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# Practical Strategies and Concepts in GPCR Allosteric Modulator Discovery: Recent Advances with Metabotropic Glutamate Receptors

**Craig W. Lindsley**[†,‡,*], **Kyle A. Emmitte**[§], **Corey R. Hopkins**[†], **Thomas M. Bridges**[†], **Karen J. Gregory**[‖], **Colleen M. Niswender**[†,⊥], and **P. Jeffrey Conn**[†,⊥,*]

[†]Vanderbilt Center for Neuroscience Drug Discovery, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, United States

[‡]Department of Chemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, United States

[⊥]Vanderbilt Kennedy Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, United States

[§]Department of Pharmaceutical Sciences, UNT System College of Pharmacy, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, Texas 76107, United States

[‖]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville VIC 3052, Australia

## Abstract

Allosteric modulation of GPCRs has initiated a new era of basic and translational discovery, filled with therapeutic promise yet fraught with caveats. Allosteric ligands stabilize unique conformations of the GPCR that afford fundamentally new receptors, capable of novel pharmacology, unprecedented subtype selectivity, and unique signal bias. This review provides a comprehensive overview of the basics of GPCR allosteric pharmacology, medicinal chemistry, drug metabolism, and validated approaches to address each of the major challenges and caveats. Then, the review narrows focus to highlight recent advances in the discovery of allosteric ligands for metabotropic glutamate receptor subtypes 1–5 and 7 (mGlu $_{1–57}$) highlighting key concepts ("molecular switches", signal bias, heterodimers) and practical solutions to enable the development of tool compounds and clinical candidates. The review closes with a section on late-breaking new advances with allosteric ligands for other GPCRs and emerging data for endogenous allosteric modulators.

## Graphical Abstract

[*]**Corresponding Authors**, craig.lindsley@vanderbilt.edu. jeff.conn@vanderbilt.edu.

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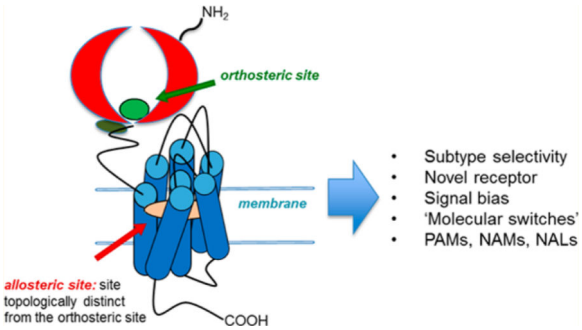


# 1. INTRODUCTION

## 1.1. Historical Overview of Allosteric Modulation

In recent years, the conformational flexibility of various proteins and receptors has been exploited to identify ligands that modulate pharmacological function by actions at topographically distinct binding sites (i.e., an allosteric site) other than the defined, regulatory site of the endogenous ligand/neurotransmitter/agonist (i.e., the orthosteric site).[1–12] There is no question that allosteric modulation is a “hot” and dynamic area of research, with new insights and innovations reported at an ever-increasing rate. The concept of allosteric modulation was posited over 50 years ago; however, the field lacked the technology and tools to capitalize on its promise until the late 1990s. Indeed, the birth of the field was 1965, with the proposal of allosterism by Monod, Wyman, and Changeux to describe the actions of ligands and conformational selection mechanisms within bacterial regulatory enzymes.[1–13] At around the same time, the benzodiazepines, or BZDs (**1**, Figure 1), were among the first approved drugs for the treatment of disorders of the central nervous system (CNS) and shown to be allosteric modulators of $GABA_A$ receptors, ligand-gated chloride-selective ion channels that are activated by γ-aminobutyric acid (GABA).[14] Whereas direct agonists of $GABA_A$ receptors are excitotoxic, allosteric modulation of $GABA_A$ receptors by the BZDs has proven to be both effective and well-tolerated.[14] Moreover, the BZDs display a wide range of molecular pharmacological profiles including positive allosteric modulators (PAMs) that potentiate $GABA_A$ receptor response to GABA, negative allosteric modulators (NAMs) that non-competitively decrease channel activity, and neutral allosteric ligands (NALs, formerly referred to as silent allosteric modulators or SAMs) that occupy the allosteric site yet elicit no functional response but can block the action of PAMs and NAMs.[1–12,14] These exciting findings fundamentally altered our thinking of target modulation beyond traditional approaches and were expanded to other challenging and/or intractable molecular targets. This novel strategy has now been applied to a diverse breadth of regulatory proteins, including ion channels, caspases, kinases, phospholipases, and G protein-coupled receptors (GPCRs) with great success, providing key proof-of-concept compounds, clinical candidates, and marketed therapeutics.[1–12] Despite its far-reaching, holistic impact, allosteric modulation has truly transformed GPCR research and GPCR-focused drug discovery. Although many excellent reviews have covered various aspects of GPCR allosteric modulation,[1–12] we focus here on new discoveries and lessons

learned en route to optimizing key in vivo proof-of-concept tool compounds and preclinical candidates.

