Biased agonism and allosteric modulation of metabotropic glutamate receptor 5

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Metabotropic glutamate receptors belong to class C G-protein-coupled receptors and consist of eight subtypes that are ubiquitously expressed throughout the central nervous system. In recent years, the metabotropic glutamate receptor subtype 5 (mGlu5) has emerged as a promising target for a broad range of psychiatric and neurological disorders. Drug discovery programs targeting mGlu5 are primarily focused on development of allosteric modulators that interact with sites distinct from the endogenous agonist glutamate. Significant efforts have seen mGlu5 allosteric modulators progress into clinical trials; however, recent failures due to lack of efficacy or adverse effects indicate a need for a better understanding of the functional consequences of mGlu5 allosteric modulation. Biased agonism is an interrelated phenomenon to allosterism, describing how different ligands acting through the same receptor can differentially influence signaling to distinct transducers and pathways. Emerging evidence demonstrates that allosteric modulators can induce biased pharmacology at the level of intrinsic agonism as well as through differential modulation of orthosteric agonist-signaling pathways. Here, we present key considerations in the discovery and development of mGlu5 allosteric modulators and the opportunities and pitfalls offered by biased agonism and modulation.

Introduction

Glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system (CNS) and elicits its activity via the ionotropic and metabotropic glutamate (mGlu) receptors. The ionotropic glutamate receptors are ligand-gated ion channels, while the mGlu receptors belong to the class C G-protein-coupled receptors (GPCRs) – a group that also includes calcium sensing receptor (CaSR), γ-aminobutyric acid receptor B (GABAB) and numerous taste, pheromone, and orphan receptors. Class C GPCRs share the characteristic 7 transmembrane (7TM) α-helical bundle architecture separated by alternating intracellular and extracellular loops. The 7TM domain links an intracellular carboxy-terminal domain and a large bilobed extracellular amino-terminus, termed the Venus flytrap (VFT) domain [1-3], where endogenous ligands bind (Figure 1A). Furthermore, the mGlu receptors are also known for their obligatory constitutive dimerization [4] mediated via a disulfide bond connecting the top of the VFT domains [5,6]. Evidence suggests that mGlu receptors can form homo- or heterodimers with other subtypes [7-12] and unrelated GPCRs [13-17]. Glutamate and other orthosteric agonists typically bind in a cleft between two lobes that comprise the VFT domain to induce active ‘closed–closed’ (both clefts of the VFT dimer closed) or ‘open–closed’ (one cleft open, one cleft closed) conformations [18]. Conformational rearrangements within the VFT domains are transmitted to the 7TM domain via a cysteine-rich region, to modulate G-protein coupling, subsequently triggering intracellular signaling [19]. Based on sequence homology, preferred signal transduction pathways and pharmacological profiles, the mGlu receptors are subclassified into group I (mGlu1 and mGlu5 (metabotropic glutamate receptor subtype 5)), group II (mGlu2 and mGlu3), and group III (mGlu4, mGlu6, mGlu7, and mGlu8) [20]. Group I mGlu...
Figure 1. Schematic representation of orthosteric agonism and allosteric modulation.

(A) Glutamate binds to the cleft between two lobes of VFT domain, whereas allosteric modulators (e.g. PAM, NAM, and NAL) bind to the common allosteric site in the 7TM domain. Simultaneous binding of an orthosteric and allosteric ligand at a receptor can have two dominant effects; affinity modulation (orange arrow) and/or efficacy modulation (green arrow). (B) Quantification of allosteric effects with operational model of allosterism [97]. In the equation, \( K_A \) and \( K_B \) denote the equilibrium dissociation constant of the orthosteric ligand A and allosteric ligand B, respectively. The parameter \( \alpha \) governs allosteric modulation of binding affinity. Allosteric modulation of orthosteric ligand efficacy is incorporated by the empirical parameter, \( \beta \) which scales from zero to infinity. The parameters \( \tau_A \) and \( \tau_B \) reflect the ability of the orthosteric and allosteric ligands, respectively, to promote receptor activation and incorporate the intrinsic efficacy of each ligand, the receptor density, and the efficiency of stimulus-response coupling. \( E_M \) denotes the maximal system response and \( n \) is the slope factor of the transducer function that links receptor occupancy to the final observed response.

Abbreviations: NAL, neutral allosteric ligand; NAM, negative allosteric modulator; PAM, positive allosteric modulators.

members are primarily located postsynaptically and preferentially couple to G\( \alpha_{q/11} \), resulting in activation of phospholipase C, production of inositol 1,4,5-trisphosphate and diacylglycerol, and subsequent mobilization of intracellular calcium (iCa\(^{2+}\)). The group II and group III receptors, with the exception of mGlu6, which is exclusively localized to retina, are typically found presynaptically and functionally linked to G\( \alpha_{i/o} \) and the inhibition of adenylyl cyclase activity [20].

