reduces anxiety and seizures (Yan et al., 2005), whilst crossbreeding of FXS mice with mGlu₅ knock-out mice suggest that a number of fragile X phenotypes can be corrected by reducing levels of mGlu₅ (Dolen et al., 2007). Indeed, fenobam treatment improved clinical behaviors and prepulse inhibition with no adverse effects in adults with FXS (Berry-Kravis et al., 2009). Furthermore, AFQ056, another mGlu₅ NAM is in phase 2 clinical trials for adults with FXS (Novartis, 2010a). Therefore, antagonists of mGlu₅ represent promising novel treatment strategies for FXS (Bear et al., 2004).

In addition to anxiety and FXS, mGlu₅ NAMs have also been suggested as treatment options for iatrogenic dystonias, substance abuse and withdrawal, chronic pain, depression, gastroesophageal reflux disorder and migraine (Slassi et al., 2005). AFQ056 is also in phase II clinical trials for L-DOPA-induced dyskinesia (LID) associated with the treatment of Parkinson's disease (PD) (Novartis, 2010b; 2010c; 2010d; Rylander et al., 2010). Furthermore, the mGlu₅ NAM, ADX10059, showed efficacy for the treatment of gastroesophageal reflux disorder (Keywood et al., 2009; Zerbib et al., 2010), however, a separate trial investigating efficacy in migraine was terminated due to liver toxicity (Addex, 2010).

An important consideration in the clinical development of mGlu₅ NAMs is the potential for target-related adverse effects. mGlu₅ plays an important role in cognitive processing and administration of MPEP has been shown to exacerbate psychosis induced by PCP (Campbell et al., 2004), therefore mGlu₅ NAMs may cause cognitive impairments and psychotomimetic effects. Indeed, psychostimulant adverse effects were reported in early fenobam clinical trials (Itil et al., 1978; Friedman et al., 1980; Pecknold et al., 1982), although whether or not this was due to interactions with mGlu₅, an unknown interaction or through the formation of active metabolites remains to be seen. However, no psychostimulant effects or cognitive impairments were reported for fenobam in a recent trial in FXS patients, nor for another mGlu₅ NAM, ADX10059 (Berry-Kravis et al., 2009; Keywood et al., 2009; Zerbib et al., 2010). In a recent study, Rodriguez et al., (2005) reported the discovery of mGlu₅ NAMs with limited negative cooperativity. These so-called partial antagonists do not completely block the activity of glutamate, despite achieving 100% receptor occupancy. NAMs with limited cooperativity could have improved clinical success, as they would retain some level of activity at the receptor, which may result in an improved adverse-effect profile.

4.2 Positive allosteric modulation of metabotropic glutamate receptor 5 for schizophrenia and cognitive disorders

Whilst mGlu₅ antagonists are an attractive strategy for a number of disorders, positive allosteric modulation of mGlu₅ has arisen as exciting new approach for the treatment of schizophrenia and cognitive disorders. Until recently, the neurochemical mechanism underlying schizophrenia has been attributed to hyperactivity of the dopaminergic system. However,

evidence has accumulated to suggest that hypofunction of glutamatergic receptors, particularly NMDA receptors, could also contribute to the underlying neurochemical cause of schizophrenia (Conn et al., 2009a). Clinical studies have shown that antagonists of the NMDA receptor, such as PCP and ketamine, induce symptoms in rats and humans that mirror the positive, negative and cognitive symptoms of schizophrenic patients (Gaspar et al., 2009). Conversely, ligands that enhance NMDA receptor function have proven to be efficacious in the treatment of schizophrenia (Lindsley et al., 2006). Interestingly, mGlu₅ has been identified as a closely associated signaling partner with NMDA receptors and may play an integral role in regulating NMDA receptor function in brain regions involved in cognitive function and implicated in the pathology of schizophrenia such as the hippocampus, striatum and prefrontal cortex (Alagarsamy et al., 1999; Ugolini et al., 1999; Ehlers et al., 1999; Awad et al., 2000; Doherty et al., 2000; Attucci et al., 2001; Mannaioni et al., 2001; Pisani et al., 2001; Marino et al., 2002; Alagarsamy et al., 2005). Therefore, selective activation of mGlu₅ and subsequent enhancement of NMDA receptor activity may provide a novel means of improving not only the positive symptoms but also the cognitive impairments associated with schizophrenia. The first selective mGlu₅ PAMs include three different chemical series: DFB, CPPHA and CDPPB (O'Brien et al., 2003, 2004; Lindsley et al., 2004; Kinney et al., 2005). All three of these compounds cause a leftward shift in the glutamate concentration response curve of approximately 10 fold for CPPHA and CDPPB and 2-4 fold for DFB, yet have no impact on the affinity of glutamate. It is also worth noting that while DFB and CDPPB and derivatives thereof are thought to interact competitively with MPEP, CPPHA acts at a second distinct allosteric site within the TM domains (see later for details).

The discovery, development and optimization of these PAMs, in particular DFB and CPPHA, highlight some of the challenges faced by medicinal chemists when pursuing allosteric modulators. The structure-activity relationship (SAR) of CPPHA was 'flat', as evidenced by the fact that out of 995 analogues only 45 were active (Zhao et al., 2007). For DFB the SAR was even more interesting, with slight modifications to the scaffold rendering not only inactive compounds but also causing significant changes in the pharmacology, switching the compounds from PAMs to NAMs or resulting in neutral cooperativity (O'Brien et al., 2003). Whilst the medicinal chemistry efforts surrounding the CDPPB scaffold were more successful, they did not yield any significant improvements overall (dePaulis et al., 2006). Despite these challenges, these initial mGlu₅ PAMs provided much needed tools study potentiation of mGlu₅ activity in electrophysiological preparations (O'Brien et al., 2003, 2004; Chen et al., 2007; Ayala et al., 2009) as well as in rodent behavioral models (Kinney et al., 2005; Balschun et al., 2006; Lecourtier et al., 2007; Gass & Olive, 2009; Uslaner et al., 2009; Stefani & Moghaddam, 2010). Recently, a fourth PAM chemotype represented by ADX47273 was reported, which also displays efficacy in rodent behavioral models similar to proven antipsychotics and importantly, improved cognitive functioning in impaired animals (Liu et al., 2008; Schlumberger et al., 2009, 2010). Thus, mGlu₅ PAMs show much promise as potential therapeutics for the treatment of schizophrenia and also as cognition enhancers.

4.3 Positive allosteric modulation of metabotropic glutamate receptor 2 for schizophrenia and anxiety disorders

There is now a large body of evidence that activation of group II mGlu_s may present a novel means of treating schizophrenia and anxiety disorders. In brain regions implicated in the pathology of schizophrenia it is hypothesized that hypofunction of NMDA receptors leads to decreased downstream activation of GABAergic neurons resulting in disinhibition and an overall increase in excitation (Swanson et al., 2005; Conn et al., 2009a). Activation of mGlu₂ has been shown to decrease excitatory amino acid transmission at a number of synapses (Macek et al., 1996; Doherty et al., 2004; Nicholls et al., 2006). Orthosteric group II mGlu agonists have efficacy in preclinical models of psychosis and anxiety (Moghaddam et al.,

1998; Cartmell et al., 1999; Lorrain et al., 2003; Swanson et al., 2005; Conn et al., 2008). In addition, there is evidence for improvement in cognitive deficits in both humans (induced by ketamine) and rats (induced by PCP) (Moghaddam et al., 1998; Cartmell et al., 1999; Krystal et al., 2005). In a recent clinical trial, LY2140023, an oral prodrug of the orthosteric agonist LY404039, improved the positive and negative symptoms of schizophrenic patients with a similar efficacy to olanzapine, without the side effects associated with typical and atypical antipsychotics (Patil et al., 2007). Currently, LY2140023 is entering phase II/III trials for schizophrenia (Eli Lilly, 2010). Similarly, LY354740 has clinical efficacy in treating panic attacks and generalized anxiety disorder (Schoepp et al., 2003; Schoepp, 2004). Whilst these orthosteric agonists have much promise as therapeutic options, there are a number of disadvantages. These orthosteric agonists activate both mGlu₂ and mGlu₃, they are all based on a similar chemical scaffold, and tolerance was induced in one rodent model used to assess anti-psychotic efficacy (Galici et al., 2005). Development of tolerance is a key concern for CNS disorders that require long-term treatment as it can result in both a loss of efficacy over time and changes in plasticity leading to adverse effects, as exemplified by the clinically efficacious orthosteric D2 dopamine receptor antagonists (Wadenberg et al., 2001; Natesan et al., 2005; Ginovart et al., 2009). Thus, mGlu₂ PAMs are an attractive alternative as they may have the capacity to overcome some of these potential shortcomings.

Numerous mGlu₂ selective PAMs have now been reported, the majority of which are related to either BINA or LY487379 (Johnson et al., 2003; Lorrain et al., 2003; Schaffhauser et al., 2003; Cube et al., 2005; Galici et al., 2005; Pinkerton et al., 2005; Galici et al., 2006). Both BINA and LY487379 induce leftward shifts of the glutamate concentration response curve, and potentiate the ability of group II selective agonists to reduce transmission at a number of glutamatergic synapses (Johnson et al., 2003; Schaffhauser et al., 2003; Poisik et al., 2005; Galici et al., 2006; Bennyworth et al., 2007). In rodent behavioral models predictive of anti-psychotic and anxiolytic efficacy, these mGlu₂ PAMs have similar efficacy to group II orthosteric agonists (Galici et al., 2005; Govek et al., 2005; Johnson et al., 2005; Pinkerton et al., 2005; Bennyworth et al., 2007). Therefore there is much excitement that selective mGlu₂ PAMs represent a novel treatment strategy for schizophrenia and anxiety that will have improved outcomes and side effect profiles over currently used anti-psychotics.

4.4 Positive allosteric modulation of metabotropic glutamate receptor 4 for Parkinson's disease

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by severe motor impairments such as bradykinesia, tremor and rigidity. These motor symptoms arise as a consequence of a progressive loss of dopaminergic neurons in the substantia nigra, which project into the striatum regulating activity through the basal ganglia. Dopamine reduces the activity of striatal neurons projecting into the globus pallidus, in PD patients the loss of dopaminergic neurons results in overactivity of these synapses (Wichmann & DeLong, 2003). Current therapeutics aim to restore dopaminergic modulation, such as administration of L-DOPA, the precursor of dopamine. However, dopamine replacement therapy is associated with increased adverse effects as the disease progresses and a loss of efficacy (Poewe et al., 1986a, 1986b). Therefore alternative therapeutic options are much in demand. At the striato-pallidal synapse, activation of mGlu₄ reduces neurotransmission (Matsui & Kita, 2003; Valenti et al., 2003; Conn et al., 2005; MacInnes & Duty, 2008). In addition, the mGlu₄ preferring orthosteric agonist LSP1-2111 has demonstrated efficacy in rodent models of PD, specifically reversal of haloperidol-induced catalepsy and 6-hydroxydopamine-induced motor deficits (Beurrier et al., 2009). This has been linked to the inhibition of striato-pallidal GABAergic transmission through mGlu₄ activation (Cuomo et al., 2009). These studies lend support to the hypothesis that mGlu₄ PAMs or allosteric agonists are a viable approach to the treatment of the symptoms of PD.

The mGlu₅ NAMs SIB-1893 and MPEP are also positive allosteric modulators of mGlu₄, potentiating the response to L-2-amino-4-phosphonobutyrate, an orthosteric agonist (Mathiesen et al., 2003). The first selective mGlu₄ PAM, PHCCC, was recently identified (Flor et al., 2002; Marino et al., 2003; Maj et al., 2003). PHCCC has no agonist activity but increases the potency of glutamate in recombinant systems as well as at several synapses including the striato-pallidal synapse (Marino et al., 2003; Valenti et al., 2005). Moreover, PHCCC decreases reserpine-induced akinesia (Marino et al., 2003), a rodent model of PD, and is neuroprotective in a model of dopaminergic cell death (Battaglia et al., 2006). A novel mGlu₄ PAM chemotype, exemplified by VU0155041, was recently identified in a high-throughput screen by Niswender and colleagues (2008). Interestingly, VU0155041 was found to be an allosteric agonist in addition to its PAM properties, with significant advantages over PHCCC, namely improved aqueous solubility, potency and selectivity. VU0155041 was also efficacious in reversing haloperidol-induced catalepsy and reserpine-induced akinesia (Niswender et al., 2008). Clearly, there is much promise for the development of mGlu₄ PAMs and allosteric agonists as novel therapeutics for PD.