### 1.2. Allosteric Modulation of GPCRs

G protein-coupled receptors (GPCRs), also commonly referred to as seven transmembrane spanning receptors (7TMRs), have been and remain staples as targets for drug discovery.[1–12,15,16] Classical approaches of target modulation focused on orthosteric ligands (agonists, competitive antagonists, and inverse agonists); these ligands constitute up to 40% of historically marketed drugs, and of the 19 top-selling drugs through 2013, seven targeted GPCRs.[1–12,15,16] The top-selling drug of 2014 was aripiprazole (**2**, Figure 1), a dopamine receptor partial agonist, with worldwide sales in excess of $9 billion.[17] Moreover, recent years have seen a plethora of fundamentally new paradigms and technologies to drive GPCR drug discovery.[18] Despite the success of GPCRs as a druggable target class, many efforts have failed to identify selective ligands based on the high evolutionary conservation of the orthosteric binding site.[1–12] In addition, for many GPCRs, agonists are not tolerated as chronic therapeutics; therefore, alternative strategies are required to safely and selectively activate receptors.[1–12] As X-ray crystal structures and cocrystals with orthosteric and/or allosteric ligands are increasingly available, GPCR ligand optimization is embracing structure-based drug design and subsequently providing the next generation of homology models for in silico screening.[10] Advances in molecular pharmacology and screening have propelled the discovery and development of allosteric modulators, biased ligands, and designer receptors exclusively activated by designer drugs (DREADDs),[19,20] while deepening our conceptual understanding and utilization of signal bias along with divergent ligand profiles targeting GPCR heterodimers.[10] Of all of these advances, allosteric modulation is the front runner, with allosteric ligands reported across the four major GPCR families (families A, B, C, and F) that overcome major limitations and liabilities of their orthosteric congeners (nondrug-like properties, limited CNS exposure, peptidic ligands, subtype and/or GPCR-nome selectivity, desensitization, down-regulation).[1–12] At present, there are two marketed drugs that allosterically modulate GPCRs, cinacalcet [Sensipar, **3**, a PAM of the calcium-sensing receptor (CaSR)][21] and maraviroc [Selzentry, **4**, a C-C chemokine receptor 5 ($CCR_5$) NAM],[22] further validating the pharmacological approach (Figure 1). Moreover, multiple allosteric modulators are in clinical development:[10] reparixin (**5**, a $CXCR_1$ and $CXCR_2$ NAM in Phase II/III); multiple $mGlu_5$ NAMs in Phase II or Phase III [mavoglurant (**6**), dipraglurant (**7**), STX107 (structure not disclosed), basimglurant (**8**), fenobam (**9**)]; several $mGlu_2$ PAMs in Phase II or Phase III [ADX71149 (**10**), JNJ-40411813 (**11**), and the tracer [$^{11}$C]-42491293 (**12**)]; a dual $mGlu_{2/3}$ PAM [AZD8529 (**13**) in Phase II]; and finally, an $M_1$ PAM (MK-7622 recruiting in Phase II, structure not disclosed).[23–26] Behind these, the preclinical pipelines of numerous academic and industrial laboratories are filled with allosteric modulators targeting various GPCRs.[1–12]

### 1.3. Pharmacological Considerations with Allosteric Modulation of GPCRs

GPCRs, highly flexible proteins that are continuously sampling new conformations within the lipid bilayer, are ideal targets for allosteric modulation.[1–12] It is important to note that, when bound by both the orthosteric and allosteric ligands, the GPCR is in effect a new receptor species, capable of diverse and potentially non-native signaling and function.[1–12]

This phenomenon can be exploited in drug discovery (vide infra), if the requisite secondary assays are in place. Moreover, allosteric ligands can induce a broad range of pharmacological modes of action upon binding to the GPCR. Like the BZDs, PAMs and NAMs stabilize conformations of the GPCR that enhance and diminish, respectively, the functional response elicited by the orthosteric ligand.[1–12] In addition, the affinity of the GPCR for the orthosteric ligand and/or its efficacy can be modulated by PAMs and NAMs, as can the activity of the receptor on downstream signaling cascades in the absence of orthosteric agonist. NALs have also been reported for GPCRs, wherein these ligands occupy the allosteric site and induce no functional response but block the functional activity of both PAMs and NAMs.[1–12] PAMs can also function as pure PAMs, devoid of receptor activation irrespective of receptor expression in cell lines or native preparations, or ago-PAMs, wherein the ligand induces allosteric agonism, to varying degrees, in the absence of orthosteric ligand but also potentiates activation of the GPCR when the orthosteric ligand binds.[1–12] In certain instances, where the basal “tone” might be low, an ago-PAM could be advantageous, whereas in other scenarios, ago-PAMs can lead to adverse events and toxicity. Allosterism has also afforded a new mode of pharmacology within the NAM manifold: partial antagonists.[27–29] These are NAMs with weak negative cooperativity that only partially block receptor signaling when fully occupying the allosteric site.[27] The inability of such ligands to induce a complete blockade of signaling might be advantageous for certain GPCR targets, where either signal ablation or inverse agonist activity can lead to adverse effect liability. Finally, allosteric ligands can bind, and activate, the GPCR in the absence of the orthosteric ligand, so-called allosteric agonists.[1–12,30–32] This includes PAMs that also exhibit an intrinsic activity (ago-PAMs), as well as allosteric agonists that activate the receptor but do not potentiate responses to orthosteric agonists. In most cases, allosteric agonists that do not also exhibit PAM activity have been found to be bitopic ligands that do bind the orthosteric site but engender functional selectivity through activation at an allosteric site.[1–12,30–32] These ligands are typically partial agonists, and their efficacy varies with receptor expression such that they appear to be nearly full agonists in high-expression/-reserve systems/tissues but weak partial agonists or even antagonists in systems with low receptor expression/reserve.[30] Thus, this mode of allosteric pharmacology has somewhat fallen out of favor for certain targets with preference afforded to PAMs.

Beyond the mode of pharmacology elicited by an allosteric ligand, numerous other considerations must be addressed and strategies put into place for a successful allosteric modulator discovery campaign.[1–12] The first consideration is the assay protocol for both the initial high-throughput screen and the primary assay. Because of the propensity of certain allosteric ligands to display pharmacological mode switching, through subtle “molecular switches” (vide infra),[33–35] a paradigm in which multiple additions of test compound and agonist are added is particularly beneficial at both stages of ligand screening. An example of such a functional assay (e.g., intracellular calcium mobilization, measured by a calcium-sensitive dye, as a surrogate for GPCR activation/inhibition) can be performed by adding the compound (1st add), followed 2 min later by a low concentration [for example, a concentration eliciting a response that is 20% of the maximal response (~$EC_{20}$)] of orthosteric agonist (2nd add), followed 1 min later by an near-maximal $EC_{80}$ concentration of orthosteric agonist (3rd add).[36] This allows identification of agonists, ago-PAMs, PAMs,

and antagonists/NAMs in a single kinetic assay, reducing the need for multiple high-throughput screening (HTS) campaigns and, importantly, capturing the propensity of a given chemotype for mode switching early. Ideally, ligands with the latter profile are deprioritized, as oxidative metabolism of these ligands can also produce metabolites with undesired or opposing modes of pharmacology (vide infra),[37] complicating both proof-of-concept studies and development.

