Published in final edited form as:

Drug Discov Today Technol. 2013 June 1; 10(2): e269–e276. doi:10.1016/j.ddtec.2012.10.007.

Allosteric Modulation of Class C GPCRs: A Novel Approach for the Treatment of CNS Disorders

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Abstract

Allosteric modulation has emerged as an innovative pharmacological approach to selectively activate or inhibit a number of Class C GPCRs. Of the Class C GPCRs, metabotropic glutamate (mGlu) receptors represent the most promising candidates for clinical success, and both positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs) of mGluRs have demonstrated therapeutic potential for a range of psychiatric and neurological disorders such as pain, depression, anxiety, cognition, Fragile X syndrome, Parkinson's disease and schizophrenia.

Keywords

G-protein coupled receptor; positive allosteric modulator (PAM); negative allosteric modulator (NAM); metabotropic glutamate receptor

Introduction

G Protein-coupled receptors (GPCRs) represent the largest class of cell surface receptors, and small molecules targeting ~50 GPCRs constitute >50% of currently marketed drugs [1–5]. Over 300 GPCRs remain to be the targets of novel therapeutics, and, despite intense efforts, viable ligands have failed to be identified [1–7]. Classical approaches afford ligands that interact with the highly conserved orthosteric binding site (OBS), leading to poor selectivity amongst GPCR subtypes, ligands with poor physiochemical properties, and potential adverse events. Therefore, alternative, functional screening approaches have been developed to identify allosteric ligands that bind at a site distinct from the OBS, that modulate (potentiate or inhibit) the binding and/or signaling of the native orthosteric ligand [1–12]. Allosteric ligands afford unprecedented GPCR subtype selectivity, attractive chemotypes and diminished potential for adverse events. This strategy has been highly successful for Class C GPCRs, providing robust *in vivo* probes and clinical candidates for a number of CNS disorders [1–12].

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Conflict of Interest

Dr. Lindsley and Dr. Engers are actively developing a wide range of Class C allosteric ligands for numerous CNS indications with corporate partners, and receive research funding from Johnson & Johnson and Seaside Therapeutics.

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Class C GPCRs and Metabotropic Glutamate Receptors

GPCRs are commonly divided into three major classes (or families) based on sequence homology and functional roles: Class A (e.g., dopamine D₁), Class B (e.g., glucagon-like peptide, GLP-1) and Class C (e.g., metabotropic glutamate receptors, mGlu₅) [1–15]. The Class C GPCRs are distinguished by two unique structural features: 1) a large extracellular, venus fly-trap amino-terminal agonist binding site linked to the heptahelical transmembrane (7TM) domain via a cysteine-rich region and 2) the formation of constitutive homo- and heterodimers that engender diverse activation modes (Fig. 1), where the allosteric and orthosteric ligands can occupy the same receptor or a canonical trans-mode (Fig. 1B). Unlike Class A and B GPCRs, the orthosteric binding site for Class C GPCRs is not located within the 7TM domain. Class C GPCRs are comprised of several members: metabotropic glutamate receptors (mGlu receptors), γ -aminobutyric acid_B receptors (GABA_B receptors), calcium sensing receptors (CaS receptors), taste (sweet and umami) receptors (T1 receptors), fish odorant receptors and a number of orphan receptors. Of these, the mGlu receptors and the GABA_B receptors represent exciting molecular targets for the development of central nervous system (CNS) therapeutic agents, with mGlu receptors showing the greatest promise [1–15].