5. Localization of allosteric sites on metabotropic glutamate receptors

5.1. Common allosteric sites within and between metabotropic glutamate receptors

One of the continuing challenges faced in drug discovery is establishing suitably subtype selective ligands. Although allosteric ligands have better specificity than orthosteric ligands, there are numerous examples of allosteric modulators that interact with multiple receptor subtypes. As mentioned above, MPEP, an mGlu₅ NAM, is an mGlu₄ PAM (Mathiesen et al., 2003), while DFB and CPPHA (mGlu₅ PAMs) are also weak mGlu₄ NAMs (O'Brien et al., 2003, 2004). Similarly, PHCCC, an mGlu₄ PAM, is also an mGlu₁ NAM (Annoura et al., 1996). Although problematic in terms of identifying new chemotypes, this lack of selectivity for some compounds across different mGlu groups suggests similarities in allosteric binding pockets on these receptors.

To identify the sites at which allosteric modulators bind mGlu, initial mutational sites were chosen based on models predicting a pocket that may resemble the site where 11-*cis*-retinal, an inverse agonist, binds in bovine rhodopsin (Palczewski et al., 2000; Teller et al., 2001). Indeed, several critical residues for *cis*-rhodopsin binding accurately predicted residues that are important for binding of mGlu allosteric ligands, validating the use of rhodopsin as a template for the transmembrane region of class C GPCRs (Malherbe et al., 2003a, 2003b). Critical residues for allosteric modulator binding and cooperativity determinants have been characterized for mGlu₁, mGlu₂ and mGlu₅ (Table 2). A complete table of mutational studies on allosteric modulators of mGlu can be found in the supplementary material.

In the characterization of potential binding pockets for mGlu allosteric modulators, within a particular receptor subtype the majority of structurally unrelated PAMs and NAMs tend to cluster in overlapping binding sites. As can be seen from Table 2 and the sequence alignment in Figure 3, the binding site for [³H]EM-TBPC in mGlu₁ is primarily located on TM 6 and 7 (Malherbe et al., 2003a), which is also where functionally important residues are located for other mGlu₁ NAMs: CFMMC, LY456066, YM298198, FTICD and CPCCOEt (Litschig et al., 1999; Surin et al., 2007; Suzuki et al., 2007; Fukuda et al., 2009). When mapped onto a 3D model of the hepta-helical transmembrane domain (using the backbone coordinates from the β_2 -adrenergic receptor X-ray crystal structure) these residues are found on the top half of TMs 3, 5, 6 and 7 on the inside faces of these helices (Figure 4A).

Key binding determinants for allosteric modulation by MPEP and fenobam at mGlu₅ are found on TMs 3, 6 and 7, as seen in Table 2 and Figure 3 (Pagano et al., 2000; Malherbe et al., 2003b; 2006). Similarly, functionally important residues for positive allosteric modulation by

DFB and VU29 show a similar distribution (Muhlemann et al., 2006; Chen et al., 2008). Figure 4C demonstrates the clustering of these residues on the top half of the TMs, again located on the inside face of the helices. Competition binding assays with radioligands provide additional support for a common binding site for allosteric modulators. In mGlu₅ studies, CDPBP inhibits allosteric binding of the MPEP analog [³H]methoxyPEPy in a competitive manner (Chen et al., 2007). In addition, point mutations reducing the binding of MPEP also decrease the ability of CDPBP to potentiate mGlu₅ response to glutamate (Chen et al., 2007). These results suggest that CDPBP, along with its derivative VU29, share a common or overlapping binding site with MPEP (Kinney et al., 2005; Chen et al., 2007).

The allosteric binding sites for the majority of allosteric modulators of group I mGlu are located in similar regions. As seen in Figure 4D, overlaying the key residues for allosteric modulation from both receptors clearly demonstrates the similarity between the two receptor subtypes. Experiments performing residue swaps provide further evidence for similar binding sites between mGlu₁ and mGlu₅. Substituting non-conserved residues of mGlu₁, known to be important for CPCCOEt and Ro 67-7476 allosteric modulation, onto mGlu₅ results in a gain of function for these mGlu₁ selective modulators at mGlu₅ (Litschig et al., 1999; Knoflach et al., 2001). Similarly, substitution of residues important for MPEP binding onto mGlu₁ results in the gain of [³H]MPEP binding (Pagano et al., 2000). Clearly, there is evidence that the location of at least one allosteric site is very similar for the group I mGlu, despite the availability of selective allosteric modulators for these sites. In contrast, the residues identified that perturb modulation by group II selective PAMs are found in TM 4 and 5 (Figures 3 & 4B) (Schaffhauser et al., 2003; Hemstapat et al., 2007; Rowe et al., 2008). The two residues identified in TM5 cluster near the common allosteric site of group I mGlu, whilst those in TM4 do not. Unlike the mutations in group I mGlu, the existing mGlu₂ data relies entirely upon functional assays, thus, it remains to be seen whether these residues are required for modulator binding or the transmission of cooperativity. More studies are required of mGlu₂ selective modulators to determine whether or not the binding pocket utilized is similar to that shared by the majority of group I mGlu allosteric modulators.

5.2 Multiple allosteric sites within a single metabotropic glutamate receptor type

While commonalities between allosteric binding sites within subtypes of mGlu have been the prevailing trend, there is evidence for multiple allosteric sites on the same receptor. Inhibition binding assays with radioligands and Schild-like analysis with neutral allosteric ligands has enabled the detection of mGlu allosteric modulators that do not interact with the common binding sites. This has been most definitively shown for CPPHA, a group I PAM. CPPHA does not displace the binding of [³H]methoxyPEPy at mGlu₅, even at concentrations several orders of magnitude higher than its potency as a PAM (O'Brien et al., 2004; Chen et al., 2008). The same occurs with the mGlu₁ radioligand [³H]R214127, which is thought to bind to site on mGlu₁ homologous with the MPEP binding site on mGlu₅ (Pagano et al., 2000; Lavreysen et al., 2003). CPPHA does not compete with [³H]R214127 (Chen et al., 2008), despite having PAM activity at both group I mGlu. Furthermore, the neutral allosteric modulator 5MPEP noncompetitively inhibits CPPHA potentiation of glutamate, suggesting that ligands at the MPEP and CPPHA sites can allosterically regulate one another (Chen et al., 2008). This is in contrast to other mGlu₅ PAMs and NAMs that display a competitive interaction with 5MPEP (Chen et al., 2007). Collectively, these data suggest that CPPHA interacts with a different site to other known allosteric ligands. In site-directed mutagenesis studies, only F585I/mGlu₅, and its equivalent at mGlu₁ (F599I), in TM1 eliminated the potentiation of mGlu₅ by 1 μM CPPHA (Chen et al., 2008). As seen in Figure 4E, this residue on TM1 is spatially in a different region of the receptor compared to the other residues implicated in allosteric modulation in both mGlu₁ and mGlu₅. Because CPPHA potentiates responses to activation of both mGlu₅ and mGlu₁ in sites that are clearly distinct from previously characterized allosteric sites, the data

suggests that multiple distinct allosteric sites exist on group I mGluR that can serve as targets for PAMs.

In a similarly designed study to the CPPHA work, two mGlu₁ selective PAMs, VU48 and VU71, were shown to be non-competitive with [³H]R214127 and were not susceptible to mutations known to affect NAMs that bind the common group I mGluR allosteric site (Hemstapat et al., 2006). Multiple binding sites have also been suggested for mGlu₄ PAMs and mGlu₂ NAMs compared to PAMs. At mGlu₂, mutations known to perturb the allosteric modulation by PAMs had no effect on NAMs from the MNI series of compounds (Hemstapat et al., 2007). However, given that these mGlu₂ data rely upon functional measures alone, it remains to be seen whether or not these differential effects correspond to different binding sites or merely reflect the manifestation of positive versus negative cooperativity. Interestingly, at mGlu₄, PHCCC was unable to influence the concentration-response curve of VU0155041, a PAM from a different chemical class, suggesting that these two ligands do not act at a single allosteric site (Niswender et al., 2008). Furthermore, VU0155041 also has agonist activity in its own right, whilst PHCCC does not (Niswender et al., 2008), such differences in pharmacology of these PAMs could be postulated to arise due to interaction with different binding sites. It is possible that allosteric modulators acting at different sites could regulate mGluR via different pathways. Indeed, CPPHA has been shown to differentially effect ERK1/2 phosphorylation relative to inositol phosphate accumulation in rat cortical astrocytes (Zhang et al., 2005). In contrast, mGlu₅ PAMs from the CDPPB series have equivalent effects in both assays. Such differences between the activities of PAMs interacting at different sites have important implications with respect to therapeutic intervention and lead compound choice in drug discovery programs.

This evidence bears the potential that instead of (or in addition to) multiple well-defined and separated allosteric binding sites a larger binding region for allosteric modulation may exist defined by the inward-facing top half of the TM helices. Allosteric modulators interact with part of that region in multiple, possibly overlapping binding modes depending on their chemotype. Such a scenario increases the possibility to design allosteric modulators with finely tuned pharmacological effects as the determinants of the receptor/ligand interaction are optimized.

6. Conclusions

Since the discovery of the first mGluR allosteric modulator in 1996, the field of mGluR allosteric modulation has seen a number of major advances. For the majority of subtypes, selective allosteric modulators have now been discovered. Positive, negative and neutral allosteric ligands have been identified for some subtypes, providing much needed pharmacological tools to probe the physiological roles of these important receptors. With the clinical validity of allosteric modulation of GPCRs as a therapeutic avenue being proven, allosteric modulators of mGluR have considerable promise as candidates for therapeutic intervention for a variety of psychiatric and neurological disorders. Much progress has also been made in our understanding of the locations of allosteric binding sites on these receptors. The studies reported to date suggest both common binding sites across the different subtypes that can be selectively targeted as well as evidence for the presence of multiple binding sites on a single subtype. With the growing number of GPCR templates, information-rich homology models can be developed in the absence of a crystal structure for the transmembrane region of a class C GPCR. As presented above homology models are successfully utilized to interpret mutagenesis-based studies, allowing 3D visualization of these regions of the receptor. Studies of mGluR have also utilized homology models to manually dock ligand-based pharmacophore maps to interpret structure activity relationships of a particular chemical series (Noeske et al., 2007, 2009). In the future, the combination of ligand-based and receptor-based pharmacophores (Kratzschwil et al.,