Another important consideration is the species of the receptor cell line employed. As allosteric sites are evolutionarily less conserved than the orthosteric site, the literature is filled with examples of allosteric ligands that display significant species differences.[1–12,38,39] Therefore, it is critical to have available cell lines for rat, dog, nonhuman primate (NHP), and human and to evaluate lead scaffolds to assess for species differences. Clearly, an allosteric ligand that is active on the human receptor, but not on either rat or dog receptors, will complicate development and preclude standard safety assessment. Accordingly, early-stage proof-of-concept programs are often driven with a rodent receptor for the primary assay, with periodic checks against human; however, in late-stage programs, structure-activity relationships (SARs) with the human receptor and counter-screen against safety species' cell lines predominate. When possible, it is advantageous to select for allosteric ligands that do not display pronounced species bias en route to clinical candidates.[1–12]

Yet another common caveat is that of ligand bias.[40,41] Certain allosteric ligands will potentiate any orthosteric ligand, whereas others will only potentiate a subset of orthosteric ligands (and can often exhibit negative cooperativity with others). This phenomenonon requires careful consideration to be made both in the HTS phase and during primary screening as to the orthosteric agonist to employ (ideally the native/endogenous ligand).[40,41] Therefore, it is critical to evaluate the response of an allosteric ligand to both native and synthetic agonists early and to deprioritize ligands with significant ligand bias, as failure to do so might require the addition of exogenous agonists in vivo to observe activity.

Signal bias is an emerging concept of significant interest with allosteric ligands.[42,43] Whereas the endogenous orthosteric agonist typically stabilizes an active conformation of the GPCR that activates its canonical downstream signaling cascades, allosteric ligands are capable of stabilizing unique activated conformations that enable selective activation of only certain downstream signaling pathways, both G protein-dependent and G protein-independent, while leaving others unaffected.[42,43] Signal bias, also referred to as stimulus bias, has been demonstrated for both PAMs and NAMs for a wide range of GPCRs including $M_1$ and $M_4$ receptors, calcium-sensing receptors, multiple mGlu receptors, and cannabinoid receptors, to list only a few.[10,44–50] An interesting case in point involves the $M_1$ PAMs VU0029767 (**15**) and VU0090157 (**16**), which both provide comparable potentiation of acetylcholine- (ACh−) induced calcium mobilization in stable, $M_1$-expressing cell lines.[51] As expected, $M_1$ PAM VU0090157 potentiates the ability of ACh to stabilize an $M_1$ receptor conformation that couples to $G\alpha_q$ [and subsequent activation of phospholipase C (PLC) to release intracellular calcium] as well as $G\alpha_{12}$ (or other small G proteins), leading to activation of phospholipase D (PLD). In contrast, $M_1$ PAM VU0029767 stabilizes a unique activated conformation that does not activate PLD. This could be mediated by stabilizing a

conformation of the receptor that is not able to productively couple to $G\alpha_{12}$ (or other small G proteins) but does productively couple to $G\alpha_q$ for the subsequent activation of PLC to ultimately release intracellular calcium.[51] Since these early proof-of-principle discoveries, signal bias has been replicated in native tissues and has been a major driver in avoiding adverse pharmacological events through a selective activation of specific signaling pathways, for example, $mGlu_5$ PAMs (vide infra).[52–55] Although requiring numerous secondary assays to detect and optimize, signal bias will undoubtedly be a major player in future allosteric modulator drug discovery programs.

Finally, one last pharmacological consideration with PAMs that should be discussed is the PAM $EC_{50}$ (potency). It is important to note that the $EC_{50}$ value for potentiation is most often based on a submaximal concentration of orthosteric agonist, typically approximately 20% of the maximal response (~$EC_{20}$), a concentration arbitrarily set to enable comparative SARs.[1–12] This is not an absolute measure of PAM potency, as endogenous agonist tone will vary across brain regions/circuits/synapses and native peripheral tissues; therefore, the $EC_{50}$ value determined with an $EC_{20}$ of agonist can either over-or underestimate in vivo PAM potency.[1–12] Thus, this measure of potency is essential to drive SARs and early pharmacokinetic (PK)/pharmacodynamic studies; however, it is not an absolute value to be held rigidly, as there are many caveats discussed in detail in the following sections.

### 1.4. Quantification of Allosteric Interactions

The binding of an allosteric ligand to a GPCR engenders a distinct subset of receptor conformations that cannot be achieved through occupancy with an orthosteric ligand alone. As a result, allosteric ligands can potentiate or inhibit the binding and/or efficacy of an orthosteric ligand. An ongoing challenge in the field is the quantification of the myriad of effects an allosteric ligand can have on the response to an orthosteric ligand.[1–12]

**1.4.1. Affinity Modulation—**The simplest framework to describe allosteric interactions at GPCRs is the allosteric ternary complex model (ATCM) (Figure 2a). In this model, the receptor (R) can be bound by orthosteric ligand (AR) or allosteric ligand (BR) as determined by the concentration of each ligand and the equilibrium dissociation constants (or affinity) of each ligand ($K_A$ and $K_B$) for the free receptor. The magnitude and direction of the change in ligand affinity when the receptor is simultaneously bound (ARB) is described by the "cooperativity factor" $\alpha$. Because the two binding sites are conformationally linked, the allosteric interaction is reciprocal.[6] Cooperativity is also saturable; therefore, allosteric ligands can offer the advantage of being safer in the case of overdose. According to the ATCM, allosteric modulators are quiescent in the absence of orthosteric ligand, and cooperativity manifests only as a consequence of the presence of an orthosteric ligand. Therefore, allosteric modulators offer the potential to modulate receptor activity in a spatial and temporal fashion. An $\alpha$ value less than 1 (but greater than 0) indicates a negative allosteric interaction, such that the binding of one ligand decreases the affinity of the other. The effect of increasing concentrations of a negative allosteric modulator on the binding of an orthosteric ligand is simulated in Figure 2a. An $\alpha$ value greater than 1 denotes positive cooperativity, such that the binding of one ligand enhances the affinity of the second. In Figure 2, the influence of a positive allosteric modulator on orthosteric ligand binding is

simulated. Neutral allosteric ligands (NALs) that occupy allosteric sites but have no net effect on orthosteric ligand affinity are described by an α value equal to 1. Neutral allosteric ligands represent important pharmacological tools to study and validate small-molecule allosteric ligands; however, they might also offer therapeutic benefit in blocking the binding of pathological endogenous allosteric modulators.[56] In such a scenario, altering endogenous agonist activity might not be necessary or desirable.