Despite being members of the same group, mGlu1 and mGlu5 receptors have distinct functional roles within the brain [21], which have been linked to their iCa\(^{2+}\) mobilization profiles. For instance, mGlu5 activation stimulates iCa\(^{2+}\) oscillations, whereas mGlu1 primarily elicits sustained and non-oscillatory Ca\(^{2+}\) responses [22,23]. The iCa\(^{2+}\) oscillations induced by mGlu5 occur via a dynamic mechanism involving phosphorylation and dephosphorylation of the receptor at Ser839 [24,25]. In addition to coupling to G\( \alpha_{q/11} \), a recent study indicated that purified mGlu5 activates G\( \alpha_q \) upon glutamate stimulation to increase intracellular cyclic adenosine monophosphate (cAMP) [26]. Further, mGlu5 activates additional non-G\( \alpha_{q/11} \)-dependent signaling cascades and regulates gene expression via its expression on intracellular membranes of the endoplasmic reticulum and nucleus [27,28]. Importantly, the distinct signaling profiles of intracellular and cell surface mGlu5 result in different physiological effects [29,30]. For example, activation of cell surface mGlu5 leads to long-term potentiation and long-term depression, whereas intracellular mGlu5 mediates only long-term depression [31]. mGlu5 is expressed ubiquitously throughout the postsynaptic densities of the cerebral cortex, corpus striatum, olfactory bulb, hippocampus, caudate nucleus, and nucleus accumbens. Further, mGlu5 is also expressed in non-neuronal cells throughout the brain, including astrocytes and microglia [32-36]. mGlu5 mediates diverse effects including synaptic transmission and plasticity, and proliferative, growth and survival responses of neurons and glial cells in the CNS [20,37-47] and periphery [48]. Thus, mGlu5 has emerged as an attractive drug target for a
Overview of allosteric modulation

Allosterism arises as a consequence of the conformational flexibility of GPCRs. The classic model of allosterism, the Monod–Wyman–Changeux model, posits that allosteric proteins exist in a dynamic equilibrium between multiple conformational states, even in the absence of ligand. Further, allosteric proteins possess multiple binding sites, and binding of a ligand (or other entity) to any of these sites favors a subset of possible conformational states [84]. In doing so, allosteric modulators engender GPCR conformational states that are less stable in the presence of an orthosteric ligand alone, influencing the interactions with transducers. Allosteric modulators are classified based on the magnitude and direction of their effect on orthosteric ligand affinity and/or efficacy, a property referred to as cooperativity. Positive allosteric modulators (PAMs) enhance the binding and/or activity of orthosteric ligands, whereas negative allosteric modulators (NAMs) inhibit the binding or response to orthosteric agonist. Last, neutral allosteric ligands (NALs) bind to the receptor, but do not alter the binding and/or function of the orthosteric ligand [85,86].

A continuing challenge for allosteric modulator-based drug discovery is the ability to detect, validate, and quantify the pharmacology of allosteric modulators. All current methods (e.g. radioligand binding and functional assays) that assess allosteric interactions require the availability of a probe to provide a relevant readout of receptor-based activity. When screening for allosteric interactions, it is imperative to consider both methods to avoid missing any behaviors of allosteric ligands [87,88]. Binding assays have the advantage of direct validation of an allosteric mode of action and reveal the site of interaction of allosteric ligands [89,90]; however, they do not assess efficacy modulation. For functional assays, the most relevant probe is the endogenous agonist; however, it is not always feasible to use the endogenous agonist. Thus, surrogate orthosteric probes are often required. Functional assays can readily detect a wider spectrum of allosteric behaviors and allow for detection of modulation of both affinity and efficacy. Further, when designing experiments to study allosterism, it is worth considering probe dependence and saturation of effect, two basic allosteric modulator properties. Probe dependence describes the phenomenon where the degree and direction of cooperativity is determined by the chemical nature of the orthosteric ligand (probe). Cooperativity might...
be apparent with one probe, but the observed interaction may be negligible or in an opposing direction when using another [91-93]. A second characteristic is the saturability of effect; that is, the influence of allosteric modulators is limited by the cooperativity between orthosteric and allosteric sites. The degree of cooperativity introduces a ceiling on the magnitude of the allosteric effect, and as such, no further modulation will be observed when saturating concentrations of allosteric modulators are present. Therefore, allosteric modulators may have reduced risk in the event of drug overdose [89,94]. Allosteric interactions can be quantified by numerous models, namely the allosteric ternary complex model (ATCM) [95], an allosteric ‘two-state’ model (ATSM) [96], or the operational model of allosterism [89,97]. Of these, the ATSM and operational model of allosterism offer the ability to quantify both affinity and efficacy modulation. However, the ATSM contains many parameters that cannot be readily fitted to experimental data; therefore, it is more feasible and practical to use the operational model of allosterism (Figure 1B), which combines the ATCM with the classic Black-Leff operational model of agonism [11]. The major advantage of this model is the capability of quantifying modulator efficacy as well as delineating modulator effects on orthosteric agonist affinity compared with efficacy [11,97-99], providing valuable information to guide lead-discovery programs.