2005; Radestock et al., 2008) is likely to aid in the identification of novel chemotypes and optimization of allosteric modulators to improve specificity and potency for use as therapeutic agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Addex Pharma. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2000. ADX10059 Migraine Prevention Study. [cited 2010 Jun 23]. Available from: <http://clinicaltrials.gov/show/NCT00820105> NLM Identifier: NCT00820105
- Alagarsamy S, Marino MJ, Rouse ST, Gereau RWt, Heinemann SF, Conn PJ. Activation of NMDA receptors reverses desensitization of mGlu5 in native and recombinant systems. *Nat Neurosci* 1999;2:234–240. [PubMed: 10195215]
- Alagarsamy S, Saugstad J, Warren L, Mansuy IM, Gereau RWt, Conn PJ. NMDA-induced potentiation of mGlu5 is mediated by activation of protein phosphatase 2B/calcineurin. *Neuropharmacology* 2005;49(Suppl 1):135–145. [PubMed: 16005030]
- Alexander GM, Godwin DW. Metabotropic glutamate receptors as a strategic target for the treatment of epilepsy. *Epilepsy Res* 2006;71:1–22. [PubMed: 16787741]
- Ametamey SM, Treyer V, Streffer J, Wyss MT, Schmidt M, Blagoev M, Hintermann S, Auberson Y, Gasparini F, Fischer UC, Buck A. Human PET Studies of Metabotropic Glutamate Receptor Subtype 5 with 11C-ABP688. *J Nucl Med* 2007;48(2):247–252. [PubMed: 17268022]
- Anderson JJ, Bradbury MJ, Giracello DR, Chapman DF, Holtz G, Roppe J, King C, Cosford ND, Varney MA. In vivo receptor occupancy of mGlu5 receptor antagonists using the novel radioligand [3H]3-methoxy-5-(pyridin-2-ylethynyl)pyridine. *Eur J Pharmacol* 2003;473:35–40. [PubMed: 12877935]
- Anderson JJ, Rao SP, Rowe B, Giracello DR, Holtz G, Chapman DF, Tehrani L, Bradbury MJ, Cosford ND, Varney MA. [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* 2002;303:1044–1051. [PubMed: 12438526]
- Annoura H, Fukunaga A, Uesugi M, Tatsuoka T, Horikawa Y. A novel class of antagonists for metabotropic glutamate receptors, 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylates. *Bioorg Med Chem Lett* 1996;6:763–766.
- Antony J, Kellershohn K, Mohr-Andra M, Kebig A, Prilla S, Muth M, Heller E, Disingrini T, Dallanocce C, Bertoni S, Schrobang J, Trankle C, Kostenis E, Christopoulos A, Holtje HD, Barocelli E, De Amici M, Holzgrabe U, Mohr K. Dualsteric GPCR targeting: a novel route to binding and signaling pathway selectivity. *FASEB J* 2009;23:442–450. [PubMed: 18842964]
- Anwyl R. Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. *Brain Res Brain Res Rev* 1999;29:83–120. [PubMed: 9974152]
- Attucci S, Carla V, Mannaioni G, Moroni F. Activation of type 5 metabotropic glutamate receptors enhances NMDA responses in mice cortical wedges. *Br J Pharmacol* 2001;132:799–806. [PubMed: 11181420]
- Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ. Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *J Neurosci* 2000;20:7871–7879. [PubMed: 11050106]
- Ayala JE, Chen Y, Banko JL, Sheffler DJ, Williams R, Telk AN, Watson NL, Xiang Z, Zhang Y, Jones PJ, Lindsley CW, Olive MF, Conn PJ. mGlu5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. *Neuropsychopharmacology* 2009;34:2057–2071. [PubMed: 19295507]
- Baldwin JM, Schertler GF, Unger VM. An alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors. *J Mol Biol* 1997;272:144–164. [PubMed: 9299344]
- Ballesteros J, Palczewski K. G protein-coupled receptor drug discovery: implications from the crystal structure of rhodopsin. *Curr Opin Drug Discov Devel* 2001;4:561–574.

- Ballesteros J, Weinstein H. Integrated methods for the construction of three dimensional models and computational probing of structure-function relations in G-protein coupled receptors. *Methods Neuroscience* 1995;25:366–428.
- Balschun D, Zuschratter W, Wetzel W. Allosteric enhancement of metabotropic glutamate receptor 5 function promotes spatial memory. *Neuroscience* 2006;142:691–702. [PubMed: 16890368]
- Battaglia G, Busceti CL, Molinaro G, Biagioni F, Traficante A, Nicoletti F, Bruno V. Pharmacological activation of mGlu4 metabotropic glutamate receptors reduces nigrostriatal degeneration in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J Neurosci* 2006;26:7222–7229. [PubMed: 16822979]
- Baumann CA, Mu L, Johannsen S, Honer M, Schubiger PA, Ametamey SM. Structure–Activity Relationships of Fluorinated (E)-3-((6-Methylpyridin-2-yl)ethynyl)cyclohex-2-enone-O-methyloxime (ABP688) Derivatives and the Discovery of a High Affinity Analogue as a Potential Candidate for Imaging Metabotropic Glutamate Receptors Subtype 5 (mGluR5) with Positron Emission Tomography (PET). *J Med Chem* 2010;53:4009–4017. [PubMed: 20411954]
- Bear MF, Dolen G, Osterweil E, Nagarajan N. Fragile X: translation in action. *Neuropsychopharmacology* 2008;33:84–87. [PubMed: 17940551]
- Bellone C, Luscher C, Mameli M. Mechanisms of synaptic depression triggered by metabotropic glutamate receptors. *Cell Mol Life Sci* 2008;65:2913–2923. [PubMed: 18712277]
- Benneyworth MA, Xiang Z, Smith RL, Garcia EE, Conn PJ, Sanders-Bush E. A selective positive allosteric modulator of metabotropic glutamate receptor subtype 2 blocks a hallucinogenic drug model of psychosis. *Mol Pharmacol* 2007;72:477–484. [PubMed: 17526600]
- Berry-Kravis E, Hessel D, Coffey S, Hervey C, Schneider A, Yuhas J, Hutchison J, Snape M, Tranfaglia M, Nguyen DV, Hagerman R. A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J Mol Genet* 2009;46:266–271.
- Bessis AS, Bertrand HO, Galvez T, De Colle C, Pin JP, Acher F. Three-dimensional model of the extracellular domain of the type 4a metabotropic glutamate receptor: new insights into the activation process. *Protein Sci* 2000;9:2200–2209. [PubMed: 11152130]
- Bessis AS, Rondard P, Gaven F, Brabet I, Triballeau N, Prezeau L, Acher F, Pin JP. Closure of the Venus flytrap module of mGlu8 receptor and the activation process: Insights from mutations converting antagonists into agonists. *Proc Natl Acad Sci U S A* 2002;99:11097–11102. [PubMed: 12151600]
- Beurrier C, Lopez S, Revy D, Selvam C, Goudet C, Lherondel M, Gubellini P, Kerkerian-LeGoff L, Acher F, Pin JP. Electrophysiological and behavioral evidence that modulation of metabotropic glutamate receptor 4 with a new agonist reverses experimental parkinsonism. *FASEB J* 2009;23(10):3619–3628. [PubMed: 19525404]
- Bhave G, Nadin BM, Brasier DJ, Glauner KS, Shah RD, Heinemann SF, Karim F, Gereau RWt. Membrane topology of a metabotropic glutamate receptor. *J Biol Chem* 2003;278:30294–30301. [PubMed: 12764131]
- Black JW, Leff P. Operational models of pharmacological agonism. *Proc R Soc Lond B Biol Sci* 1983;220:141–162. [PubMed: 6141562]
- Bordi F, Ugolini A. Group I metabotropic glutamate receptors: implications for brain diseases. *Prog Neurobiol* 1999;59:55–79. [PubMed: 10416961]
- Brauner-Osborne H, Jensen AA, Krogsgaard-Larsen P. Interaction of CPCCOEt with a chimeric mGlu1b and calcium sensing receptor. *Neuroreport* 1999;10:3923–3925. [PubMed: 10716234]
- Campbell UC, Lalwani K, Hernandez L, Kinney GG, Conn PJ, Bristow LJ. The mGlu5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) potentiates PCP-induced cognitive deficits in rats. *Psychopharmacology (Berl)* 2004;175:310–318. [PubMed: 15024550]
- Carroll FY, Stolle A, Beart PM, Voerste A, Brabet I, Mauler F, Joly C, Antonicek H, Bockaert J, Muller T, Pin JP, Prezeau L. BAY36-7620: a potent non-competitive mGlu1 receptor antagonist with inverse agonist activity. *Mol Pharmacol* 2001;59:965–973. [PubMed: 11306677]
- Cartmell J, Monn JA, Schoepp DD. The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine versus d-amphetamine motor behaviors in rats. *J Pharmacol Exp Ther* 1999;291:161–170. [PubMed: 10490900]
- Chen Y, Goudet C, Pin JP, Conn PJ. 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* 2008;73:909–918. [PubMed: 18056795]
- Chen Y, Nong Y, Goudet C, Hemstapat K, de Paulis T, Pin JP, Conn PJ. 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* 2007;71:1389–1398. [PubMed: 17303702]
- Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* 2007;318:1258–1265. [PubMed: 17962520]
- Chojnacka-Wojcik E, Tatarczynska E, Pilc A. The anxiolytic-like effect of metabotropic glutamate receptor antagonists after intrahippocampal injection in rats. *Eur J Pharmacol* 1997;319:153–156. [PubMed: 9042585]
- Conn PJ, Battaglia G, Marino MJ, Nicoletti F. Metabotropic glutamate receptors in the basal ganglia motor circuit. *Nat Rev Neurosci* 2005;6:787–798. [PubMed: 16276355]
- Conn PJ, Christopoulos A, Lindsley CW. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat Rev Drug Discov* 2009b;8:41–54. [PubMed: 19116626]
- Conn PJ, Lindsley CW, Jones CK. Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. *Trends Pharmacol Sci* 2009a;30:25–31. [PubMed: 19058862]
- Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol* 1997;37:205–237. [PubMed: 9131252]
- Conn PJ, Tamminga C, Schoepp DD, Lindsley C. Schizophrenia: moving beyond monoamine antagonists. *Mol Interv* 2008;8:99–107. [PubMed: 18403654]
- Cosford ND, Roppe J, Tehrani L, Schweiger EJ, Seiders TJ, Chaudary A, Rao S, Varney MA. [3H]-methoxymethyl-MTEP and [3H]-methoxy-PEPy: potent and selective radioligands for the metabotropic glutamate subtype 5 (mGlu5) receptor. *Bioorg Med Chem Lett* 2003b;13:351–354. [PubMed: 12565928]
- Cosford ND, Tehrani L, Roppe J, Schweiger E, Smith ND, Anderson J, Bristow L, Brodtkin J, Jiang X, McDonald I, Rao S, Washburn M, Varney MA. 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: a potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. *J Med Chem* 2003a;46:204–206. [PubMed: 12519057]
- Coutinho V, Knopfel T. Metabotropic glutamate receptors: electrical and chemical signaling properties. *Neuroscientist* 2002;8:551–561. [PubMed: 12467377]
- Crawford DC, Acuna JM, Sherman SL. FMR1 and the fragile X syndrome: human genome epidemiology review. *Genet Med* 2001;3:359–371. [PubMed: 11545690]
- Cube RV, Vernier JM, Hutchinson JH, Gardner MF, James JK, Rowe BA, Schaffhauser H, Daggett L, Pinkerton AB. 3-(2-Ethoxy-4-{4-[3-hydroxy-2-methyl-4-(3-methylbutanoyl)phenoxy]butoxy} phenyl)propanoic acid: a brain penetrant allosteric potentiator at the metabotropic glutamate receptor 2 (mGlu2). *Bioorg Med Chem Lett* 2005;15:2389–2393. [PubMed: 15837331]
- Cuomo D, Martella G, Barabino E, Platania P, Vita D, Madeo G, Selvam C, Goudet C, Oueslati N, Pin JP, Acher F, Pisani A, Beurrier C, Melon C, Kerkerian-Le Goff L, Gubellini P. Metabotropic glutamate receptor subtype 4 selectively modulates both glutamate and GABA transmission in the striatum: implications for Parkinson's disease treatment. *J Neurochem* 2009;109:1096–1105. [PubMed: 19519781]
- de Paulis T, Hemstapat K, Chen Y, Zhang Y, Saleh S, Alagille D, Baldwin RM, Tamagnan GD, Conn PJ. Substituent effects of N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamides on positive allosteric modulation of the metabotropic glutamate-5 receptor in rat cortical astrocytes. *J Med Chem* 2006;49:3332–3344. [PubMed: 16722652]
- Doherty AJ, Palmer MJ, Bortolotto ZA, Hargreaves A, Kingston AE, Ornstein PL, Schoepp DD, Lodge D, Collingridge GL. A novel, competitive mGlu(5) receptor antagonist (LY344545) blocks DHPG-induced potentiation of NMDA responses but not the induction of LTP in rat hippocampal slices. *Br J Pharmacol* 2000;131:239–244. [PubMed: 10991916]
- Doherty JJ, Alagarsamy S, Bough KJ, Conn PJ, Dingledine R, Mott DD. Metabotropic glutamate receptors modulate feedback inhibition in a developmentally regulated manner in rat dentate gyrus. *J Physiol* 2004;561:395–401. [PubMed: 15513941]