Glutamate, L-glutamic acid, is the major excitatory transmitter in the mammalian central nervous system (CNS), exerting its effects through both ionotropic and metabotropic glutamate receptors [1–15]. Glutamate, the orthosteric agonist, along with the other known agonists and competitive antagonists of the mGlu receptors, bind within the extracellular *N*-terminal region; in contrast, all of the positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs) described thus far bind within the 7TM domain (Fig. 1). To date, eight mGluRs have been cloned and sequenced, and have been assigned to three groups based on their structure, preferred coupling to effector mechanisms and pharmacology [1–15]. Group I receptors (mGlu₁ and mGlu₅) are predominantly located post-synaptically and couple to G_q, resulting in increases in intracellular calcium. Group II (mGlu₂ and mGlu₃) and Group III (mGlu₄, mGlu₆, mGlu₇ and mGlu₈) receptors are coupled through G_i/G_o resulting in decreases in cAMP levels. Group II and III mGlu receptors are located presynaptically and generally have inhibitory effects on neurotransmitter release [1–15].

Orthosteric and Allosteric GPCR Pharmacology

Classical orthosteric GPCR ligands modulate signaling by directly stimulating a receptor response (agonist), blocking the binding of the native agonist (competitive antagonist) or blocking constitutive activity (inverse agonist) of the GPCR, and have typically been discovered and optimized using orthosteric radioligand binding methods [1–15]. While some of these agonists and antagonists are group-selective, it has been difficult to identify subgroup-selective ligands, presumably due to evolutionary pressure to conserve the glutamate binding site across the mGlu receptors. For the mGlu receptors, a number of synthetic orthosteric agonists and competitive antagonists, analogs of glutamate **1** [1–15], have been identified and include the clinically validated mGlu_{2/3} agonist LY354740 (**2**) [16] and the mGlu₄ preferring agonist LSP3-2074-I (**3**) [17] (Fig. 1). Moreover, poor oral bioavailability, limited CNS exposure and poor physical properties for these amino acid analogs have limited their utility in the study of the therapeutic potential of individual mGlu pharmacology and often require pro-drug formulations to achieve appropriate exposure *in vivo* [1–18].

The advent of high-throughput functional assays has enabled scientists to screen for compounds capable of modulating the activity of a receptor without regard to the binding

site(s), and ideally identify modulators that bind at distinct allosteric sites [1–15]. This functional screening strategy constitutes a major shift from historical ligand discovery efforts, and has successfully identified a number of allosteric modulators (PAMs, NAMs, ago-PAMs, silent allosteric modulators (SAMs), allosteric agonists and partial antagonists) of GPCRs that can modify receptor function without necessarily affecting radioligand binding [1–15]. Key to the success of functional screening was the advent of the ‘triple add’ protocol (compound add, 2 min later EC₂₀ glutamate add, 1 min later EC₈₀ glutamate add), allowing for the identification of agonists, ago-PAMs, PAMs and NAMs in a single kinetic assay (Fig. 1) [2,19]. This works well for group I mGluRs, which natively couple to G_q to release intracellular calcium. For group II and group III mGluRs, cell lines must be generated that co-express the desired group II and III mGlu receptor with a chimeric G protein, such as G_{q15}, which links the G_{i/o}-coupled group II and III mGlu receptor to the phospholipase C/Ca²⁺ pathway, and enables the ‘triple add’ kinetic assay platform [14]. In the case of the mGlu receptors, the first small molecules that were clearly shown to exert allosteric effects were negative allosteric modulators of mGlu₁ (CPCCOEt, **4**) and mGlu₅ (MPEP, **5**) [1–15], and recently, mGlu₃ and mGlu₄ NAMs [20,21] have been identified as well (Fig. 2). Soon after, selective positive allosteric modulators, small molecules that enhance the agonist response without intrinsic agonist activity, were reported for mGlu₁ [22–24], mGlu₂ [25], mGlu₄ [26] and mGlu₅ [27]. The ability to identify sub-type selective allosteric modulators suggests that unlike the case of the agonist binding site, where there is significant evolutionary pressure to conserve residues across mGluRs, the allosteric binding sites are under less evolutionary pressure for their conservation [1–15, 23–28]. In addition to greater receptor sub-type selectivity, allosteric ligands offer numerous advantages: 1) the effects of an allosteric modulator are saturable (ie., a ‘ceiling effect’) 2) a modulator that lacks agonistic activity will only exert its effects when the endogenous agonist is present, resulting in temporal and spatial activity, also known as state dependence, of the endogenous ligand and 3) improved chemical tractability. In addition, ago-PAMs and partial antagonists provide unique modes of pharmacology, while ligand-biased signaling phenomena can be both welcomed and admonished [1–15, 28].