### mGlu₅ allosteric pharmacology

Allosteric modulators of mGlu₅ are attractive putative therapeutics for a number of reasons. As previously mentioned, allosteric modulators may achieve subtype selectivity due to sequence divergence between allosteric pockets across receptor subtypes, which is important given that there are eight mGlu subtypes. Further, in the absence of intrinsic efficacy, allosteric modulators have the potential to maintain the spatial and temporal profile of endogenous neurotransmitters. Pure allosteric modulators only elicit an effect when and where the endogenous agonist is present, thereby tuning up, or down, the existing responses in proportion to normal physiological tone. In contrast, orthosteric ligands continuously stimulate GPCR activity, or block the action of the endogenous agonist, for the duration of their presence. This profile may be particularly detrimental in the CNS where receptor signaling must be precisely controlled. It is worth noting that some allosteric modulators may possess intrinsic efficacy to either activate or inhibit GPCR-mediated signaling in the absence of orthosteric ligands and are referred to as agonist-PAMs (ago-PAMs) or inverse agonist-NAMs. Last, allosteric modulators can have differential cooperativity with respect to orthosteric agonist affinity compared with efficacy. For instance, the mGlu₅ NAM, 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester (CPCCOEt), completely inhibits glutamate efficacy in chinese hamster ovary (CHO) cells but does not impact glutamate binding, suggesting that CPCCOEt has neutral cooperativity with respect to glutamate affinity and negative cooperativity at the level of efficacy [100].

Discovery of mGlu₅ allosteric ligands has been particularly successful with multiple diverse chemotypes disclosed covering the full spectrum of pharmacology including, pure PAMs, ago-PAMs, NALs, partial and full NAMs, largely classified based on glutamate-mediated iCa²⁺ mobilization assays (see Table 1, Figure 2). The first mGlu₅ allosteric modulators discovered were NAMs, with subsequent intense discovery programs by both academic and industrial researchers yielding diverse chemotypes [20,101,102]. Inhibition of mGlu₅ has been confirmed as a potential mechanism for treatment of depression, anxiety, Parkinson’s disease levodopa-induced dyskinesias (PD-LID), FXS and Alzheimer’s disease, with mGlu₅ NAMs showing efficacy in preclinical models [44,55,57,103-108]. Despite significant efforts in preclinical settings, several mGlu₅ NAMs were reported to have detrimental effects, such as cognitive impairments and psychotomimetic effects in rodents [53,109-112]. Moreover, enthusiasm for mGlu₅ NAMs has waned given recent failures in clinical trials for depression and FXS, although ongoing studies are being pursued for PD-LID and dystonia [59,106]. Recent studies have shown that the partial mGlu₅ NAMs (where cooperativity between the allosteric ligand and glutamate is limited), 2-(2-(3-methoxyphenyl)ethynyl)-5-methylpyridine (M-5MPEP) and 2-(2-(5-bromopyridin-3-yl)ethynyl)-5-methylpyridine, reduce cocaine-mediated addiction behaviors and have comparable anxiolytic and antidepressant activity to the full NAM, 3-(2-Methyl-4-thiazolyl)ethynyl)pyridine (MTEP) [112]. Importantly, the partial NAMs did not exhibit psychotomimetic adverse effects. N,N-diethyl-5-((3-fluorophenyl)ethynyl)picolinamide (VU0477573), another partial mGlu₅ NAM, displays robust efficacy in the anxiolytic behavioral model as comparable with MTEP [113]. Therefore, partial NAM activity may provide one means to achieve a desirable therapeutic window for mGlu₅ NAMs via fine-tuning rather than complete blockade of mGlu₅ activity.

Enhancement or activation of mGlu₅ is a promising strategy to treat psychosis, cognitive disorders and drug addiction, with mGlu₅ PAMs demonstrating efficacy in preclinical models [110,114-118]. However, there is growing evidence for CNS-related adverse effect liability for certain mGlu₅ PAMs and ago-PAMs, such as the seizure liability caused by N-cyclobutyl-5-((3-fluorophenyl)ethynyl)picolinamide (VU0403602) [119].
Table 1 Allosteric pharmacology and bias evidence for selected mGlu5 allosteric ligands