- Dolen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, Bear MF. Correction of fragile X syndrome in mice. *Neuron* 2007;56:955–962. [PubMed: 18093519]
- Dorr P, Westby M, Dobbs S, Griffin P, Irvine B, Macartney M, Mori J, Rickett G, Smith-Burchnell C, Napier C, Webster R, Armour D, Price D, Stammen B, Wood A, Perros M. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob Agents Chemother* 2005;49:4721–4732. [PubMed: 16251317]
- Ehlers MD. Synapse structure: glutamate receptors connected by the shanks. *Curr Biol* 1999;9:R848–850. [PubMed: 10574750]
- Eli Lilly. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2000. A Long-Term, Open-Label, Study on Schizophrenia. [cited 2010 Jun 23b] Available from: <http://clinicaltrials.gov/show/NCT01129674> NLM Identifier: NCT01129674
- Ferraguti F, Shigemoto R. Metabotropic glutamate receptors. *Cell Tissue Res* 2006;326:483–504. [PubMed: 16847639]
- Friedmann CTH, Davis LJ, Ciccone PE, Rubin RT. Phase II double blind controlled study of a new anxiolytic, fenobam (McN-3377) vs placebo. *Curr Ther Res* 1980;27:144–151.
- Flor PJ, Maj M, Dragic Z, Bruno V, Battaglia G, Inderbitzin W, van der Putten H, Kuhn R, Nicoletti F, Gasparini F. Positive allosteric modulators of metabotropic glutamate receptor subtype 4: pharmacological and molecular characterization. *Neuropharmacology* 2002;43:286.
- Fukuda J, Suzuki G, Kimura T, Nagatomi Y, Ito S, Kawamoto H, Ozaki S, Ohta H. Identification of a novel transmembrane domain involved in the negative modulation of mGlu1 using a newly discovered allosteric mGlu1 antagonist, 3-cyclohexyl-5-fluoro-6-methyl-7-(2-morpholin-4-ylethoxy)-4H-chromen-4-one. *Neuropharmacology* 2009;57:438–445. [PubMed: 19559036]
- Galandrin S, Oligny-Longpre G, Bouvier M. The evasive nature of drug efficacy: implications for drug discovery. *Trends Pharmacol Sci* 2007;28:423–430. [PubMed: 17659355]
- Galici R, Echemendia NG, Rodriguez AL, Conn PJ. A selective allosteric potentiator of metabotropic glutamate (mGlu) 2 receptors has effects similar to an orthosteric mGlu2/3 receptor agonist in mouse models predictive of antipsychotic activity. *J Pharmacol Exp Ther* 2005;315:1181–1187. [PubMed: 16123306]
- Galici R, Jones CK, Hemstapat K, Nong Y, Echemendia NG, Williams LC, de Paulis T, Conn PJ. 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* 2006;318:173–185. [PubMed: 16608916]
- Galvan A, Smith Y, Wichmann T. Continuous monitoring of intracerebral glutamate levels in awake monkeys using microdialysis and enzyme fluorometric detection. *J Neurosci Methods* 2003;126:175–185. [PubMed: 12814842]
- Garber KB, Visotsak J, Warren ST. Fragile X syndrome. *Eur J Hum Genet* 2008;16:666–672. [PubMed: 18398441]
- Gaspar PA, Bustamante ML, Silva H, Aboitiz F. Molecular mechanisms underlying glutamatergic dysfunction in schizophrenia: therapeutic implications. *J Neurochem* 2009;111:891–900. [PubMed: 19686383]
- Gasparini F, Andres H, Flor PJ, Heinrich M, Inderbitzin W, Lingenhohl K, Muller H, Munk VC, Omilusic K, Stierlin C, Stoehr N, Vranesic I, Kuhn R. [(3)H]-M-MPEP, a potent, subtype-selective radioligand for the metabotropic glutamate receptor subtype 5. *Bioorg Med Chem Lett* 2002;12:407–409. [PubMed: 11814808]
- Gasparini F, Floersheim P, Flor PJ, Heinrich M, Inderbitzin W, Ott D, Pagano A, Stierlin C, Stoehr N, Vranesic I, Kuhn R. Discovery and characterization of non-competitive antagonists of group I metabotropic glutamate receptors. *Farmacology* 2001;56:95–99. [PubMed: 11347975]
- Gasparini F, Lingenhohl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I, Biollaz M, Allgeier H, Heckendorn R, Urwyler S, Varney MA, Johnson EC, Hess SD, Rao SP, Sacca AI, Santori EM, Velicelebi G, Kuhn R. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology* 1999;38:1493–1503. [PubMed: 10530811]
- Gass JT, Olive MF. Positive allosteric modulation of mGlu5 receptors facilitates extinction of a cocaine contextual memory. *Biol Psychiatry* 2009;65:717–720. [PubMed: 19100966]

- Ginovart N, Wilson AA, Hussey D, Houle S, Kapur S. D2-Receptor upregulation is dependent upon temporal course of D2-occupancy: A longitudinal [¹¹C]-Raclopride PET study in cats. *Neuropsychopharmacology* 2009;34:662–671. [PubMed: 18688210]
- Goldberg ME, Salama AI, Patel JB, Malick JB. Novel non-benzodiazepine anxiolytics. *Neuropharmacology* 1983;22:1499–1504. [PubMed: 6142427]
- Goudet C, Gaven F, Kniazeff J, Vol C, Liu J, Cohen-Gonsaud M, Acher F, Prezeau L, Pin JP. Heptahelical domain of metabotropic glutamate receptor 5 behaves like rhodopsin-like receptors. *Proc Natl Acad Sci U S A* 2004;101:378–383. [PubMed: 14691258]
- Govek SP, Bonnefous C, Hutchinson JH, Kamenecka T, McQuiston J, Pracitto R, Zhao LX, Gardner MF, James JK, Daggett LP, Rowe BA, Schaffhauser H, Bristow LJ, Campbell UC, Rodriguez DE, Vernier JM. Benzazoles as allosteric potentiators of metabotropic glutamate receptor 2 (mGlu2): efficacy in an animal model for schizophrenia. *Bioorg Med Chem Lett* 2005;15:4068–4072. [PubMed: 16005222]
- Gravius A, Pietraszek M, Schäfer D, Schmidt WJ, Danysz W. Effects of mGlu1 and mGlu5 receptor antagonists on negatively reinforced learning. *Behav Pharmacol* 2005;16(2):113–21. [PubMed: 15767847]
- Hall DA. Modeling the Functional Effects of Allosteric Modulators at Pharmacological Receptors: An Extension of the Two-State Model of Receptor Activation. *Mol Pharmacol* 2000;58:1412–1423. [PubMed: 11093781]
- Hayashi Y, Sekiyama N, Nakanishi S, Jane DE, Sunter DC, Birse EF, Udvarhelyi PM, Watkins JC. Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. *J Neurosci* 1994;14:3370–3377. [PubMed: 8182479]
- Hemstapat K, Da Costa H, Nong Y, Brady AE, Luo Q, Niswender CM, Tamagnan GD, Conn PJ. A novel family of potent negative allosteric modulators of group II metabotropic glutamate receptors. *J Pharmacol Exp Ther* 2007;322:254–264. [PubMed: 17416742]
- Hemstapat K, de Paulis T, Chen Y, Brady AE, Grover VK, Alagille D, Tamagnan GD, Conn PJ. 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* 2006;70:616–626. [PubMed: 16645124]
- Holst B, Frimurer TM, Mokrosinski J, Halkjaer T, Cullberg KB, Underwood CR, Schwartz TW. Overlapping binding site for the endogenous agonist, small-molecule agonists, and ago-allosteric modulators on the ghrelin receptor. *Mol Pharmacol* 2009;75:44–59. [PubMed: 18923064]
- Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci U S A* 2002;99:7746–7750. [PubMed: 12032354]
- Itil TM, Seaman BA, Huque M, Mukhopadhyay S, Blasucci DNKT, Ciccone PE. The clinical and quantitative EEG effects and plasma levels of fenobam (McN-3377) in subjects with anxiety: an open rising dose tolerance and efficacy study. *Curr Ther Res* 1978:708–724.
- Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, Ijzerman AP, Stevens RC. The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science* 2008;322:1211–1217. [PubMed: 18832607]
- Johnson KA, Conn PJ, Niswender CM. Glutamate receptors as therapeutic targets for Parkinson's disease. *CNS Neurol Disord Drug Targets* 2009;8:475–491. [PubMed: 19702565]
- Johnson MP, Baez M, Jagdmann GE Jr, Britton TC, Large TH, Callagaro DO, Tizzano JP, Monn JA, Schoepp DD. Discovery of allosteric potentiators for the metabotropic glutamate 2 receptor: synthesis and subtype selectivity of N-(4-(2-methoxyphenoxy)phenyl)-N-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine. *J Med Chem* 2003;46:3189–3192. [PubMed: 12852748]
- Johnson MP, Barda D, Britton TC, Emkey R, Hornback WJ, Jagdmann GE, McKinzie DL, Nisenbaum ES, Tizzano JP, Schoepp DD. Metabotropic glutamate 2 receptor potentiators: receptor modulation, frequency-dependent synaptic activity, and efficacy in preclinical anxiety and psychosis model(s). *Psychopharmacology (Berl)* 2005;179:271–283. [PubMed: 15717213]
- Jones CK, Brady AE, Davis AA, Xiang Z, Bubser M, Tantawy MN, Kane AS, Bridges TM, Kennedy JP, Bradley SR, Peterson TE, Ansari MS, Baldwin RM, Kessler RM, Deutch AY, Lah JJ, Levey AI, Lindsley CW, Conn PJ. Novel selective allosteric activator of the M1 muscarinic acetylcholine

- receptor regulates amyloid processing and produces antipsychotic-like activity in rats. *J Neurosci* 2008;28:10422–10433. [PubMed: 18842902]
- Kenakin T. Functional selectivity through protean and biased agonism: who steers the ship? *Mol Pharmacol* 2007;72:1393–1401. [PubMed: 17901198]
- Keywood C, Wakefield M, Tack J. 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* 2009;58(9):1192–1199. [PubMed: 19460767]
- Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, Chen TB, Wisnoski DD, Lindsley CW, Tiller PR, Smith S, Jacobson MA, Sur C, Duggan ME, Pettibone DJ, Conn PJ, Williams DL Jr. 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* 2005;313:199–206. [PubMed: 15608073]
- Knoflach F, Mutel V, Jolidon S, Kew JN, Malherbe P, Vieira E, Wichmann J, Kemp JA. Positive allosteric modulators of metabotropic glutamate 1 receptor: characterization, mechanism of action, and binding site. *Proc Natl Acad Sci U S A* 2001;98:13402–13407. [PubMed: 11606768]
- Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov* 2004;3:711–715. [PubMed: 15286737]
- Kratochwil NA, Malherbe P, Lindemann L, Ebeling M, Hoener MC, Muhlemann A, Porter RH, Stahl M, Gerber PR. An automated system for the analysis of G protein-coupled receptor transmembrane binding pockets: alignment, receptor-based pharmacophores, and their application. *J Chem Inf Model* 2005;45:1324–1336. [PubMed: 16180909]
- Krystal JH, Abi-Saab W, Perry E, D'Souza DC, Liu N, Gueorguieva R, McDougall L, Hunsberger T, Belger A, Levine L, Breier A. Preliminary evidence of attenuation of the disruptive effects of the NMDA glutamate receptor antagonist, ketamine, on working memory by pretreatment with the group II metabotropic glutamate receptor agonist, LY354740, in healthy human subjects. *Psychopharmacology (Berl)* 2005;179:303–309. [PubMed: 15309376]
- Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, Nakanishi S, Jingami H, Morikawa K. Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* 2000;407:971–977. [PubMed: 11069170]
- Langmead CJ, Fry VA, Forbes IT, Branch CL, Christopoulos A, Wood MD, Herdon HJ. Probing the molecular mechanism of interaction between 4-n-butyl-1-[4-(2-methylphenyl)-4-oxo-1-butyl]-piperidine (AC-42) and the muscarinic M(1) receptor: direct pharmacological evidence that AC-42 is an allosteric agonist. *Mol Pharmacol* 2006;69:236–246. [PubMed: 16207821]
- Lapierre YD, Tremblay A, Gagnon A, Monpremier P, Berliss H, Oyewumi LK. A therapeutic and discontinuation study of clobazam and diazepam in anxiety neurosis. *J Clin Psychiatry* 1982;43:372–374. [PubMed: 6126475]
- Lavreysen H, Janssen C, Bischoff F, Langlois X, Leysen JE, Lesage AS. [3H]R214127: a novel high-affinity radioligand for the mGlu1 receptor reveals a common binding site shared by multiple allosteric antagonists. *Mol Pharmacol* 2003;63:1082–1093. [PubMed: 12695537]
- Lazareno S, Dolezal V, Popham A, Birdsall NJ. Thiochrome enhances acetylcholine affinity at muscarinic M4 receptors: receptor subtype selectivity via cooperativity rather than affinity. *Mol Pharmacol* 2004;65:257–266. [PubMed: 14722259]
- Leach K, Sexton PM, Christopoulos A. Allosteric GPCR modulators: taking advantage of permissive receptor pharmacology. *Trends Pharmacol Sci* 2007;28:382–389. [PubMed: 17629965]
- Lebois EP, Bridges TM, Lewis LM, Dawson ES, Kane AS, Xiang Z, Jadhav SB, Yin H, Kennedy JP, Meiler J, Niswender CM, Jones CK, Conn PJ, Weaver CD, Lindsley C. Discovery and Characterization of Novel Subtype-Selective Allosteric Agonists for the Investigation of M1 Receptor Function in the Central Nervous System. *ACS Chemical Neuroscience* 2010;1:104–121.
- Lecourtier L, Homayoun H, Tamagnan G, Moghaddam B. Positive allosteric modulation of metabotropic glutamate 5 (mGlu5) receptors reverses N-Methyl-D-aspartate antagonist-induced alteration of neuronal firing in prefrontal cortex. *Biol Psychiatry* 2007;62:739–746. [PubMed: 17511968]
- Lee HG, Zhu X, O'Neill MJ, Webber K, Casadesus G, Marlatt M, Raina AK, Perry G, Smith MA. The role of metabotropic glutamate receptors in Alzheimer's disease. *Acta Neurobiol Exp (Wars)* 2004;64:89–98. [PubMed: 15190683]