While many advantages over orthosteric ligands have been realized (Table 1), allosteric modulation is far from a panacea for GPCR drug discovery, and many issues have arisen as the field matures [1–15]. First, the SAR for allosteric modulators can be extremely ‘steep’ (subtle modifications to a ligand leads to complete loss of activity), and coupled with ‘molecular switch’ events (subtle structural changes that modulate modes of pharmacology or subtype selectivity), chemical optimization can be complex; however, this can also provide entry into ligands for other mGlu receptors. Thus, the ‘triple add’ screening technology is critical both for HTS campaigns and for the routine primary assay driving the optimization, along with periodic mGlu selectivity screens. Second, optimization must consider EC₅₀/IC₅₀, fold-shift and efficacy (% Glu Max) as well as standard *in vitro* and *in vivo* DMPK parameters. Third, the lack of evolutionary pressure on allosteric sites can lead to significant species differences, which can complicate IND-enabling studies. Finally, the state-dependence of allosteric modulators could be a liability in degenerative diseases, such as Alzheimer’s disease, where there is a progressive loss of endogenous orthosteric cholinergic tone [1–15].

Therapeutic potential for CNS disorders

The therapeutic potential of mGlu receptor allosteric modulation is quite broad, and dysfunction in glutamatergic systems has been implicated in a number of CNS pathologies including pain, anxiety, depression, Parkinson’s disease, cognition, addiction, Fragile X syndrome and schizophrenia [1–15,22–28]. A diverse array of allosteric ligands have been reported for mGlu₁, mGlu₂, mGlu₃, mGlu₄, and mGlu₅; however, selective allosteric ligands

are lacking for mGlu₆ and only few examples exist for mGlu₇ and mGlu₈ (Figs. 2–4) [1–15,22–31]. Here we will summarize key data and therapeutic potential for the known allosteric mGlu receptor ligands.

Group I mGlu Receptors (mGlu₁ and mGlu₅)

The mGlu₁ receptor controls the post-synaptic release of the key neurotransmitters GABA and glutamate, and also interacts with the NMDA receptor [21–24]. mGlu₁ knock-out mice display motor dysfunction and diminished learning and memory capabilities [24]. Recent genetic data indicates loss of function mutation in the GRM1 gene, which encodes mGlu₁, in patients suffering from schizophrenia and bipolar disorder [32,33], and impaired mGlu₁ signaling has also been shown in cocaine treated mice [34]. Thus, mGlu₁ PAMs may represent novel treatment approaches for both schizophrenia and addiction; however, few mGlu₁ PAMs have been described, and the known ligands represent two distinct scaffolds (Fig. 2). In contrast, a large number of mGlu₁ NAMs with tremendous structural diversity have been reported, with robust *in vivo* efficacy in preclinical models of pain, stroke, epilepsy, drug addiction, Huntington's disease, anxiety and neurodegeneration by increasing neuronal excitability and modulation of multiple ion channels (Fig. 2) [22–24,32–34].

Allosteric modulation of mGlu₅ affords opportunities for the treatment of a wide range of CNS disorders [1–15]. With respect to mGlu₅ PAMs, the major therapeutic interests are focused on schizophrenia and cognition, *via* potentiation of the *N*-methyl-D-aspartate (NMDA) receptor and the NMDA hypofunction hypothesis of schizophrenia [1–15,35]. mGlu₅ physically and functionally interacts with NMDA receptors, and multiple mGlu₅ PAMs (Fig. 2) have been shown to potentiate NMDA currents, and afford robust efficacy in preclinical models predictive of antipsychotic activity. Moreover, several series of mGlu₅ PAMs have demonstrated pro-cognitive profiles in a variety of preclinical cognition assays and have also shown potential to treat absence epilepsy [1–15,27,35]. However, no clinical data has yet to be reported. For mGlu₅ PAMs, ligands have been developed that bind at one of three distinct allosteric sites, further adding complexity, as the MPEP binding site is the only characterized allosteric site on mGlu₅. It is not yet clear if the unique conformations stabilized by ligands at the other allosteric sites offer advantages or disadvantages over modulation via the MPEP site. Regardless, pure mGlu₅ PAMs are essential, as ago-PAMs, may suffer the same adverse events as orthosteric Group I agonists [1–15,35,36].