**1.4.2. Efficacy Modulation—**It has become increasingly apparent that allosteric ligands can exhibit an intrinsic efficacy (positive or inverse), in addition to, or exclusive of, cooperativity with orthosteric ligands. Furthermore, the binding of an allosteric ligand can also influence the orthosteric agonist efficacy.[57] Importantly, efficacy modulation can occur independently of affinity modulation, as is commonly observed for small-molecule allosteric modulators interacting with family C GPCRs.[51–65] Moreover, efficacy modulation need not be in the same direction as α.[67–69] To accommodate this increased complexity, multiple models have been proposed.[69–75] The most widely adopted framework is an operational model of allosterism that combines an operational model of agonism[76] with the ATCM (Figure 2b).[77] The advantage of this quantitative framework is that efficacy modulation (of a specific functional readout/effect) is distilled to a single cooperativity factor, β, which is derived experimentally. As simulated in Figure 2b, efficacy modulation can manifest as changes in agonist potency and maximal response; in contrast, α influences only agonist potency. In practice, the relative contributions of α and β to the allosteric interaction observed in a functional assay can be delineated where there is a change in the agonist maximal response[63,68] or by constraining α to the value determined through radioligand binding assays.[78–80] Alternatively, where there is no change in agonist maximal response, which can occur when an orthosteric agonist has high coupling efficiency, a composite αβ parameter has been reported to quantify the interaction.[48,81] It is apparent that assessment of allosteric interactions using functional assays introduces considerable complexity and analytical challenges. The unique receptor conformations engendered by allosteric ligands gives rise to further complexity, such as the phenomena of probe dependence and biased agonism/modulation.

**1.4.3. Probe Dependence of Cooperativity—**The degree and direction of the cooperativity observed is determined by the chemical nature of the two ligands simultaneously bound to the receptor, referred to as probe dependence. It is therefore important to consider probe dependence when assessing allosteric interactions and classifying ligand pharmacology. Probe dependence can manifest as variations in the degree of positive or negative cooperativity depending on the orthosteric ligand employed. In addition, there are multiple instances (for example, at muscarinic acetylcholine receptors) where the direction of cooperativity will switch depending on the orthosteric ligand probe.[78,82–85] To successfully translate the pharmacology observed in cell-based assays to the native system and ultimately the whole animal, the influence of probe dependence needs to be considered in systems where it is impractical to use the endogenous agonist and a surrogate agonist is required.

Moreover, certain GPCRs have multiple endogenous ligands; for such receptors, considerations of probe dependence must be included early within the drug discovery pipeline. For example, GLP-1 receptors have at least six endogenous ligands including oxyntomodulin, full-length GLP-1(1–36)$NH_2$, and its metabolite GLP-1(9–36)$NH_2$. Early small-molecule GLP-1 receptor allosteric ligands had probe dependent effects, being weak positive modulators ($\alpha\beta < 2$) of GLP-1(1–36)$NH_2$ and robust potentiators of oxyntomodulin ($\alpha\beta = 10–30$) and GLP-1(9–36)$NH_2$ ($\alpha\beta > 100$) activity in cAMP assays.[81,86,87] A recent example of this is represented by PAMs of the glucagon-like peptide 1 receptor, a family B GPCR, where the native agonist is a 39-amino acid peptide, GLP-1.[88,89] Several PAM chemotypes have been discovered that potentiate either endogenous GLP-1 (or the related splice variants) or the therapeutically relevant synthetic peptides liraglutide or exendin-4. Thus, these allosteric ligands either rely on endogenous GLP-1 tone or are coadministered with a synthetic peptide for potentiator activity at the GLP-1 receptor. A more versatile and useful allosteric ligand would potentiate both endogenous and synthetic peptide ligands equally. A functional HTS screen and subsequent optimization effort identified VU0453379 (**14**), a GLP-1 receptor PAM without ligand bias, as affording comparable $EC_{50}$ values and efficacy for the GLP-1 receptor as well as synthetic peptides luraglatide and exendin-4.[88,89] Clearly, for targets where multiple endogenous orthosteric ligands exist, such marked differences in allosteric ligand pharmacology can have significant effects when translating cell-based studies to the whole animal. Probe dependence of different endogenous ligands could be exploited as a means of driving selectivity; however, if not given due consideration, it could also result in unanticipated on-target biological effects.

### 1.5. The Problem with Potency

The vast majority of drug discovery efforts rely on a single functional assay (most often intracellular $Ca^{2+}$ mobilization) and allosteric modulator titration curves to a single concentration of orthosteric agonist and associated potency estimates to inform SARs. However, the potency of an allosteric modulator is dependent on the concentration of agonist and the coupling efficiency of the agonist and system.[64] Additionally, the potency derived from a modulator titration curve represents a composite of modulator affinity as well as efficacy and affinity cooperativity. The shortcomings in relying on potency values alone as a means of informing allosteric modulator SARs are exemplified in Figure 3. Numerous diverse small-molecule allosteric ligands have been revealed for $mGlu_5$, including both negative and positive allosteric modulators of glutamate.[23,90,91] In addition, allosteric radioligands are available for $mGlu_5$, allowing determination of novel allosteric ligand affinity using simple inhibition binding assays.[92,93] Comparison of negative modulator potencies with affinity estimates from binding assays shows that only 59% of potency values are within a factor of 3 of affinity estimates (Figure 3a). For $mGlu_5$ NAMs, in 17% of cases, potency values overestimate affinity, and in 23% of cases, potency values underestimate affinity by more than a factor of 3. For an assessment of the ability of $mGlu_5$ PAMs to potentiate responses to glutamate/quisqualate, the lack of concordance between affinity and potency is even more pronounced. In this case, only 10% of PAM potencies are within 3 times affinity estimates, and the majority of $mGlu_5$ PAMs (86%) have higher potency than affinity (Figure 3b). The larger discordance for $mGlu_5$ PAMs might be due, in part, to the smaller data set available; however, more likely, this reflects the fact that $mGlu_5$ PAM