- Lee HJ, Mun HC, Lewis NC, Crouch MF, Culverston EL, Mason RS, Conigrave AD. Allosteric activation of the extracellular Ca²⁺-sensing receptor by L-amino acids enhances ERK1/2 phosphorylation. *Biochem J* 2007;404:141–149. [PubMed: 17212589]
- Lindberg JS, Culleton B, Wong G, Borah MF, Clark RV, Shapiro WB, Roger SD, Husserl FE, Klassen PS, Guo MD, Albizem MB, Coburn JW. Cinacalcet HCl, an oral calcimimetic agent for the treatment of secondary hyperparathyroidism in hemodialysis and peritoneal dialysis: a randomized, double-blind, multicenter study. *J Am Soc Nephrol* 2005;16:800–807. [PubMed: 15689407]
- Lindsley CW, Wisnoski DD, Leister WH, O'Brien JA, Lemaire W, Williams DL Jr, Burno M, Sur C, Kinney GG, Pettibone DJ, Tiller PR, Smith S, Duggan ME, Hartman GD, Conn PJ, Huff JR. Discovery of positive allosteric modulators for the metabotropic glutamate receptor subtype 5 from a series of N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamides that potentiate receptor function in vivo. *J Med Chem* 2004;47:5825–5828. [PubMed: 15537338]
- Lindsley CW, Zhao Z, Leister WH, O'Brien J, Lemaire W, Williams DL Jr, Chen TB, Chang RS, Burno M, Jacobson MA, Sur C, Kinney GG, Pettibone DJ, Tiller PR, Smith S, Tsou NN, Duggan ME, Conn PJ, Hartman GD. Design, synthesis, and in vivo efficacy of glycine transporter-1 (GlyT1) inhibitors derived from a series of [4-phenyl-1-(propylsulfonyl)piperidin-4-yl]methyl benzamides. *ChemMedChem* 2006;1:807–811. [PubMed: 16902933]
- Litschig S, Gasparini F, Rueegg D, Stoehr N, Flor PJ, Vranesic I, Prezeau L, Pin JP, Thomsen C, Kuhn R. CPCCOEt, a noncompetitive metabotropic glutamate receptor 1 antagonist, inhibits receptor signaling without affecting glutamate binding. *Mol Pharmacol* 1999;55:453–461. [PubMed: 10051528]
- Liu F, Grauer S, Kelley C, Navarra R, Graf R, Zhang G, Atkinson PJ, Popielek M, Wantuch C, Khawaja X, Smith D, Olsen M, Kouranova E, Lai M, Pruthi F, Pulicicchio C, Day M, Gilbert A, Pausch MH, Brandon NJ, Beyer CE, Comery TA, Logue S, Rosenzweig-Lipson S, Marquis KL. ADX47273 [S-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone]: a novel metabotropic glutamate receptor 5-selective positive allosteric modulator with preclinical antipsychotic-like and procognitive activities. *J Pharmacol Exp Ther* 2008;327:827–839. [PubMed: 18753411]
- Liu X, He Q, Studholme DJ, Wu Q, Liang S, Yu L. NCD3G: a novel nine-cysteine domain in family 3 GPCRs. *Trends Biochem Sci* 2004;29:458–461. [PubMed: 15337118]
- Lorrain DS, Schaffhauser H, Campbell UC, Baccei CS, Correa LD, Rowe B, Rodriguez DE, Anderson JJ, Varney MA, Pinkerton AB, Vernier JM, Bristow LJ. Group II mGlu receptor activation suppresses norepinephrine release in the ventral hippocampus and locomotor responses to acute ketamine challenge. *Neuropsychopharmacology* 2003;28:1622–1632. [PubMed: 12825094]
- Macek TA, Winder DG, Gereau RWt, Ladd CO, Conn PJ. Differential involvement of group II and group III mGlu as autoreceptors at lateral and medial perforant path synapses. *J Neurophysiol* 1996;76:3798–3806. [PubMed: 8985877]
- MacInnes N, Duty S. Group III metabotropic glutamate receptors act as hetero-receptors modulating evoked GABA release in the globus pallidus in vivo. *Eur J Pharmacol* 2008;580:95–99. [PubMed: 18035348]
- Maj M, Bruno V, Dragic Z, Yamamoto R, Battaglia G, Inderbitzin W, Stoehr N, Stein T, Gasparini F, Vranesic I, Kuhn R, Nicoletti F, Flor PJ. (-)-PHCCC, a positive allosteric modulator of mGlu4: characterization, mechanism of action, and neuroprotection. *Neuropharmacology* 2003;45:895–906. [PubMed: 14573382]
- Malherbe P, Kratochwil N, Knoflach F, Zenner MT, Kew JN, Kratzeisen C, Maerki HP, Adam G, Mutel V. 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* 2003a;278:8340–8347. [PubMed: 12509432]
- Malherbe P, Kratochwil N, Muhlemann A, Zenner MT, Fischer C, Stahl M, Gerber PR, Jaeschke G, Porter RHP. 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* 2006;98:601–615. [PubMed: 16805850]
- Malherbe P, Kratochwil N, Zenner MT, Piussi J, Diener C, Kratzeisen C, Fischer C, Porter RH. Mutational analysis and molecular modeling of the binding pocket of the metabotropic glutamate 5 receptor

negative modulator 2-methyl-6-(phenylethynyl)-pyridine. *Mol Pharmacol* 2003b;64:823–832. [PubMed: 14500738]

Mannaioni G, Marino MJ, Valenti O, Traynelis SF, Conn PJ. Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. *J Neurosci* 2001;21:5925–5934. [PubMed: 11487615]

Marino MJ, Conn PJ. Direct and indirect modulation of the N-methyl D-aspartate receptor. *Curr Drug Targets CNS Neurol Disord* 2002;1:1–16. [PubMed: 12769631]

Marino MJ, Conn PJ. Glutamate-based therapeutic approaches: allosteric modulators of metabotropic glutamate receptors. *Curr Opin Pharmacol* 2006;6:98–102. [PubMed: 16368268]

Marino MJ, Williams DL Jr, O'Brien JA, Valenti O, McDonald TP, Clements MK, Wang R, DiLella AG, Hess JF, Kinney GG, Conn PJ. Allosteric modulation of group III metabotropic glutamate receptor 4: a potential approach to Parkinson's disease treatment. *Proc Natl Acad Sci U S A* 2003;100:13668–13673. [PubMed: 14593202]

Mathiesen JM, Svendsen N, Brauner-Osborne H, Thomsen C, Ramirez MT. Positive allosteric modulation of the human metabotropic glutamate receptor 4 (hmGlu4) by SIB-1893 and MPEP. *Br J Pharmacol* 2003;138:1026–1030. [PubMed: 12684257]

Matsui T, Kita H. Activation of group III metabotropic glutamate receptors presynaptically reduces both GABAergic and glutamatergic transmission in the rat globus pallidus. *Neuroscience* 2003;122:727–737. [PubMed: 14622916]

May LT, Avlani VA, Langmead CJ, Herdon HJ, Wood MD, Sexton PM, Christopoulos A. Structure-function studies of allosteric agonism at M2 muscarinic acetylcholine receptors. *Mol Pharmacol* 2007a;72:463–476. [PubMed: 17525129]

May LT, Leach K, Sexton PM, Christopoulos A. Allosteric modulation of G protein-coupled receptors. *Annu Rev Pharmacol Toxicol* 2007b;47:1–51. [PubMed: 17009927]

Miedlich SU, Gama L, Seuwen K, Wolf RM, Breitwieser GE. Homology modeling of the transmembrane domain of the human calcium sensing receptor and localization of an allosteric binding site. *J Biol Chem* 2004;279:7254–7263. [PubMed: 14660633]

Mitsukawa K, Yamamoto R, Ofner S, Nozulak J, Pescott O, Lukic S, Stoehr N, Mombereau C, Kuhn R, McAllister KH, van der Putten H, Cryan JF, Flor PJ. A selective metabotropic glutamate receptor 7 agonist: activation of receptor signaling via an allosteric site modulates stress parameters in vivo. *Proc Natl Acad Sci U S A* 2005;102:18712–18717. [PubMed: 16339898]

Moghaddam B, Adams BW. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science* 1998;281:1349–1352. [PubMed: 9721099]

Mohler H, Fritschy JM, Rudolph U. A new benzodiazepine pharmacology. *J Pharmacol Exp Ther* 2002;300:2–8. [PubMed: 11752090]

Muhlemann A, Ward NA, Kratochwil N, Diener C, Fischer C, Stucki A, Jaeschke G, Malherbe P, Porter RH. Determination of key amino acids implicated in the actions of allosteric modulation by 3,3'-difluorobenzaldazine on rat mGlu5 receptors. *Eur J Pharmacol* 2006;529:95–104. [PubMed: 16352303]

Muto T, Tsuchiya D, Morikawa K, Jingami H. Expression, purification, crystallization and preliminary X-ray analysis of the ligand-binding domain of metabotropic glutamate receptor 7. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 2007;63:627–630.

Natesan S, Reckless GE, Nobrega JN, Fletcher PJ, Kapur S. Dissociation between In Vivo Occupancy and Functional Antagonism of Dopamine D2 Receptors: Comparing Aripiprazole to Other Antipsychotics in Animal Models. *Neuropsychopharmacology* 2005;31:1854–1863. [PubMed: 16319908]

Nawaratne V, Leach K, Suratman N, Loiacono RE, Felder CC, Armbruster BN, Roth BL, Sexton PM, Christopoulos A. New insights into the function of M4 muscarinic acetylcholine receptors gained using a novel allosteric modulator and a DREADD (designer receptor exclusively activated by a designer drug). *Mol Pharmacol* 2008;74:1119–1131. [PubMed: 18628403]

Nicholls RE, Zhang XL, Bailey CP, Conklin BR, Kandel ER, Stanton PK. mGlu2 acts through inhibitory Gα subunits to regulate transmission and long-term plasticity at hippocampal mossy fiber-CA3 synapses. *Proc Natl Acad Sci U S A* 2006;103:6380–6385. [PubMed: 16606834]

- Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol* 2010;50:295–322. [PubMed: 20055706]
- Niswender CM, Johnson KA, Miller NR, Ayala JE, Luo Q, Williams R, Saleh S, Orton D, Weaver CD, Conn PJ. Context-dependent pharmacology exhibited by negative allosteric modulators of metabotropic glutamate receptor 7. *Mol Pharmacol* 2010;77:459–468. [PubMed: 20026717]
- Niswender CM, Johnson KA, Weaver CD, Jones CK, Xiang Z, Luo Q, Rodriguez AL, Marlo JE, de Paulis T, Thompson AD, Days EL, Nalywajko T, Austin CA, Williams MB, Ayala JE, Williams R, Lindsley CW, Conn PJ. Discovery, characterization, and antiparkinsonian effect of novel positive allosteric modulators of metabotropic glutamate receptor 4. *Mol Pharmacol* 2008;74:1345–1358. [PubMed: 18664603]
- Niswender CM, Jones CK, Conn PJ. New therapeutic frontiers for metabotropic glutamate receptors. *Curr Top Med Chem* 2005;5:847–857. [PubMed: 16178730]
- Noeske T, Jirgensons A, Starchenkova I, Renner S, Jaunzeme I, Trifanova D, Hechenberger M, Bauer T, Kauss V, Parsons CG, Schneider G, Weil T. Virtual screening for selective allosteric mGlu1 antagonists and structure-activity relationship investigations for coumarine derivatives. *ChemMedChem* 2007;2:1763–1773. [PubMed: 17868161]
- Noeske T, Trifanova D, Kauss V, Renner S, Parsons CG, Schneider G, Weil T. Synergism of virtual screening and medicinal chemistry: identification and optimization of allosteric antagonists of metabotropic glutamate receptor 1. *Bioorg Med Chem* 2009;17:5708–5715. [PubMed: 19574055]
- Novartis. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2000. Efficacy, Safety and Tolerability of AFQ056 in Fragile X Patients. [cited 2010 Jun 23a] Available from: <http://clinicaltrials.gov/ct2/show/NCT00718341> NLM Identifier: NCT00718341
- Novartis. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2000. Efficacy and Safety of AFQ056 in Reducing L-dopa Induced Dyskinesias in Parkinson's Disease Patients, and Safety in Combination With L-dopa. [cited 2010 Jun 23b] Available from: <http://clinicaltrials.gov/show/NCT00582673> NLM Identifier: NCT00582673
- Novartis. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2000. Efficacy and Safety of AFQ056 in Reducing L-dopa Induced Dyskinesias in Parkinson's Disease Patients. [cited 2010 Jun 23c] Available from: <http://clinicaltrials.gov/show/NCT00888004> NLM Identifier: NCT00888004
- Novartis. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2000. Evaluation of the Efficacy and Safety of AFQ056 in Reducing Moderate to Severe L-dopa Induced Dyskinesias in Patients With Parkinson's Disease. [cited 2010 Jun 23d] Available from: <http://clinicaltrials.gov/show/NCT00986414> NLM Identifier: NCT00986414
- O'Brien JA, Lemaire W, Chen TB, Chang RS, Jacobson MA, Ha SN, Lindsley CW, Schaffhauser HJ, Sur C, Pettibone DJ, Conne PJ, Williams DL Jr. A family of highly selective allosteric modulators of the metabotropic glutamate receptor subtype 5. *Mol Pharmacol* 2003;64:731–740. [PubMed: 12920211]
- O'Brien JA, Lemaire W, Wittmann M, Jacobson MA, Ha SN, Wisnoski DD, Lindsley CW, Schaffhauser HJ, Rowe B, Sur C, Duggan ME, Pettibone DJ, Conn PJ, Williams DL Jr. A novel selective allosteric modulator potentiates the activity of native metabotropic glutamate receptor subtype 5 in rat forebrain. *J Pharmacol Exp Ther* 2004;309:568–577. [PubMed: 14747613]
- Ott D, Floersheim P, Inderbitzin W, Stoehr N, Francotte E, Lecis G, Richert P, Rihs G, Flor PJ, Kuhn R, Gasparini F. 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* 2000;43:4428–4436. [PubMed: 11087567]
- Pagano A, Ruegg D, Litschig S, Stoehr N, Stierlin C, Heinrich M, Floersheim P, Prezeau L, Carroll F, Pin JP, Cambria A, Vranesic I, Flor PJ, Gasparini F, Kuhn R. The noncompetitive antagonists 2-methyl-6-(phenylethynyl)pyridine and 7-hydroxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester interact with overlapping binding pockets in the transmembrane region of group I metabotropic glutamate receptors. *J Biol Chem* 2000;275:33750–33758. [PubMed: 10934211]
- Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Trong IL, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M. Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor. *Science* 2000;289:739–745. [PubMed: 10926528]

- Palucha A, Pilc A. Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. *Pharmacol Ther* 2007;115:116–147. [PubMed: 17582504]
- Parmentier ML, Prezeau L, Bockaert J, Pin JP. A model for the functioning of family 3 GPCRs. *Trends Pharmacol Sci* 2002;23:268–274. [PubMed: 12084632]
- Patel JB, Martin C, Malick JB. Differential antagonism of the anticonflict effects of typical and atypical anxiolytics. *Eur J Pharmacol* 1982;86:295–298. [PubMed: 6130960]
- Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, Avedisova AS, Bardenstein LM, Gurovich IY, Morozova MA, Mosolov SN, Neznanov NG, Reznik AM, Smulevich AB, Tochilov VA, Johnson BG, Monn JA, Schoepp DD. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. *Nat Med* 2007;13:1102–1107. [PubMed: 17767166]
- Pecknold JC, McClure DJ, Appeltauer L, Wrzesinski L, Allan T. Treatment of anxiety using fenobam (a nonbenzodiazepine) in a double-blind standard (diazepam) placebo-controlled study. *J Clin Psychopharmacol* 1982;2:129–133. [PubMed: 7042771]
- Pelkey KA, Yuan X, Lavezzari G, Roche KW, McBain CJ. mGlu7 undergoes rapid internalization in response to activation by the allosteric agonist AMN082. *Neuropharmacology* 2007;52:108–117. [PubMed: 16914173]
- Pietraszek M, Sukhanov I, Maciejak P, Szyndler J, Gravius A, Wisłowska A, Płaźnik A, Bessalov AY, Danysz W. Anxiolytic-like effects of mGlu1 and mGlu5 receptor antagonists in rats. *Eur J Pharmacol* 2005;514(1):25–34. [PubMed: 15878321]
- Pin JP, Galvez T, Prezeau L. Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol Ther* 2003;98:325–354. [PubMed: 12782243]
- Pinheiro PS, Mulle C. Presynaptic glutamate receptors: physiological functions and mechanisms of action. *Nat Rev Neurosci* 2008;9:423–436. [PubMed: 18464791]
- Pinkerton AB, Cube RV, Hutchinson JH, James JK, Gardner MF, Rowe BA, Schaffhauser H, Rodriguez DE, Campbell UC, Daggett LP, Vernier JM. Allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2). Part 3: Identification and biological activity of indanone containing mGlu2 receptor potentiators. *Bioorg Med Chem Lett* 2005;15:1565–1571. [PubMed: 15745798]
- Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G, Calabresi P. Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. *Neuroscience* 2001;106:579–587. [PubMed: 11591458]
- Poewe WH, Lees AJ, Stern GM. Low-dose L-dopa therapy in Parkinson's disease: a 6-year follow-up study. *Neurology* 1986;36:1528–1530. [PubMed: 3762973]
- Poewe WH, Lees AJ, Stern GM. Treatment of motor fluctuations in Parkinson's disease with an oral sustained-release preparation of L-dopa: clinical and pharmacokinetic observations. *Clin Neuropharmacol* 1986;9:430–439. [PubMed: 3768865]
- Poisik O, Raju DV, Verreault M, Rodriguez A, Abeniyi OA, Conn PJ, Smith Y. Metabotropic glutamate receptor 2 modulates excitatory synaptic transmission in the rat globus pallidus. *Neuropharmacology* 2005;49(Suppl 1):57–69. [PubMed: 15993439]
- Porter RH, Jaeschke G, Spooren W, Ballard TM, Buttelmann B, Kolczewski S, Peters JU, Prinssen E, Wichmann J, Vieira E, Muhlemann A, Gatti S, Mutel V, Malherbe P. Fenobam: a clinically validated nonbenzodiazepine anxiolytic is a potent, selective, and noncompetitive mGlu5 receptor antagonist with inverse agonist activity. *J Pharmacol Exp Ther* 2005;315:711–721. [PubMed: 16040814]
- Price MR, Baillie GL, Thomas A, Stevenson LA, Easson M, Goodwin R, McLean A, McIntosh L, Goodwin G, Walker G, Westwood P, Marrs J, Thomson F, Cowley P, Christopoulos A, Pertwee RG, Ross RA. Allosteric modulation of the cannabinoid CB1 receptor. *Mol Pharmacol* 2005;68:1484–1495. [PubMed: 16113085]
- Radestock S, Weil T, Renner S. Homology model-based virtual screening for GPCR ligands using docking and target-biased scoring. *J Chem Inf Model* 2008;48:1104–1117. [PubMed: 18442221]
- Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature* 2007;450:383–387. [PubMed: 17952055]

- Rodrigues SM, Bauer EP, Farb CR, Schafe GE, LeDoux JE. The group I metabotropic glutamate receptor mGlu5 is required for fear memory formation and long-term potentiation in the lateral amygdala. *J Neurosci* 2002;22:5219–5229. [PubMed: 12077217]
- Rodriguez AL, Nong Y, Sekaran NK, Alagille D, Tamagnan GD, Conn PJ. 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* 2005;68:1793–1802. [PubMed: 16155210]
- Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW. Distribution of metabotropic glutamate receptor mGlu5 immunoreactivity in rat brain. *J Comp Neurol* 1995;355:455–469. [PubMed: 7636025]
- Romano C, Yang WL, O'Malley KL. Metabotropic glutamate receptor 5 is a disulfide-linked dimer. *J Biol Chem* 1996;271:28612–28616. [PubMed: 8910492]
- Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC, Kobilka BK. GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science* 2007;318:1266–1273. [PubMed: 17962519]
- Rondard P, Liu J, Huang S, Malhaire F, Vol C, Pinaut A, Labesse G, Pin JP. Coupling of agonist binding to effector domain activation in metabotropic glutamate-like receptors. *J Biol Chem* 2006;281:24653–24661. [PubMed: 16787923]
- Rowe BA, Schaffhauser H, Morales S, Lubbers LS, Bonnefous C, Kamenecka TM, McQuiston J, Daggett LP. Transposition of three amino acids transforms the human metabotropic glutamate receptor (mGlu)-3-positive allosteric modulation site to mGlu2, and additional characterization of the mGlu2-positive allosteric modulation site. *J Pharmacol Exp Ther* 2008;326:240–251. [PubMed: 18430863]
- Rylander D, Iderberg H, Li Q, Dekundy A, Zhang J, Li H, Baishen R, Danysz W, Bezard E, Cenci MA. A mGluR5 antagonist under clinical development improves L-DOPA-induced dyskinesia in parkinsonian rats and monkeys. *Neurobiol Dis*. 2010 in press.
- Sachpatzidis A, Benton BK, Manfredi JP, Wang H, Hamilton A, Dohlman HG, Lolis E. Identification of Allosteric Peptide Agonists of CXCR4. *J Biol Chem* 2003;278:896–907. [PubMed: 12417595]
- Schaffhauser H, Rowe BA, Morales S, Chavez-Noriega LE, Yin R, Jachec C, Rao SP, Bain G, Pinkerton AB, Vernier JM, Bristow LJ, Varney MA, Daggett LP. Pharmacological characterization and identification of amino acids involved in the positive modulation of metabotropic glutamate receptor subtype 2. *Mol Pharmacol* 2003;64:798–810. [PubMed: 14500736]
- Schlumberger C, Pietraszek M, Gravius A, Danysz W. Effects of a positive allosteric modulator of mGlu5 ADX47273 on conditioned avoidance response and PCP-induced hyperlocomotion in the rat as models for schizophrenia. *Pharmacol Biochem Behav* 2010;95:23–30. [PubMed: 19995568]
- Schlumberger C, Pietraszek M, Gravius A, Klein KU, Greco S, More L, Danysz W. Comparison of the mGlu(5) receptor positive allosteric modulator ADX47273 and the mGlu(2/3) receptor agonist LY354740 in tests for antipsychotic-like activity. *Eur J Pharmacol* 2009;623:73–83. [PubMed: 19765575]
- Schoepp DD. Case Study: utility of metabotropic glutamate agonists in psychiatric illness. 5th World Conference on Stress; 2004. Abs 120
- Schoepp DD, Wright RA, Levine LR, Gaydos B, Potter WZ. LY354740, an mGlu2/3 receptor agonist as a novel approach to treat anxiety/stress. *Stress* 2003;6:189–197. [PubMed: 13129812]
- Schulz B, Fendt M, Gasparini F, Lingenhohl K, Kuhn R, Koch M. The metabotropic glutamate receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) blocks fear conditioning in rats. *Neuropharmacology* 2001;41:1–7. [PubMed: 11445180]
- Schwartz TW, Holst B. Allosteric enhancers, allosteric agonists and ago-allosteric modulators: where do they bind and how do they act? *Trends Pharmacol Sci* 2007;28:366–373. [PubMed: 17629958]
- Slassi A, Isaac M, Edwards L, Minidis A, Wensbo D, Mattsson J, Nilsson K, Raboisson P, McLeod D, Stormann TM, Hammerland LG, Johnson E. Recent advances in noncompetitive mGlu5 receptor antagonists and their potential therapeutic applications. *Curr Top Med Chem* 2005;5:897–911. [PubMed: 16178734]
- Spalding TA, Ma JN, Ott TR, Friberg M, Bajpai A, Bradley SR, Davis RE, Brann MR, Burstein ES. Structural requirements of transmembrane domain 3 for activation by the M1 muscarinic receptor