<table>
<thead>
<tr>
<th>Ligand name</th>
<th>Pharmacology</th>
<th>Biological/clinical significance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PAMs and PAM-agonists (ago-PAMs)</strong></td>
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<tr>
<td>SPAM523</td>
<td>PAM: glu efficacy for iCa²⁺ (CHO-mGlu5 &amp; rat cortical astrocytes); [³H]quisqualate binding (CHO-mGlu5); DHPP efficacy for NMDA-evoked currents (rat hippocampus)</td>
<td>Antipsychotic-like activity with adverse effects (seizures, neurotoxicity) in rodents</td>
<td>[120,121]</td>
</tr>
<tr>
<td>ADX47273</td>
<td>PAM: glu, quisqualate efficacy for iCa²⁺ and IP₁ (HEK-mGlu5 &amp; rat cortical astrocytes); [³H]quisqualate binding (CHO-mGlu5); DHPP efficacy for NMDA-evoked currents (rat hippocampal CA1 pyramidal cells)</td>
<td>Antipsychotic-like and procognitive effects</td>
<td>[115,121,139]</td>
</tr>
<tr>
<td>CDPPB</td>
<td>PAM: glu, quisqualate efficacy for iCa²⁺ and IP₁ (CHO-mGlu5 &amp; rat astrocytes); Ago-PAM: glu efficacy for iCa²⁺ (CHO-mGlu5); pERK1/2 (BACHD mice); p-Akt (mouse striatal neurons)</td>
<td>Antipsychotic-like and procognitive, HD, no reported adverse effects in rodents</td>
<td>[91,99,114,120,139-142]</td>
</tr>
<tr>
<td>CPPHA</td>
<td>PAM: glu, quisqualate, DHPP efficacy for iCa²⁺ and IP₁ (CHO-mGlu5 and rat astrocytes) Ago-PAM: glu efficacy for pERK1/2 (HEK-mGlu5)</td>
<td>Bias: agonism (relative to DHPG): IP₁ &gt; pERK1/2 and iCa²⁺ (HEK-mGlu5 &amp; mouse cortical neurons)</td>
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<td></td>
<td></td>
<td></td>
<td>[99,137,139,143,144]</td>
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<tr>
<td>DPFE</td>
<td>PAM: glu, DHPP efficacy for iCa²⁺, IP₁, pERK1/2 (HEK-mGlu5 and mouse cortical neurons)</td>
<td>Bias: agonism (relative to DHPG): IP₁ &gt;&gt; iCa²⁺, biphasic pERK1/2 (mouse cortical neurons)</td>
<td>Antipsychotic-like effects, no reported adverse effects in rodents</td>
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<tr>
<td>VU0360172</td>
<td>PAM: glu, DHPP efficacy for iCa²⁺ (HEK-mGlu5); Ago-PAM: glu, DHPP efficacy for IP₁, pERK1/2 (HEK-mGlu5 &amp; mouse cortical neurons)</td>
<td>Bias: agonism (relative to DHPG): IP₁ &gt;&gt; iCa²⁺ and pERK1/2 (mouse cortical neurons)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Antipsychotic-like effects, no known adverse effects in rodents</td>
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<tr>
<td>VU0409551</td>
<td>PAM: glu efficacy for iCa²⁺ and pERK1/2 (HEK-mGlu5 and cortical astrocytes), DHPP-LTD (hippocampal slices)</td>
<td>Bias: agonism (relative to DHPG): IP₁ &gt;&gt; iCa²⁺ (HEK-mGlu5 and mouse cortical neurons)</td>
<td>Antipsychotic-like, HD, no reported adverse effects in mice</td>
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<tr>
<td>VU0424465</td>
<td>Ago-PAM: glu, DHPP efficacy for iCa²⁺, IP₁, and cAMP accumulation, pERK1/2 (HEK-mGlu5, rat cortical astrocytes, neurons), DHPP efficacy for hippocampal LTD</td>
<td>Bias: agonism (relative to DHPG): IP₁ ≫ pERK1/2 &gt;&gt; iCa²⁺ (HEK- mGlu5 and mouse cortical neurons)</td>
<td>Seizurogenic and neurotoxic in rats</td>
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<tr>
<td><strong>NAMs and partial NAMs</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Basimglurant/RG7090</td>
<td>NAM: quisqualate efficacy for iCa²⁺ and IP₁ (HEK-mGlu5); inverse agonism: IP₁</td>
<td>Depression, FXS</td>
<td>[55-58]</td>
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<td>CTEP</td>
<td>NAM: quisqualate efficacy for iCa²⁺ and IP₁ (HEK-mGlu5); inverse agonism: IP₁</td>
<td>Alzheimer’s disease, FXS</td>
<td>[108,147,148]</td>
</tr>
<tr>
<td>Dipraglurant/ADX48621</td>
<td>NAM: glu efficacy for iCa²⁺ (HEK-mGlu5)</td>
<td>PD-LID</td>
<td>[67,149]</td>
</tr>
<tr>
<td>Fenobam</td>
<td>NAM: quisqualate efficacy for iCa²⁺ and IP₁ (HEK-mGlu5); inverse agonism: IP₁</td>
<td>FXS</td>
<td>[53,54]</td>
</tr>
<tr>
<td>GET73</td>
<td>NAM: quisqualate, glu, CHPP efficacy for iCa²⁺, IP₁, pCREB (rat cortical astrocytes and neurons, hippocampal slices)</td>
<td>Alcohol dependence, anxiety</td>
<td>[150,151]</td>
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<td>Mavoglurant/AFQ056</td>
<td>NAM: glu efficacy for iCa²⁺ (Ltk⁻-mGlu5) and IP₁ (CHO-mGlu5)</td>
<td>FXS, PD-LID, GERD, HD</td>
<td>[59-63,104,152]</td>
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<tr>
<td>MTEP</td>
<td>NAM: quisqualate, glu efficacy for iCa²⁺ (Ltk⁻-, HEK-mGlu5); inverse agonism: IP₁</td>
<td>Alzheimer’s disease, addiction, anxiety, depression, epilepsy, FXS, GERD, pain, HD, PD-LID, psychotomimetic, and cognitive adverse effects</td>
<td>[113,153,154]</td>
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**Continued over**
Table 1 Allosteric pharmacology and bias evidence for selected mGlu5 allosteric ligands (Continued)