The most advanced mGlu allosteric ligands, in terms of clinical trials in humans, are the mGlu₅ NAMs [1–15,28]. At present, nine chemically distinct mGlu₅ NAMs are in clinical testing for a wide range of indications including anxiety, Fragile X syndrome, migraine, chronic neuropathic pain, treatment resistant depression, L-DOPA-induced dyskinesia (PD-LID) and gastroesophageal reflux disease (GERD); moreover, positive results have been reported [2]. Preclinically, a wide range of mGlu₅ NAM chemotypes have demonstrated efficacy in rodent models of the same CNS disorders listed above [1–15]. Interestingly, mGlu₅ partial antagonists, allosteric ligands that fully occupy the allosteric site but only partially block signaling, have been discovered, and represent a new mode of pharmacology that may limit potential adverse events associated with ablation of receptor signaling with NAMs (also referred to as non-competitive antagonists) [37]. Only recently have non-MPEP (non-acetylene containing) mGlu₅ NAM chemotypes been reported, which display improved ancillary pharmacology and DMPK profiles [1–15].

Group II mGlu Receptors (mGlu₂ and mGlu₃)

Group II mGlu receptors, mGlu₂ and mGlu₃, inhibit further release of glutamate once levels accumulate in the perisynaptic extracellular region, and thus modulate glutamatergic neurotransmission [1–15,25]. Recent preclinical rodent and human clinical data provide

strong evidence that the mGlu_{2/3} agonist (**3**) is effective in the treatment of the positive symptoms of schizophrenia. Data from knock-out mice, coupled with the fact that the efficacy of **3** has been recapitulated preclinically with mGlu₂ PAMs, has resulted in an enormous efforts by multiple pharmaceutical companies to develop mGlu₂ PAMs (Fig. 3). Beyond schizophrenia, mGlu₂ PAMs have also displayed efficacy in anxiety assays, drug dependence (cocaine) and sleep-wake architecture (normalizing REM states characteristic of depression) [1–15,25]. As of this writing, no selective mGlu₃ PAMs have been reported; therefore the therapeutic potential of selective mGlu₃ activation remains unclear.

Until recently, the only known Group II NAMs were dual mGlu_{2/3} NAMs, representing only two chemotypes (Fig. 3). In 2012, a selective mGlu₃ NAM (**24**) was developed starting from a potent mGlu₅ PAM, highlighting the value of ‘molecular switches’ [20,38]. Therapeutically, Group II NAMs have shown efficacy in reversing hyperlocomotion and in improving memory; however, it is not yet clear if this is mGlu₂ or mGlu₃ driven, and additional small molecule tools are required to dissect the contribution of the two receptors to the observed efficacy [1–15,25].

Group III mGlu Receptors (mGlu₄, mGlu₆, mGlu₇ and mGlu₈)

Of the Group III mGlu receptors, mGlu₄ is the most advanced, with a diverse array of PAMs and ago-PAMs (Fig. 4) affording robust efficacy in multiple preclinical models of Parkinson’s disease (PD), both for symptomatic motor disturbances and for neuroprotection of dopaminergic neurons [26]. Recent studies have demonstrated that mGlu₄ PAMs also synergize with A_{2A} antagonists (in haloperidol-induced catalepsy) and with L-DOPA (in 6-OHDA rats), offering additional co-treatment options for PD patients and reducing the necessary dosage of L-DOPA [39]. In parallel, new roles for mGlu₄ PAMs in the treatment of pain, anxiety and mood disorders are being developed. Thus far, only one report of an mGlu₄ NAM has been disclosed [21], but the therapeutic potential of inhibiting mGlu₄ is not clear.