potency is determined by both cooperativity and affinity, so that compounds with high cooperativity can fully potentiate receptor responses when occupying only a small fraction of the receptors. In addition to the influence of agonist concentration, modulator affinity, and cooperativity between ligands, the differences in assay kinetics between functional and binding assays can also be a contributing factor to potency/affinity discrepancies. Binding assays are performed at equilibrium (or a close approximation thereof), whereas functional assays are often not; this is particularly so for intracellular $Ca^{2+}$ mobilization, where the response measured occurs within seconds of exposure to agonist. A final contributing factor is that modulator titration curves in functional assays are limited in that they will only detect allosteric ligands that inhibit or enhance the activity of the agonist. Therefore, there is the possibility that ligands designated as inactive might in fact include NALs. Indeed, this property was recently exploited in the rational discovery of a high-affinity NAL for $mGlu_5$.[94] Although convenient, relying on allosteric titration curves and potency estimates alone has significant limitations because there is no way to delineate whether chemical modifications are changing affinity, cooperativity, or efficacy.

### 1.6. Homo- versus Heterodimers

For GPCRs of families A and B, it has been shown that specific GPCR pairs can interact when expressed in vitro and that the pharmacology of ligands interacting at heteromers can be distinct.[95] For example, in vitro coexpression of μ- and δ-opioid receptors results in changes in the absolute potencies and rank orders of potency of various ligands[95,96] compared to the expression of each receptor alone. Similarly, heteromers of various GPCRs have been shown to couple to distinct G proteins or signal-transduction cascades, at times even engaging in completely new pathways, as shown in the coexpression of $D_1$ and $D_2$ dopamine receptors.[97] This obviously presents significant biological complexity, as unique heteromers have the potential to differentially interact with ligands; couple to unique signaling components; and undergo distinct mechanisms of receptor trafficking, regulation, and internalization.[95,98–102] Both family A and family B GPCRs have been reported to be subject to heterointeractions that can involve higher-order oligomerization rather than strict dimerization.[95,103]

Allosteric modulator pharmacology can also be impacted by the complexation of GPCRs with other GPCRs, as well as other cellular interacting proteins, such as G proteins themselves, scaffolding proteins such as those found in synaptic terminals such as postsynaptic density proteins, and other signaling components. In the case of family B receptors that are responsive to ligands such as calcitonin gene-related peptide (CGRP), adrenomedullin, intermedin, amylin, and secretin, interactions with receptor-activity-modifying proteins (RAMPs) are essential for full receptor function and interaction with various RAMPs (i.e., RAMP1-RAMP3) can dictate signaling, pharmacology, and trafficking.[104–108] For example, both CGRP and adrenomedullin act through a common receptor, the calcitonin receptor-like receptor (CLR); specificity, however, is directed by RAMPs. In this case, CLR complexed with RAMP1 leads to a high-affinity receptor for CGRP, whereas CLR complexed to RAMP2, although able to bind CGRP with lower affinity, also responds to adrenomedullin.[104] Although the majority of the above-mentioned complexes are not heterodimerizations between two GPCRs, these interactions with cellular

proteins are almost certainly cell-type-specific and might be responsible for differential ligand interactions with the same GPCR when a receptor is expressed in distinct cellular backgrounds or within different endogenous cell populations.

In contrast to the class A GPCRs, the class C GPCRs, including the $GABA_B$ receptors, calcium-sensing receptors, taste receptors, and metabotropic glutamate receptors, function as constitutive dimers.[109–116] $GABA_B$ receptors are obligate heterodimers composed of distinct $GABA_{B1}$ and $GABA_{B2}$ subunits that are required to assemble into heteromeric form for signal transduction and membrane trafficking.[109,117,118] Although agonists for the receptor bind within the $GABA_{B1}$ subunit, signaling does not occur in the absence of $GABA_{B2}$, and this protomer both enhances the binding of agonists to $GABA_{B1}$ and contains the region of the heteromeric receptor that is responsible for coupling to G proteins. A positive allosteric modulator of the $GABA_B$ receptor, termed CGP7930 (**17**), has been shown to bind within the $GABA_{B2}$ TM domain to potentiate the effects of GABA.[119] As the $GABA_{B1}$ and $GABA_{B2}$ subunits are not functional when each is expressed alone, this result indicates that CGP7930 is essentially a heterodimer-specific allosteric modulator. Similarly, the three identified taste receptors [taste receptor type 1 members 1, 2, and 3 (T1R1, T1R2, T1R3)] do not function when expressed alone or in homodimeric form.[120] Another example of heteromer-specific regulation is seen in the case of a T1R2/T1R3 heteromer: Whereas the T1R2 subunit binds the agonist aspartame, cyclamate (PAM) and lactisole (NAM) regulate the activity of aspartame by binding to T1R3.[121–125] This suggests that these modulators are influencing the interaction of aspartame with its T1R2 binding site by transactivation or transinhibition across the protomers.