<table>
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<th>Biological/clinical significance</th>
<th>References</th>
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<tbody>
<tr>
<td>MPEP</td>
<td>NAM: quisqualate and DHPG efficacy for IP1; gluu efficacy for iCa2+ and pERK1/2 (HEK-mGlu5); gluu, DHPG and quisqualate induced iCa2+ oscillations and IP1; (rat astrocytes); inverse agonism; iPr1</td>
<td>Addiction, anxiety, depression, no psychotomimetic effects</td>
<td>[99,112,139,159]</td>
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<tr>
<td>M-5MPEP</td>
<td>NAM: DHPG, and quisqualate mediated iCa2+ oscillations (rat cortical astrocytes); gluu efficacy for pERK1/2 (HEK-mGlu5) Partial NAM: gluu efficacy for iCa2+; quisqualate and DHPG efficacy for IP1; (HEK-mGlu5, rat cortical astrocytes and cortex membranes)</td>
<td>Anxiety disorders</td>
<td>[113]</td>
</tr>
<tr>
<td>VU0477573</td>
<td>NAM: gluu efficacy for pERK1/2 (HEK-mGlu5) Partial NAM: gluu efficacy for iCa2+ and IP1 (HEK-mGlu5 and cortical neurons)</td>
<td>Pharmacological tool compound</td>
<td>[139,144,159,161]</td>
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<td>NALs</td>
<td>MPEP</td>
<td>NAM: gluu, DHPG efficacy for iCa2+; IP1 (rat cortical astrocytes)</td>
<td>Alzheimer's disease</td>
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<td>SMPEP</td>
<td>NAM: gluu, DHPG efficacy for iCa2+; IP1 (rat cortical astrocytes)</td>
<td>Pharmacological tool compound</td>
<td>[139,144,159,161]</td>
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<tr>
<td>BMS-984923</td>
<td>NAM: gluu, DHPG efficacy for iCa2+ (HEK-mGlu5 and rat cortical neurons)</td>
<td>Alzheimer's disease</td>
<td>[162,163]</td>
</tr>
</tbody>
</table>

Underlined text in the ‘biological/clinical significance’ column denotes human clinical evidence associated with these allosteric ligands. The following abbreviations are used in the table:

5PAM523, 5-fluoro-2-(1H-oxadiazol-5-yl)pyridine;
CPPHA, N-[4-Chloro-2-((1,3-dihydro-1,3-dioxo-2H-isindol-2-yl)methyl)phenyl]-2-hydroxybenzamide;
pCREB, phosphorylation of cAMP response element binding protein;
CTEP, 2-Chloro-4-[(2,5-dimethyl-1-[4-(trifluoromethoxy)phenyl]-1H-imidazol-4-yl)ethyl]pyridine;
DPFE, 1-(4-(2,4-difluorophenyl)piperazin-1-yl)-2-((4-fluorobenzyl)oxy)ethenone;
HD, Huntington’s disease;
IP1, inositol monophosphate;
L(tk-), mouse fibroblast;
NMDA, N-methyl-D-aspartate;
VU0409551, ((4-fluorophenyl)(2-(phenoxymethyl)-6,7-dihydroxyazolo[5,4-c]pyridin-5(4H)-yl)methanone);
VU0360172, N-cyclobutyl-6-((3-fluorophenyl)ethynyl)picolinamide;
VU0424465, (R)-5-((3-fluorophenyl)ethynyl)-N-(3-hydroxy-3-methylbutan-2-yl)picolinamide.