Of the remaining Group III mGlu receptors, mGlu₆ is restricted to the retina, and therefore not relevant to CNS disorders; moreover, no selective ligands for mGlu₆ have been disclosed [1–15]. Selective activation of mGlu₇ (Fig. 4), with the lone mGlu₇ allosteric agonist AMN082 (**32**) has been shown to reduce the rewarding effects of cocaine and ethanol and facilitate the extinction of conditioned fear; thus, mGlu₇ has potential in addiction, anxiety and post-traumatic stress disorder (PTSD) [29]. In addition, two selective mGlu₇ antagonists/NAMs, **33** and **34**, has also been described [2,30]. Similarly, there is only one selective mGlu₈ PAM, AZ12216052 (**35**), reported thus far, and data from animal models indicate activation of mGlu₈ may have anxiolytic effects (Fig. 4) [31]. Additional data suggests that mGlu₇ and mGlu₈, either alone or in combination, may have efficacy in neurodegeneration, mood disorders, pain, addiction and epilepsy [29–31].

Conclusion

The advent of kinetic, functional assay paradigms has led to a revolution in drug discovery aimed at Class C GPCRs, providing new opportunities to modulate receptor function through novel, allosteric mechanisms. These novel modes of pharmacology lead to small molecules that can function as PAMs, ago-PAMs, NAMs, silent allosteric modulators, allosteric agonists and partial allosteric antagonists, highlight the unprecedented potential for fine tuning cellular signaling processes in either a positive or negative manner. Emerging from this new paradigm is CinacalcetTM, a PAM of the CaS receptor, and the first class C GPCR to become a marketed therapeutic [40]. A number of mGluR allosteric modulators are now in clinical development for a range of CNS disorders, and many are showing robust efficacy in a number of CNS pathologies. However, despite the positive attributes of

allosteric modulation, it is not a panacea, and lead optimization of mGlu receptor allosteric clinical candidates is burdened with many caveats and challenges. Application of technology, both within pharmacology and medicinal chemistry are critical for the successful optimization of class C allosteric ligands. Functional ‘triple add’ assays allow allosteric ligands to be identified as well as capturing mode switching, ie., PAM to NAM. In contrast, classical radioligand binding assays, employing orthosteric radioligands, will only provide traditional orthosteric ligands, and require additional functional assays to determine if potent binders have any functional activity. Allosteric ligands are best optimized through iterative parallel synthesis, as opposed to classical single compound synthesis, due to the ‘steep’ SAR and propensity for ‘molecular switches’ to modulate modes of pharmacology and subtype selectivity. Yet, it remains to be definitively proven if class C allosteric ligands will prove more successful in the clinic than the second generation of orthosteric ligands; however, subtype selectivity, temporal control and improved physiochemical properties bode well for allosteric ligands to be a driving force in class C GPCR drug discovery for decades to come.

Glossary

Orthosteric Site	The binding site of the receptor’s endogenous agonist
Allosteric Site	A ligand binding site topographically distinct from the orthosteric binding site
Positive Allosteric Modulator (PAM)	A ligand that can increase the action of an orthosteric ligand by binding at an allosteric site. The PAM may enhance the affinity and/or efficacy of the orthosteric ligand without any intrinsic activity. The PAM may also enhance coupling to the G-Protein
Negative Allosteric Modulator (NAM)	A ligand that can decrease the action of an orthosteric ligand by binding at an allosteric site. The NAM may also inhibit coupling to the G-protein. Also known as a non-competitive antagonist
Silent Allosteric Modulator (SAM)	A ligand that fully occupies an allosteric site, but exerts no pharmacological function on its own, but can block the allosteric activity of both PAMs and NAMs. Also referred to as neutral or pharmacologically silent ligands
Partial Antagonist (PA)	A NAM that fully occupies the allosteric binding site, but induces only a partial blockade of receptor signaling
Allosteric Agonist (AA)	A ligand capable of receptor activation alone, in the absence of the orthosteric ligand, by binding at an allosteric site and engendering an active conformation of the receptor
Ago-Potentiator (ago-PAM)	An allosteric ligand that functions as both a PAM and an allosteric agonist; however, the agonism is usually observed at concentrations higher than that for the PAM activity

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Figure 1.