Until recently, the mGlu receptors were reported to function as disulfide-linked, constitutive homodimers.[110,126–128] This family of eight related receptors is further classified into three groups based on sequence homology, G protein coupling profile, and receptor pharmacology:[129] Group I contains $mGlu_1$ and $mGlu_5$; group II encompasses $mGlu_2$ and $mGlu_3$; and group III consists of $mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$. In a recent and elegant study by Doumazane et al., it was shown that, in vitro, members of different mGlu groups can heterodimerize as assessed using time-resolved fluorescent resonance energy transfer (FRET) techniques.[130] In these studies, group I receptors could dimerize together but not with members of the other two groups. In contrast, these in vitro studies showed that members of group II and III could heterodimerize both within their group as well as with members of the other group.[130] These studies also demonstrated that, in this system used for the assessment of receptor activity, mGlu receptors formed strict heterodimers rather than higher-order oligomers. Kammermeier coexpressed $mGlu_2$ and $mGlu_4$ in rat superior cervical ganglion cells and reported results examining the ability of allosteric modulators to regulate heteromeric mGlus (Figure 4).[131] In these experiments, activation of one side of the putative heterodimer was not sufficient to induce receptor activation; in contrast, coapplication of $mGlu_2$ and $mGlu_4$ orthosteric agonists activated responses when $mGlu_2$ and $mGlu_4$ were coexpressed. In these studies, the $mGlu_2$ NAM Ro 64-5229 (**18**) did not antagonize glutamate responses in $mGlu_{2/4}$-expressing cells (but see results below for a separate study with a distinct $mGlu_2$ NAM). As it has previously been suggested that both halves of an mGlu receptor homomer need to be occupied with a NAM to block glutamate-

mediated activation, the finding that a NAM that binds to only one side of the heterodimer does not block receptor activation was predicted.[132,133] In contrast to their effects on homomeric receptor forms, PAMs of either $mGlu_2$ [biphenylindanone A, BINA (**19**)] or $mGlu_4$ [(−)-*N*-phenyl-7-(hydroxyimino)-cyclopropa[*b*]chromen-1a-carboxanide, PHCCC (**20**), or *N*-(4-chloro-3-methoxyphenyl)-2-pyridine carboxamide, VU0361737 (**21**)] no longer potentiated responses mediated by heterodimer activation.[131] Consistent with a pharmacologically distinct profile for an $mGlu_{2/4}$ heterodimer, application of both an $mGlu_2$ PAM and an $mGlu_4$ PAM also did not restore potentiation.

Work from our own laboratories has built on and extended these studies into native tissues, and the results are consistent with $mGlu_{2/4}$ heteromer expression in the brain.[134] We first performed studies in HEK293 (human embryonic kidney) cells in which we expressed either $mGlu_2$ alone, $mGlu_4$ alone, or $mGlu_2$ and $mGlu_4$ together. Studies in HEK293 cells confirmed that certain $mGlu_4$ PAMs, such as PHCCC (**20**) and *N*-(4-(*N*-(2-chlorophenyl)sulfamoyl)phenyl)picolinamide (4-PAM2, **22**), induced robust potentiation of responses to $mGlu_4$ when expressed alone but did not potentiate responses when $mGlu_2$ and $mGlu_4$ were coexpressed. These two PAMs are known to bind to an overlapping site on $mGlu_4$.[135] We then used two structurally distinct $mGlu_4$ PAMs, represented by *cis*-2-[[(3,5-dichlorophenyl)amino]carbonyl]cyclohexanecarboxylic acid (**23**, VU0155041) and (1*S*,2*R*)-*N*1-(3,4-dichlorophenyl)-cyclohexane-1,2-dicarboxamide (**24**, Lu AF29134),[136] which are known to bind to a distinct binding site on $mGlu_4$ when compared to PHCCC (**20**) and 4-PAM2.[135,137] To our surprise, VU0155041 and Lu AF21934 retained the ability to potentiate glutamate-mediated responses; when an agonist was used that only activated the $mGlu_4$ side of the heterodimer, VU0155041 and Lu AF21934 potentiated responses to a greater extent than when interacting with $mGlu_4$ homomers. Fitting the data using an operational model to assess ligand affinity and cooperativity, we observed that PHCCC (**20**) and 4-PAM2 exhibited the same affinity when interacting with a homodimer of $mGlu_4$ or an $mGlu_{2/4}$ heteromer; their reduced efficacy appeared to be due to a loss of cooperativity with an orthosteric agonist.[134] Interestingly, VU0155041 and Lu AF21934, the two compounds able to potentiate $mGlu_{2/4}$-dimer-mediated responses, exhibited enhanced cooperativity but reduced predicted affinity when interacting with $mGlu_{2/4}$ heteromers.[138] In further support of heteromer formation, VU0155041 and Lu AF29134 significantly potentiated responses induced by a selective $mGlu_2$ agonist only when $mGlu_2$ and $mGlu_4$ were coexpressed. Similarly to observations with cyclamate and lactisole at the taste receptors, these results suggest that there is transactivation between the subunits of the dimer, permitting the orthosteric site of one protomer to communicate with, and be influenced by, occupation of an allosteric site on the other protomer. In contrast to Kammermeier,[131] we found that an $mGlu_2$ NAM, MNI-137 (**25**), could noncompetitively block the response to an $mGlu_4$ agonist;[134] this finding provides additional support for the transactivation hypothesis.

It should be noted that, in these studies, $mGlu_2$ and $mGlu_4$ were simply cotransfected in HEK293 cells together using equal amounts of DNA. It was, therefore, somewhat surprising that responses to PHCCC (**20**) and, in particular, 4-PAM2 (as it is highly efficacious in potentiating $mGlu_4$ when expressed alone), were completely lost when glutamate was used as the orthosteric agonist. In these studies, no attempt was made to force dimerization.

Therefore, it was predicted that there should be three distinct populations of receptors in these cells: $mGlu_{2/2}$ homomers, $mGlu_{4/4}$ homomers, and $mGlu_{2/4}$ heteromers. However, the pharmacology results, particularly those performed with an agonist that activates only the $mGlu_4$ side of the dimer, suggested that almost all of the $mGlu_4$ expressed in these cells was in heteromeric form. This prompted an exploration of the stoichiometry of this apparent dominance of heteromer formation. Remarkably, when transfected in a 1:10 ratio ($mGlu_2$/$mGlu_4$), PHCCC (**20**) or 4-PAM2 still did not potentiate the activity of glutamate.[134] These results suggest that heteromerization appears to dominate, causing $mGlu_4$ to assemble almost exclusively in heterodimer form when the two are coexpressed. Future studies examining this dominant effect and the intracellular/membrane trafficking of $mGlu_{4/4}$ receptors versus $mGlu_{2/4}$ receptors are clearly warranted.