and (R)-5-((3-fluorophenyl)ethynyl)-N-(3-hydroxy-3-methylbutan-2-yl)picolinamide (VU0424465) [119] and forebrain neurotoxicity induced by 5-fluoro-2-(1H-oxadiazol-5-yl)pyridine (5PAM523) [120]. The observed side effects have been attributed to excessive mGlu5 activation by PAMs with intrinsic efficacy, such that it may be prudent to optimize mGlu5 PAMs devoid of intrinsic agonist activity. However, some ‘pure’ mGlu5 PAMs can still cause seizures and cytotoxicity after chronic administration [120], highlighting that intrinsic agonism may not be solely responsible for ontarget adverse effects. Indeed, the recent discovery of ((4-fluorophenyl)(2-(phenoxymethyl)-6,7-dihydroxyazolo[5,4-c]pyridin-5(4H)-yl)methanone) (VU0409551) challenges this idea and suggests that not all mGlu5 ago-PAMs are equal. VU0409551 has ago-PAM activity with glutamate in extracellular signal-regulated kinases 1 and 2 (ERK1/2) phosphorylation assays in recombinant cells but is a pure PAM in iCa2+ mobilization assays [91,121]. Further, VU0409551 is distinguished from other mGlu5 PAM chemotypes in slice electrophysiology preparations from rat hippocampus where it has no efficacy for enhancement of N-methyl-D-aspartate (NMDA) receptor dependent long-term potentiation [121]. Collectively, these data demonstrate that reliance on iCa2+ mobilization assays to classify mGlu5 PAMs has likely resulted in an underappreciation of the full scope of mGlu5 PAM activity and the optimal pharmacological profile to achieve therapeutic benefit.

Emerging concept: mGlu5 allosteric modulators engender biased signaling

While discovery of mGlu5 allosteric modulators has been successful, these drugs have failed to progress to market. One of the major underlying issues is a reliance on limited in vitro characterization and therefore a failure to capture the complete pharmacological profile of a particular compound. Over recent decades, it has become increasingly appreciated that different ligands acting at the same receptor preferentially stabilize different receptor conformations, such that each ligand may engage a subset of intracellular transducers, to the relative exclusion of others (Figure 3) [122,123]. Now referred to as biased agonism, this phenomenon has also been known as functional selectivity [124],
Figure 2. Representative chemotypes for mGlu5 allosteric modulators

Figure 3. Schematic depicting the concept of biased agonism and allosteric modulation

The binding of endogenous ligand (gray square) activates a receptor and results in intracellular signal transduction. The surrogate orthosteric agonist (green circle) may favor stimulation of one response over another when compared with the endogenous ligand. The binding of an allosteric modulator (blue) may modulate functional responses to an agonist equally across all signaling pathways. However, the interaction of another modulator (orange) may differentially modulate the response to the same agonist in a pathway dependent fashion. In this scenario, response 1 is potentiated whereas response 2 is inhibited.
Biased agonism in mGlu receptors

Biased agonism has been demonstrated for orthosteric agonists at mGlu1 [72,134], mGlu4, mGlu7, and mGlu8 [135]. For instance, when compared with two synthetic orthosteric agonists (L-2-amino-4-phosphonobutyrate and (2S)-2-amino-4-((4-(carboxymethoxy)phenyl)(hydroxy)methyl)(hydroxy)phosphoryl)butanoic acid), glutamate is biased toward iCa\(^{2+}\) mobilization over G-protein-coupled inwardly rectifying potassium channel activation at mGlu4 and mGlu8. Intriguingly, the reverse is true at mGlu7 [135]. At closely related mGlu1, orthosteric agonists including DHPG, a surrogate agonist often used in native tissue studies of mGlu5 allosteric modulators, are biased relative to glutamate [72]. The potential for surrogate orthosteric agonists to induce biased agonism relative to the endogenous ligand is a critical consideration for allosteric modulator discovery programs and a potential confound when classifying modulator pharmacology.