- agonists AC-42, AC-260584, clozapine, and N-desmethylozapine: evidence for three distinct modes of receptor activation. *Mol Pharmacol* 2006;70:1974–1983. [PubMed: 16959945]
- Spalding TA, Trotter C, Skjaerbaek N, Messier TL, Currier EA, Burstein ES, Li D, Hacksell U, Brann MR. Discovery of an ectopic activation site on the M(1) muscarinic receptor. *Mol Pharmacol* 2002;61:1297–1302. [PubMed: 12021390]
- Spooren W, Gasparini F. mGlu5 receptor antagonists: a novel class of anxiolytics? *Drug News Perspect* 2004;17:251–257. [PubMed: 15334174]
- Spooren WP, Schoeffter P, Gasparini F, Kuhn R, Gentsch C. Pharmacological and endocrinological characterisation of stress-induced hyperthermia in singly housed mice using classical and candidate anxiolytics (LY314582, MPEP and NKP608). *Eur J Pharmacol* 2002;435:161–170. [PubMed: 11821022]
- Spooren WP, Vassout A, Neijt HC, Kuhn R, Gasparini F, Roux S, Porsolt RD, Gentsch C. Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. *J Pharmacol Exp Ther* 2000;295:1267–1275. [PubMed: 11082464]
- Steckler T, Lavreysen H, Oliveira AM, Aerts N, Craenendonck H, Prickaerts J, Megens A, Lesage AS. Effects of mGlu1 receptor blockade on anxiety-related behaviour in the rat lick suppression test. *Psychopharmacology* 2005a;179:198–206. [PubMed: 15821950]
- Steckler T, Oliveira AF, Van Dyck C, Van Craenendonck H, Mateus AM, Langlois X, Lesage AS, Prickaerts J. Metabotropic glutamate receptor 1 blockade impairs acquisition and retention in a spatial Water maze task. *Behav Brain Res* 2005b;164:52–60. [PubMed: 16043241]
- Stefani MR, Moghaddam B. Activation of type 5 metabotropic glutamate receptors attenuates deficits in cognitive flexibility induced by NMDA receptor blockade. *Eur J Pharmacol* 2010;639:26–32. [PubMed: 20371234]
- Surin A, Pshenichkin S, Grajkowska E, Surina E, Wroblewski JT. Cyclothiazide selectively inhibits mGlu1 receptors interacting with a common allosteric site for non-competitive antagonists. *Neuropharmacology* 2007;52:744–754. [PubMed: 17095021]
- Suzuki G, Kimura T, Satow A, Kaneko N, Fukuda J, Hikichi H, Sakai N, Maehara S, Kawagoe-Takaki H, Hata M, Azuma T, Ito S, Kawamoto H, Ohta H. 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* 2007;321:1144–1153. [PubMed: 17360958]
- Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. *Nat Rev Drug Discov* 2005;4:131–144. [PubMed: 15665858]
- Takahashi K, Tsuchida K, Tanabe Y, Masu M, Nakanishi S. Role of the large extracellular domain of metabotropic glutamate receptors in agonist selectivity determination. *J Biol Chem* 1993;268:19341–19345. [PubMed: 8103516]
- Tatarczynska E, Klodzinska A, Krocza B, Chojnacka-Wojcik E, Pilc A. The antianxiety-like effects of antagonists of group I and agonists of group II and III metabotropic glutamate receptors after intrahippocampal administration. *Psychopharmacology (Berl)* 2001;158:94–99. [PubMed: 11685389]
- Teller DC, Okada T, Behnke CA, Palczewski K, Stenkamp RE. Advances in determination of a high-resolution three-dimensional structure of rhodopsin, a model of G-proteincoupled receptors (GPCRs). *Biochemistry* 2001;40:7761–7772. [PubMed: 11425302]
- Tones MA, Bendali N, Flor PJ, Knopfel T, Kuhn R. The agonist selectivity of a class III metabotropic glutamate receptor, human mGlu4a, is determined by the N-terminal extracellular domain. *Neuroreport* 1995;7:117–120. [PubMed: 8742431]
- Treyer V, Streffer J, Ametamey SM, Bettio A, Bläuenstein P, Schmidt M, Gasparini F, Hock C, Buck A. Radiation dosimetry and biodistribution of ¹¹¹C-ABP688 measured in healthy volunteers. *Eur J Nucl Med Mol Imaging* 2008;35(4):766–770. [PubMed: 18071702]
- Tsuchiya D, Kunishima N, Kamiya N, Jingami H, Morikawa K. Structural views of the ligand-binding cores of a metabotropic glutamate receptor complexed with an antagonist and both glutamate and Gd³⁺. *Proc Natl Acad Sci U S A* 2002;99:2660–2665. [PubMed: 11867751]

- Tu H, Rondard P, Xu C, Bertaso F, Cao F, Zhang X, Pin JP, Liu J. Dominant role of GABAB2 and Gbetagamma for GABAB receptor-mediated-ERK1/2/CREB pathway in cerebellar neurons. *Cell Signal* 2007;19:1996–2002. [PubMed: 17582742]
- Ugolini A, Corsi M, Bordi F. Potentiation of NMDA and AMPA responses by the specific mGlu5 agonist CHPG in spinal cord motoneurons. *Neuropharmacology* 1999;38:1569–1576. [PubMed: 10530818]
- Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, Javitch JA, Roth BL, Christopoulos A, Sexton PM, Miller KJ, Spedding M, Mailman RB. Functional Selectivity and Classical Concepts of Quantitative Pharmacology. *J Pharmacol Exp Ther* 2007;320:1–13. [PubMed: 16803859]
- Ure J, Baudry M, Perassolo M. Metabotropic glutamate receptors and epilepsy. *J Neurol Sci* 2006;247:1–9. [PubMed: 16697014]
- Uslaner JM, Parmentier-Batteur S, Flick RB, Surles NO, Lam JS, McNaughton CH, Jacobson MA, Hutson PH. Dose-dependent effect of CDPPB, the mGlu5 positive allosteric modulator, on recognition memory is associated with GluR1 and CREB phosphorylation in the prefrontal cortex and hippocampus. *Neuropharmacology* 2009;57:531–538. [PubMed: 19627999]
- Valant C, Gregory KJ, Hall NE, Scammells PJ, Lew MJ, Sexton PM, Christopoulos A. A novel mechanism of G protein-coupled receptor functional selectivity. Muscarinic partial agonist McN-A-343 as a bitopic orthosteric/allosteric ligand. *J Biol Chem* 2008;283:29312–29321. [PubMed: 18723515]
- Valenti O, Conn PJ, Marino MJ. Distinct physiological roles of the Gq-coupled metabotropic glutamate receptors Co-expressed in the same neuronal populations. *J Cell Physiol* 2002;191:125–137. [PubMed: 12064455]
- Valenti O, Mannaioni G, Seabrook GR, Conn PJ, Marino MJ. Group III metabotropic glutamate-receptor-mediated modulation of excitatory transmission in rodent substantia nigra pars compacta dopamine neurons. *J Pharmacol Exp Ther* 2005;313:1296–1304. [PubMed: 15761115]
- Valenti O, Marino MJ, Wittmann M, Lis E, DiLella AG, Kinney GG, Conn PJ. Group III metabotropic glutamate receptor-mediated modulation of the striatopallidal synapse. *J Neurosci* 2003;23:7218–7226. [PubMed: 12904482]
- Vanejevs M, Jatzke C, Renner S, Muller S, Hechenberger M, Bauer T, Klochkova A, Pyatkin I, Kazyulkin D, Aksenova E, Shulepin S, Timonina O, Haasis A, Gutcaits A, Parsons CG, Kauss V, Weil T. Positive and negative modulation of group I metabotropic glutamate receptors. *J Med Chem* 2008;51:634–647. [PubMed: 18173231]
- Varney MA, Cosford ND, Jachec C, Rao SP, Sacca A, Lin FF, Bleicher L, Santori EM, Flor PJ, Allgeier H, Gasparini F, Kuhn R, Hess SD, Velicelebi G, Johnson EC. SIB-1757 and SIB-1893: selective, noncompetitive antagonists of metabotropic glutamate receptor type 5. *J Pharmacol Exp Ther* 1999;290:170–181. [PubMed: 10381773]
- Wadenberg ML, Soliman A, VanderSpek SC, Kapur S. Dopamine D(2) receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. *Neuropsychopharmacology* 2001;25:633–641. [PubMed: 11682246]
- Wichmann T, DeLong MR. Functional neuroanatomy of the basal ganglia in Parkinson's disease. *Adv Neurol* 2003;91:9–18. [PubMed: 12442660]
- Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGlu5 antagonist MPEP. *Neuropharmacology* 2005;49:1053–1066. [PubMed: 16054174]
- Yarnitzky T, Levit A, Niv MY. Homology modeling of G-protein-coupled receptors with X-ray structures on the rise. *Curr Opin Drug Discov Devel* 2010;13(3):317–325.
- Yu M. Recent developments of the PET imaging agents for metabotropic glutamate receptor subtype 5. *Curr Top Med Chem* 2007;7:1800–1805. [PubMed: 17979788]
- Zerbib F, Keywood C, Strabach G. 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*. 2010 in press.

- Zhang Y, Rodriguez AL, Conn PJ. Allosteric potentiators of metabotropic glutamate receptor subtype 5 have differential effects on different signaling pathways in cortical astrocytes. *J Pharmacol Exp Ther* 2005;315:1212–1219. [PubMed: 16135701]
- Zhao Z, Wisnoski DD, O'Brien JA, Lemaire W, Williams DL Jr, Jacobson MA, Wittman M, Ha SN, Schaffhauser H, Sur C, Pettibone DJ, Duggan ME, Conn PJ, Hartman GD, Lindsley CW. Challenges in the development of mGlu5 positive allosteric modulators: the discovery of CPPHA. *Bioorg Med Chem Lett* 2007;17:1386–1391. [PubMed: 17210250]

Figure 1. Models of allosteric interactions

A) Allosteric Ternary Complex Model, **B)** Operational Model of Allosterism. Refer to text for definitions of parameters.

Figure 2. Prototypical allosteric modulators of mGlu

Pictured are representative allosteric modulators for mGlu₁, mGlu₂, mGlu₄, mGlu₅ and mGlu₇. The full chemical names and subtype selectivity of these compounds are provided in Table 1.