Upon discovering that different allosteric modulators exhibited distinctions in their ability to potentiate $mGlu_4$ homomers versus heteromers, we sought to expand our studies to native tissue populations and probe for the potential presence of functional heterodimers in endogenous cell populations, such as neurons in which $mGlu_2$ and $mGlu_4$ can be coexpressed. One such cell population is neurons projecting from the cortex to the striatum (corticostriatal) synapses; these synapses will respond to both $mGlu_4$ and $mGlu_2$ agonists.[134,136,139–143] After validating antibody specificity in vitro using cell lines coexpressing $mGlu_2$ and $mGlu_4$, we performed coimmunoprecipitation studies showing that that $mGlu_2$ and $mGlu_4$ can be coimmunoprecipated from cortical and striatal brain tissue from mice and rats, suggesting the potential interaction of the proteins in these regions. Previous studies had shown that Lu AF21934, a PAM that we had noted to activate $mGlu_{2/4}$ heteromers, was effective in potentiating responses at corticostriatal synapses. This led us to test the hypothesis of whether PHCCC (**20**), a “homomeric $mGlu_4$-selective PAM”, would also potentiate corticostriatal responses. Consistent with the hypothesis that an $mGlu_{2/4}$ heteromer is responsible for contributing to presynaptic responses in these neurons, PHCCC (**20**) did not potentiate the effects of $mGlu_4$ agonists at the corticostriatal synapse.[136] In contrast, and in confirmation of the results with Lu AF21934 reported by Bennouar et al.,[136] VU0155041 induced robust potentiation of the response to the $mGlu_4$ agonist L-AP4.[134] Consistent with our observations in cell lines, the lack of potentiation with PHCCC (**20**) suggested that, again, the $mGlu_{2/4}$ interaction dominates when $mGlu_2$ and $mGlu_4$ are expressed together. As a final experiment providing further validation of the expression of an $mGlu_{2/4}$ heteromer at corticostriatal synapses, the group II mGlu antagonist MNI-137 blocked responses to L-AP4, a group III agonist. This again suggests that there is a functional heteromer, responsive to both $mGlu_2$ and certain $mGlu_4$ ligands, expressed at this location.

It should be noted that PHCCC (**20**) has been shown to potentiate responses at numerous other synaptic locations in the brain, including the striatopallidal synapse, the lateral olfactory tract-piriform cortex projections, and neurons projecting from the subthalamic nucleus to the substantia nigra pars compacta.[126–128] This suggests that there might be differential expression of $mGlu_4$ homomers versus heteromers throughout the brain and potentially in other non-neuronal tissues. Although complicating from a biology and pharmacology perspective, the mGlu receptors are the targets of intense study for the

development of therapeutics for a number of disorders, particularly those of the CNS. For example, activation of $mGlu_4$ has been proposed to be a novel, nondopaminergic strategy to treat Parkinson's disease (PD).[144,145] To date, numerous $mGlu_4$ PAMs from different research groups all appear to show consistent antiparkinsonian activity in rodent PD models such as haloperidol-induced catalepsy. [126,146–149] However, the finding that some compounds affect neurotransmitter release from cortical projections into the striatum and others do not could have important implications for PD therapy, from standpoints of both efficacy and side effects. Whereas efficacy in acute, symptomatic PD models is induced by multiple PAMs and might not directly involve corticostriatal synapses, there are other complicating factors in PD treatment where changes in corticostriatal function might have implications. For example, in dopamine-depleted animals, corticostriatal synapses are overactive,[150,151] and this has been proposed to contribute to the loss of striatal medium spiny neurons in PD.[152] Dysregulation of plasticity at these synapses, such as changes in long-term depression (LTD) and long-term potentiation (LTP), has been proposed to contribute to the development of L-3,4-dihydroxyphenylalanine)- (L-DOPA−) induced dyskinesias, a debilitating and irreversible complication of prolonged L-DOPA treatment.[153,154] In this case, it is possible that heteromer-potentiating $mGlu_4$ PAMs could provide additional therapeutic benefits, such as restoring the morphology of striatal neurons and reversing L-DOPA-induced dyskinesias. Homomer-selective PAMs, in contrast, might be useful in treating the motor symptoms of PD with a reduced side-effect profile that might come from the lack of potentiation of $mGlu_4$, or changes in the regulation of its activity, when it is complexed with other mGlus. Obviously, this exciting area of GPCR pharmacology and biology will require detailed future studies. Mapping of different heteromer and homomer combinations would be helpful not only in thinking about therapeutics development but also in deconvoluting potentially confusing in vivo results induced by allosteric modulators with distinct profiles at homomeric and heteromeric forms of a target receptor.

## 2. OPTIMIZATION OF GPCR ALLOSTERIC MODULATORS

In a word, chemical lead optimization of GPCR allosteric modulators is *complex*.[1–12] The medicinal chemist must consider ligand bias, signal bias, PAM versus ago-PAM pharmacology, molecular switches, species differences, variations in affinity versus efficacy modulation, and notoriously steep SARs, all while optimizing potency, efficacy, and the drug metabolism and pharmacokinetic profiles. Within the PAM manifold, one must also consider whether low efficacy or low fold-shift (i.e., low cooperativity) is a desired profile to drive the SARs toward a candidate.[1–12] For instance, preclinical in vitro and/or in vivo data might suggest that PAMs with relatively high affinity and low cooperativity (or, conversely, low affinity and high cooperativity) will exhibit more favorable safety-toxicity and/or efficacy profiles. Therefore, efficient determination or estimation of PAM affinity and cooperativity [and intrinsic efficacy ($\tau_B$), if applicable] can prove vital to an optimization campaign. Also, as suggested above, heterodimers must also be considered, as activation of homo- versus heterodimers can result in dramatically different behavioral outcomes and/or efficacy outcomes. Fortunately, despite the gravity of each caveat, approaches have been developed to address each of these issues and enable the development of robust in vivo tool compounds

and clinical candidates. As “chance favors the prepared mind”, the discovery team must develop a workflow that incorporates a multiple-add screening paradigm, iterative parallel synthesis, and matrix libraries, as well as generating or acquiring cell lines across relevant species (e.g., mouse, rat, dog, nonhuman primate, and human) and with inducible expression.[1–12] Furthermore, key secondary assays must be in place to assess signal bias or to drive a program toward a discrete signaling transduction pathway, as well as elucidate the role of homo- versus heterodimers. However, successful navigation of these caveats provides entry into drug-like small molecules with unprecedented levels of subtype selectivity and opportunities for unique receptor pharmacology, while mimicking the most desirable aspects of native systems.