Biased mGlu5 allosteric modulators: an emerging concept

Biased agonism does not apply exclusively to orthosteric agonists but is also observed for mGlu5 allosteric modulators, with respect to both agonism and cooperativity. Comparison of the allosteric agonist activity of early mGlu5 ago-PAMs, N-(1,3-diphenyl-1H-pyrazolo-5-yl)-4-nitrobenzamide (VU29), and CDPPB, with glutamate, showed that in recombinant cells VU29 and CDPPB have higher intrinsic efficacy for signaling to ERK1/2 phosphorylation relative to iCa\(^{2+}\) mobilization, while glutamate has higher intrinsic efficacy for iCa\(^{2+}\) mobilization over ERK1/2 phosphorylation [99]. Multiple reports have since shown compounds classified as pure mGlu5 PAMs based on iCa\(^{2+}\) mobilization to be ago-PAMs with respect to ERK1/2 phosphorylation [91,121,136]. Recent bias profiling for diverse ago-PAMs across multiple measures of mGlu5 activity in both recombinant and native cells revealed distinct stimulus trafficking [125], and ligand-directed signaling [126]. Biased agonism and allosteric modulation are interlinked concepts, offering the potential to fine-tune GPCR activity to preference therapeutically beneficial signaling pathways and avoid those associated with adverse effects. This concept is attractive because it could provide a new avenue for the development of drugs that are not only ‘receptor subtype-selective’, but also ‘pathway-selective’. Although biased agonism offers great clinical potential, there are significant challenges to translation. First, there is a limited understanding of specific or combined pathways leading to therapeutically relevant outcomes, and hence the rational design of biased ligands remains difficult [85]. The identification of signaling pathways responsible for therapeutic compared with adverse effects is not straightforward. Therefore, selection of appropriate end point experiments is complex since the desirable signaling profile for most drug targets has not yet been well-defined. Second, biased agonism is highly dependent on the ligand and cellular background in which it acts (ligand and system bias, respectively). A particular bias profile in a heterologous system does not guarantee the same profile will be observed in endogenous systems or in vivo [127]. Third, since ligand bias is influenced by cellular context (context-dependent), biased agonism may conceivably change with alterations in membrane composition, proteins and signaling partners as a consequence of disease progression or the natural differences between different tissue/organ environments [128]. For example, in the setting of Alzheimer’s disease accumulation of pathological protein aggregates (e.g. amyloid β and hyperphosphorylated tau) and inflammation can change how cells respond to mGlu5 stimulation [129-131]. In recent years, the concept of biased agonism has been extended to include biased allosteric modulation. A notable example is revealed by an autoantibody that targets the CaSR, which enhances Goq/11 coupling to inositol phosphate accumulation but inhibits ERK1/2 phosphorylation [132]. Such modulators engendering biased agonism offer great potential to dissect the physiology linked to therapeutic and adverse effects and to tailor receptor activity for improved therapeutic effects.

Despite the challenges, the development of ligands with desired selectivity toward particular signaling pathways is of considerable interest. The ability to experimentally detect ligand bias has necessitated the development of methods to quantify bias in order to guide structure–activity studies and drug candidate selection. Historically, comparisons of agonist potency or maximal effects between different pathways were used as indicators of biased agonism; however, these approaches are suboptimal. One such method to quantify bias is the calculation of ‘transduction coefficients’ [133]. This approach, which is based on the Black and Leff (1983) operational model of agonism [11], can be applied to concentration-response curves to obtain a single parameter that describes bias between signaling pathways in a system independent manner [123]. However, even if transduction coefficients are used to establish true bias, it is still imperative to consider that agonist responsiveness may be influenced by ligand-independent factors, such as differing receptor-transducer coupling efficiencies and observational bias resulting from differing assay conditions and sensitivities. Therefore, a critical aspect for biased quantification is the need to exclude both system and observational bias by comparing bias factors to a reference ligand and pathway.
bias fingerprints that are postulated to be linked to different preclinical profiles. VU0424465, an mGlut5 ago-PAM with known adverse effect liability, showed the most extreme biased profile in both cell types relative to DHPG [91]. 1-(4-(2,4-difluorophenyl)piperazin-1-yl)-2-((4-fluorobenzyl)oxy)ethenone (DPFE) and VU0409551, which have not been associated with adverse effects, were also biased agonists relative to DHPG, but their bias fingerprints were distinct from VU0424465 and from one another.

Beyond biased agonism, several studies have revealed bias of mGlut5 allosteric modulators at the level of cooperativity, referred to as biased modulation (Figure 3). For instance, in rat cortical astrocytes N-[4-Chloro-2-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)methyl]phenyl]-2-hydroxybenzamide is a PAM in iCa\textsuperscript{2+} mobilization but a NAM for ERK1/2 phosphorylation [137]. More recently, diverse mGlut5 PAMs and ago-PAMs with different chemical scaffolds have exhibited differential cooperativity with DHPG depending on the measure of receptor activation (inositol monophosphate (IP\textsubscript{1}) accumulation, ERK1/2 phosphorylation, or iCa\textsuperscript{2+} mobilization) in primary neuronal cultures [91]. Importantly, the differences in cooperativity were not uniform for all PAMs; N-cyclobutyl-6-((3-fluorophenyl)ethyl)pyridinamide (VU0360172) had similar cooperativity with DHPG between iCa\textsuperscript{2+} mobilization and pERK1/2, whereas DPFE was a PAM for iCa\textsuperscript{2+} but neutral or negative for pERK1/2 [91]. Therefore, relative to VU0360172, DPFE is a biased allosteric modulator of mGlut5. Unappreciated bias may also be a contributing factor to unanticipated adverse effects or non-selective actions, with a recent study demonstrating that the apparent selectivity of allosteric ligands for class C GPCRs is largely driven by cooperativity and bias [88]. Furthermore, bias fingerprints are distinct between recombinant and native cells, highlighting the contribution of cellular context, which can confound classification of allosteric modulator pharmacology. An excellent example is VU0409551, which does not potentiate NMDA receptor dependent synaptic plasticity in the hippocampus of wild type animals [121], but does enhance hippocampal NMDA receptor activity in the serine racemase knockout mouse model of schizophrenia [138]. The increasing evidence for biased modulation by diverse mGlut5 chemotypes underscores the paucity of our understanding about chemical structure and functional consequences of mGlut5 allosteric modulation. These knowledge gaps are largely attributable to minimal characterization of the effects of allosteric ligands on different receptor-mediated behaviors. Thus, to identify biased mGlut5 allosteric modulators, future drug discovery campaigns should consider multiple measures of mGlut5 activation. Collectively, the emerging concept of biased allosteric modulation highlights the inherent complexity and challenges for allosteric modulator discovery and translation, but if harnessed effectively has the potential to provide new avenues for the development of highly selective agents with improved therapeutic indices.