	TM1		TM2	
mGluR1	Y L E W S N I E S I I A I A F S C L G I L V T L F V T L I F V L 616	SSSREL CY I I L A G I F L G Y V C P F T L I A K P T . 653		
mGluR2	Y I R W G D A W A V G P V T I A C L G A L A T L F V L G V F V R 591	ASGRELCY I I L L G G V F L G Y C M T F I F I A K P S . 628		
mGluR3	Y I R W E D A W A I G P V T I A C L G F M C T C M V V T V F I K 600	ASGRELCY I I L L F G V G L S Y C M T F F F I A K P S . 637		
mGluR4	K L E W G S P W A V L P L F L A V V G I A A T L F V V I T F V R 611	ASGRELSY V L L A G I F L C Y A T T F L M I A E P D . 646		
mGluR5	Y L R W G D P E P I A A V V F A C L G L L A T L F V V T V F I I 603	SSSREL CY I I L A G I C L G Y L C T F C L I A K P K . 640		
mGluR6	R L S W S S P W A P P L L L A V L G I V A T T T V V A T F V R 609	ASGRELSY V L L T G I F L I Y A I T F L M V A E P G . 646		
mGluR7	K L E W H S P W A V I P V F L A M L G I A T I F V M A T F I R 614	ASGRELSY V L L T G I F L C Y I I T F L M I A K P D . 651		
mGluR8	K L E W H S P W A V P V F V A I L G I A T T F V I V T F V R 607	ASGRELSY V L L T G I F L C Y S I T F L M I A A P D . 644		
CaSR	F L S W T E P F F G I A L T L F A V L G I F L T A F V L G V F I K 636	A T N R E L S Y L L L F S L L C C F S S L F F I G E P Q . 673		
b2adrenergic (2RH1)	D E V W V V G M G I V M S L I V L A I V F G N V L V I T A I A K 60	V T N Y F I T S L A C A D L V M G L A V P P F G A A H I L M 96		
rhodopsin (1U19)	E P W Q F S M L A A Y M F L L I M L G F P I N F L T L Y V T V Q 64	P L N Y I L L N L A V A D L F M V F G G F T T T L Y T S L H 100		
	TM3		TM4	
mGluR1	T T S C Y L O R L L V G L S S A M C Y S A L V T K T N R I A R I L A 687	I A S I L I S V Q L T L V V T L I I M E P P M P I 733		
mGluR2	T A V C T L R R L L G L T A F S V C Y S A L L T K T N R I A R I F G 682	I C L A L I S G Q L L I V V A W L V V E A P G T G 706		
mGluR3	P V I C A L R R L L G L G S S F A I C Y S A L L T K T N C I A R I F D 671	I C L G L I L V O I V M V S V W L I L E A P G T R 715		
mGluR4	L G T C S L R R I F L G L G M S I S Y A A L L T K T N R I Y R I F E 682	I T F S L I S L Q L L G I C V W F V V D P S H S V 726		
mGluR5	Q I Y C Y L Q I I G I G L S A M E S Y S A L V T K T N R I A R I L A 674	I A F I L I C I O L G I I V A L F I M E P P D I M 720		
mGluR6	A A V C A A R R L F L G L G T T S Y S A L L T K T N R I Y R I F E 680	I T F S L T S L O V V Q M I A W L G A R P P H S V 724		
mGluR7	V A V C S F R R V F L G L G M C I S Y A A L L T K T N R I Y R I F E 685	I T S S L I S V Q L L G V F I W F G V D P P N I I 729		
mGluR8	T I I C S F R R V F L G L G M C F S Y A A L L T K T N R I H R I F E 678	I T F S L I S V Q L L G V F V W F V D P P H I I 722		
CaSR	D W T C R L R O P A F G I S F V L G I S C I L V K T N R V L L V F E 707	L V F L C T F M Q I V I C V I W L Y T A P S S Y 751		
b2adrenergic (2RH1)	N F W C E F W T S I D V L C V T A S I E T L C V I A V D R Y F A I T 136	K N K A R V I I L M V W I V S G L T S F L P I Q M 171		
rhodopsin (1U19)	P T G C N L E G F F A T L G G E I A L S L V L A I E R Y V V V C 140	E N H A I M G V A F T W V M A L A C A A P L V G 174		
	TM5		TM6	
mGluR1	... G V V A P G G Y N G L L I M S C T Y Y A F K T R N V P 778	A N F N E A K Y I A F T M Y T T C I I L A F V P I F G S N . 809		
mGluR2	... S M L G S L A Y N V L L I A L C T L Y A F K T R K C P 753	E N F N E A K F I G F T M Y T T C I I W L A F L P I F Y V T S S 785		
mGluR3	... S M L I S L T Y D V I L C T V Y A F K T R K C P 762	E N F N E A K F I G F T M Y T T C I I W L A F L P I F Y V T S S 794		
mGluR4	... S L I C L L G Y S M L L M V T C T V A I K T R G V P 778	E T F N E A K P I G F T M Y T T C I I W L A F I P I F F G T S Q 810		
mGluR5	... G V V T P G G Y N G L L I L S C T F Y A F K T R N V P 765	A N F N E A K Y I A F T M Y T T C I I L A F V P I F G S N . 796		
mGluR6	... S L I G C L G Y S L L L M V T C T V A I K A R G V P 776	E T F N E A K P I G F T M Y T T C I I W L A F I P I F F G T A Q 808		
mGluR7	... Q I I C S L G Y S I L L M V T C T V A I K T R G V P 781	E N F N E A K P I G F T M Y T T C I I W L A F I P I F F G T A Q 813		
mGluR8	... S L I C S L G Y S I L L M V T C T V A I K T R G V P 774	E T F N E A K P I G F T M Y T T C I I W L A F I P I F F G T A Q 830		
CaSR	... A L G F L I G Y T C L L A A I C F F F A K S R K L P 798	E N F N E A K F I T F S M L I F F I V W I S F I P A Y A S T Y G 833		
b2adrenergic (2RH1)	Q A Y A I A S S I V S F Y V P L V I M V F Y S R V F Q E A K R 239	K E H K A L K T L G I M G T F T L C W L P F F I V N I V H V I 298		
rhodopsin (1U19)	S F V I Y M F V V H F I I P L I V I F F C Y G Q L V F T V K E A 233	A E K E V T R M V I I M V I A F L I C W L P Y A G V A F Y I F T 277		
	TM7			
mGluR1	R C F A V S L S V T V A L G C M F T P K M Y I I 838			
mGluR2	M C V S V S L S G S V L G C L F A P K L H I I 817			
mGluR3	M C I S V S L S G F V V L G C L F A P K V H I I 826			
mGluR4	L T V S V S L S A S V S L G M L Y M P K V Y I I 845			
mGluR5	M C F S V S L S A T V A L G C M F V P K V Y I I 825			
mGluR6	L T V S L S L S A S V S L G M L Y M P K T V Y I I 843			
mGluR7	L T I S M N L S A S V A L G M L Y M P K V Y I I 848			
mGluR8	L T V S M S L S A S V S L G M L Y M P K V Y I I 841			
CaSR	E V I A I L A A S F L L A C I F F N K I Y I I 860			
b2adrenergic (2RH1)	K E V Y I L L N W I G Y V N S G F N P L I Y C R 296			
rhodopsin (1U19)	P I F M T I P A F F A K T S A V Y N P V I Y I M 308			

Figure 3. Alignment of the 7TMD in the human mGluR and Calcium-sensing receptor (CaSR) sequences (aligned with CLUSTALW) relative to β_2 -adrenergic receptor (2RH1) and bovine rhodopsin (1U19) sequences (aligned with MUSTANG)

Alignment of TM regions between class C GPCRs and bovine rhodopsin were directly adopted from Malherbe et al., 2006, except TM2, 4 and 7, which were based on the alignment of CaSR with bovine rhodopsin from Miedlich et al., 2004. Highlighted are: residues functionally important for CPPHA (blue) or other PAMs (cyan), residues important for NAMs functionally (orange) and through binding (red), and residues important for both PAMs and NAMs functionally (light green) or both PAM function and NAM binding (dark green). The full alignment is provided in the supplementary material.

Figure 4. Functionally critical residues and binding determinants for mGlu allosteric modulators mapped onto the hepta-helical transmembrane domain of β_2 -adrenergic receptor X-ray crystal structure (2RH1) backbone

Shown in sticks is the backbone beta-carbon of functionally important residues & binding determinants. **A)** mGlu₁, **B)** mGlu₂, **C)** mGlu₅, **D)** residues from mGlu₁, mGlu₂ and mGlu₅ and **E)** side view of residues from mGlu₁, mGlu₂ and mGlu₅. Highlighted are: residues functionally important for CPPHA (blue) or other PAMs (cyan), residues important for NAMs functionally (orange) and through binding (red), and residues important for both PAMs and NAMs functionally (light green) or both PAM function and NAM binding (dark green). In **D** and **E**, residues important for both mGlu₁ and mGlu₅ allosteric modulation are in black.

Table 1

Therapeutic indications for metabotropic glutamate receptors in CNS disorders and examples of allosteric modulators

mGlu subtype	Therapeutic indication	Intervention	Representative Allosteric Modulators	
			Compound	Chemical Name
1	Pain	NAM	CPCCOEt	7-(Hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester
			EM-TBPC	1-ethyl-2-methyl-6-oxo-4-(1,2,4,5-tetrahydro-benzo[d]azepin-3-yl)-1,6-dihydro-pyrimidine-5-carbonitrile
2	Anxiety, schizophrenia	Agonist/PAM	BINA	Biphenyl-indanone A
			LY487379	2,2,2-Trifluoro-N-[4-(2-methoxyphenoxy)phenyl]-N-(3-pyridinylmethyl)ethanesulfonamide
4	Parkinson's disease, movement disorders	Agonist/PAM	PHCCC	N-Phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxamide
			VU0155041	cis-2-[[{(3,5-Dichlorophenyl)amino]carbonyl} cyclohexanecarboxylic acid
5	Anxiety, Fragile X Syndrome, chronic pain, depression, migraine, Parkinson's disease levodopa-induced dyskinesia	NAM	MPEP	2-Methyl-6-(phenylethynyl)pyridine
			fenobam	N-(3-chlorophenyl)-N'-(4,5-dihydro-1-methyl-4-oxo-1H-imidazole-2-yl)urea
5	Schizophrenia, cognition disorders	PAM	ADX47273	S-(4-fluoro-phenyl)-[3-[3-(4-fluoro-phenyl)-[1,2,4]oxadiazol-5-yl]-piperidin-1-yl]-methanone
			CDPPB	3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide
			CPPHA	N-{4-chloro-2-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide
7	Depression, anxiety	NAM	MMPiP	6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo [4,5-c]pyridine-4(5H)-one hydrochloride
7	Epilepsy, depression, anxiety	Agonist/PAM	AMN082	N,N'-Bis(diphenylmethyl)-1,2-ethanediamine
8	Epilepsy, anxiety, drug abuse, pain	Agonist/PAM	no selective allosteric ligands reported	

Table 2
Functionally critical residues and binding determinants of positive and negative allosteric modulation of mGluR receptors.

Positive Allosteric Modulators (PAMs)												Negative Allosteric Modulators (NAMs)			
mGluR1		mGluR2		mGluR5		mGluR1		mGluR5		mGluR1		mGluR5			
7TM position**	CPPHA	Ro 67-7476	LY487379, NRLSD-650, BINA	CPPHA	VU-29	DFB	EM-TBPC	CFMMC	LY45606, YM298198	FTICD	CPCCOEt	MPEP	fenobam		
TM1															
1.42	F599		F585												
TM3															
3.29												R648	R648		
3.36		S668				P655						P655*	P655*		
3.39		C671				S658						S658*	S658*		
3.40							Y672					Y659*	Y659*		
TM4															
4.45			S688												
4.46			G689												
4.55							I725								
EC2															
45.51							N747								
45.54						N734	N750								
TM5															
5.47		L757				L744	L757						L744*		
5.48			A733 [£]												
5.50			N735				N760			N760 [‡]					
TM6															

Positive Allosteric Modulators (PAMs)										Negative Allosteric Modulators (NAMs)						
mGluR1			mGluR2			mGluR5				mGluR1			mGluR5			
7TM position	CPPHA	Ro 67-7476	LY487379	NRLSD-650	BINA	CPPHA	VU-29	DFB	EM-TBPC	CFMMC	LY45606	YM298198	FTICD	CPCCOEt	MPEP	fenobam
6.43								T781							T781	T781*
6.47								W785	W798	W798			W798 [†]		W785*	W785*
6.51								F788	F801*	F801			F801		F788*	F788*
6.55								Y792	Y805*	Y805			Y805		Y792	Y792*
TM7																
7.32								M802	T815*	T815			T815		M802*	
7.35															S805*	
7.40								A810					A810		A810*	A810*

* Residues implicated in binding have been determined using selective allosteric radioligands

** The position of each residue in the mGluR 7TMD is given by the numbering system proposed by Ballesteros and Weinstein (1995), which allows for the comparison of equivalent positions within GPCR's. The first number represents the TM helix and the second number is its position relative to a highly conserved residue in the group A GPCRs from that TM, assigned the number 50. Residues in the extracellular loop E2 are labeled '45' to indicate their location between helix 4 and 5. Highly conserved residues (assigned to position 50) are from the bovine rhodopsin sequence: N55^{1.50}, D83^{2.50}, R135^{3.50}, W161^{4.50}, C187^{4.50}, P215^{5.50}, P267^{6.50}, P303^{7.50}.

[†] Effects LY456066 only.

[‡] BINA not tested at mutations of this residue.