A) A schematic representation of a Class C GPCR highlighting the large, extracellular bi-lobed ('Venus fly-trap') *N*-terminal agonist binding domain connected to the *N*-terminal domain via a cysteine-rich region to the 7TM domain. B) mGlu receptors function as homodimers. Unlike Class A and B GPCRs, the orthosteric binding site is not located within the 7TM; in contrast, glutamate, the orthosteric ligand, binds in the *N*-terminal extracellular bi-lobed domain. Thus far, the 7TM domain contains the binding sites for all known positive, negative and silent allosteric modulators of the mGlu receptors. C) Representative endogenous (glutamate, 1) and synthetic orthosteric agonists 2 and 3, along with the first mGlu allosteric ligands 4 and 5. D) The 'triple add screening' paradigm in a kinetic calcium fluorescence assay as a surrogate for GPCR activation. Compound is added (1st add), followed 2 minutes later by an EC₂₀ of glutamate (2nd add) and then 1 minute later by an EC₈₀ of glutamate (3rd add). E) An agonist will elicit fluorescence upon compound addition. A PAM will have no effect on receptor activation alone, but will potentiate the EC₂₀. A NAM will block both the EC₂₀ and EC₈₀.

Figure 2.
Representative Group I mGlu allosteric modulator ligands. mGlu₁ NAMs **6** and **7**, mGlu₁ PAMs **8** and **9**, mGlu₅ NAMs **10–13** and mGlu₅ PAMs **14–18**.

mGlu₂ PAM **19**, J&J/Addex **20**, Merck **21**, Eli Lilly **22**, CBiPESmGlu_{2/3} NAM **23**, RO4491533 **24**, Hoffmann-La RochemGlu₃ NAM **25**, VU0463597
(ML289)**Figure 3.**

Representative Group II mGlu allosteric modulator ligands. mGlu₂ PAMs **19–22**, mGlu_{2/3} NAMs **23** and **24** and an mGlu₃NAM **25**.

Figure 4. Representative Group III mGlu allosteric modulator ligands. mGlu₄ PAMs **26–30**, mGlu₄ NAM **31**, mGlu₇ allosteric agonist **32**, mGlu₇ NAMs **33** and **34** and mGlu₈ PAM, **35**.

Table 1

Technology Summary Table.

Name of technology	Technology 1	Technology 2	Technology 3	Technology 4
	Radioligand binding assay	Kinetic Functional Assay	Orthosteric ligands	Allosteric Ligands
Pros	Established approach Know binding site of ligand, typically orthosteric	High-throughput, identifies allosteric ligands 'Triple Add' Identifies PAMs, NAMs, ago-PAMs, SAMs, allosteric agonists in single assay	Radioligands typically available for occupancy studies High conservation afford little or no species differences	Excellent subtype selectivity amongst Class C GPCR families Ligands with excellent physiochemical properties and CNS penetration
Cons	Only orthosteric agonists and antagonists Radioactive ligands Only provides binding data – no function	Requires expensive kinetic plate readers and robotic addition arms. No information on site of binding	Poor subtype selectivity amongst Class C GPCR families Ligands with poor physiochemical properties and poor CNS penetration	Often no radioligand or PET tracers Unpredictable, shallow SAR 'Molecular Switches' Do not know if ligands bind to same or different allosteric sites Ligand biased signaling Potential species differences
references	[1–15]	[1–15]	[1–15]	[1–15]