**Concluding remarks**

Allosteric modulators offer preferred therapeutic modalities within the context of CNS disorders, and as such have been the focus of intense research efforts by academia and industry. While several mGlut5 PAMs and NAMs have demonstrated robust efficacy in preclinical models of multiple diseases, such as schizophrenia, cognitive dysfunction, anxiety, depression, Parkinson’s and Alzheimer’s diseases, clinical failures highlight key knowledge gaps that persist with respect to our understanding of the full scope of allosteric modulator pharmacology. Robust quantification, detection, and validation remain critical elements to successful allosteric modulator discovery programs. In this respect, biased agonism and modulation offer both opportunities and challenges. Unappreciated bias likely underscores observations that not all PAMs or NAMs are the same when translating from in vitro systems to in vivo models. With an enhanced understanding of the intracellular signaling and regulatory partners engaged by mGlut5, how these are perturbed in the disease context, and how different partners contribute to beneficial compared with adverse effects, we may be able to rationally design and develop more selective and effective mGlut5 targetting therapeutics for multiple CNS disorders.

**Competing interests**

The author declares that there are no competing interests associated with the manuscript.

**Abbreviations**

SPAM523, 5-fluoro-2-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)methyl]phenyl]-2-hydroxybenzamide; 7TM, seven transmembrane; Ago-PAM, agonist-PAM; ATCM, allosteric ternary complex model; ATSM, allosteric two-state model; cAMP, cyclic adenosine monophosphate; CaSR, calcium sensing receptor; CDPPB, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide; CHO, Chinese hamster ovary; CHPPG, (S)-3-chloro-5-hydroxyphenylglycine; CNS, central nervous system; CPCCOEt, 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester; DHPG, (S)-3,5-dihydroxyphenylglycine; DPFE, 1-(4-(2,4-difluorophenyl)piperazin-1-yl)-2-((4-fluorobenzyl)oxy)ethenone; ERK1/2, extracellular signal-regulated kinases 1 and 2; FXS, fragile X syndrome; GABA\textsubscript{A}, \(\gamma\)-aminobutyric acid receptor B;
GERD, gastroesophageal reflux disease; GPCR, G-protein-coupled receptors; iCa2+, intracellular calcium; HEK, human embryonic kidney; IP1, inositol monophosphate; LTD, long-term depression; M-5MPEP, 2-[(3-methoxyphenyl)ethyl]yl]-5-methylpyridine; mGlu, metabotropic glutamate; mGlu5, metabotropic glutamate receptor subtype 5; MPEP, 2-methyl-6-(phenylethynyl) pyridine; MTERP, 3-{[(2-Methyl-4-thiazolyl)ethyl]yl}pyridine; NAL, neutral allosteric ligand; NAM, negative allosteric modulator; NMDA, N-methyl-D-aspartate; PAM, positive allosteric modulator; pCREB, phosphorylated cAMP response element binding protein; PD-LID, Parkinson’s disease levodopa-induced dyskinesias; VFT, Venus flytrap; VU0360172, N-cyclobutyl-6-[(3-fluorophenyl)ethyl]pyridoline; VU0409551, (4-fluorophenyl)(2-(phenoxymethyl)-6,7-dihydrooxazolo[5,4-c]pyridin-5(4H)-yl)methanone; VU0424465, (R)-5-[(3-fluorophenyl)ethyl]yl-N-(3-hydroxy-3-methylbutan-2-yl)pyridoline; VU0477573, N,N-diethyl-5-[(3-fluorophenyl)ethyl]pyridoline; VU29, N-(1,3-diphenyl-1H-pyrazolo-5-yl)-4-nitrobenzamide.

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