### 2.1. Steep Structure–Activity Relationships

Although robust SARs have been noted for allosteric ligands in discovery programs, the bulk of campaigns detail extremely “steep” SARs (also sometimes referred to as “flat” or “shallow”) wherein a potent allosteric modulator, more routinely noted for PAMs, loses all activity with a very modest structural modification.[1–12] This common occurrence requires that the classical medicinal chemistry approach of single-target designs be replaced with iterative parallel synthesis and matrix libraries,[1–12,155] both of which allow for serendipitous SAR discoveries and ensure that hypotheses are fully tested before a given chemotype is abandoned (Figure 5). The issue with steep SARs[156] can be even more pronounced when disposition is involved, as steric or electronic modulation of metabolic hot spots might not be tolerated in terms of allosteric modulator activity, complicating translational science and target validation beyond in vitro cell-based assays.

Recently, optimization of $M_5$ NAM chemotype ML375 (**26**) proved very challenging with a steep SAR (Figure 6).[157,158] After five rounds of iterative parallel synthesis, with few actives, a 3 × 7 matrix library approach was undertaken with a racemic core, wherein the vast majority of synthesized compounds displayed $M_5$ NAM activity in the mid- to high micromolar range; however, one lone analogue (**27**) emerged with potent $M_5$ NAM activity ($IC_{50}$ = 517 nM). Resolution then afforded the active (*S*)-enantiomer, VU6000181 (**28**) with an $M_5$ $IC_{50}$ of 264 nM and an improved in vivo disposition relative to that of **26**.[157,158] Of note, very close analogues to **27** were weak to inactive, and a deliberate, single-compound strategy would have unlikely identified **27**, as the 26 other analogues in the matrix library were too weak to be of interest. This example with $M_5$ NAM exemplifies another key challenge: addressing metabolic “hot spots” when so little structural or electronic modification is tolerated.[157,158] To overcome this limitation, employing the kinetic isotope effect has proven effective. For example, an $mGlu_3$ NAM, ML337, contains a key *p*-OMe moiety on an aromatic ring that is critical for mGlu subtype selectivity and activity but is also the major $P_{450}$ route of metabolism (*O*-dealkylation) [159,160]. All attempts to sterically or stereoelectronically shunt metabolism resulted in the complete loss of $mGlu_3$ NAM activity. Ultimately, replacement of the $OCH_3$ group with $OCD_3$ afforded an equipotent analogue, but both the in vitro and in vivo clearances were significantly lower (~50%), enabling in vivo studies to be performed.[160] Thus, in allosteric modulator series with steep SARs and drug metabolism and pharmacokinetics (DMPK) issues, the kinetic isotope effect can be an

invaluable tool in the medicinal chemist's arsenal to improve disposition while maintaining activity.

At the onset of a program, the "fluorine walk" has proven time and again to be an effective means for quickly identifying regions of an allosteric ligand that are tolerant to functionalization and then held constant for subsequent productive lead optimization.[1–12] Here, fluorine atoms are "walked" around a core and sampled for their ability to retain or enhance pharmacological activity by enhancing lipophilicity, accepting a hydrogen bond, and/or filling a small pocket. When attempted with other moieties (Me, Cl, CN, etc.), this strategy often fails. Once optimal positions for fluorine incorporation are identified, traditional optimization generally affords tractable, robust SARs.[1–12]

As mentioned previously, the $EC_{50}$ value for an allosteric modulator is a conglomerate, arbitrarily determined at a low (e.g., $EC_{20}$) concentration of orthosteric agonist, and reflects the impacts of intrinsic efficacy ($\tau_B$), cooperativity ($\alpha$ and $\beta$), and affinity ($pK_B$) modulation by the allosteric ligand.[1–12] Christopoulos and colleagues[161] recently demonstrated that, for a series of $M_1$ PAMs with steep SARs, dissecting the relative contributions of intrinsic efficacy ($\tau_B$), cooperativity ($\alpha$ and $\beta$), and affinity ($pK_B$) to the conglomerate $EC_{50}$ exposed deep, textured SARs. This approach should be considered when a very steep SAR is encountered to rationalize the disparities. Additionally, when coupled with aggregate data from in vivo efficacy studies with numerous compounds, heuristic modeling of in vivo concentration effects (and/or longer-time-scale pharmacokinetic-pharmacodynamic relationships) that incorporates aspects of modulator pharmacology from in vitro assays (e.g., in vivo $C_{max}$ values in an efficacy paradigm adjusted for the compounds' respective $K_B$, $\tau_B$, $\alpha$, and/or $\beta$ values or some combination thereof) might reveal evidence suggesting which modulator parameter(s) are most relevant to the particular efficacy paradigm and/or key insight(s) into the biology of the target mechanism/system (e.g., endogenous orthosteric ligand tone).[1–12,161] Furthermore, with the increasing number of X-ray crystal structures of GPCRs, with and without both orthosteric and allosteric ligands bound, there is hope that, in time, a deeper understanding of binding modes will be developed, as well as the potential for structure-based design of allosteric ligands.[10]

### 2.2. Molecular Switches

The propensity of a given allosteric chemotype to afford a broad range of pharmacology (PAM, ago-PAM, NAM, partial antagonist, NAL) and/or a dynamic range of receptor-subtype selectivity profiles with very subtle structural modifications have been termed "molecular switches".[33–35] Although they have been described across multiple class A GPCRs, they are most prevalent in metabotropic glutamate receptors (mGlus), especially $mGlu_5$ ligands.[35] Before the concept of molecular switches was formalized, it was observed in the first series of benzaldazine-based $mGlu_5$ PAMs (Figure 7).[62] Here, 3,3′-difluorobenzaldazine (DFB, **29**) was the first reported $mGlu_5$ PAM, but SAR studies found that the analogous dimethoxy congener (DMeOB, **30**) was an equipotent $mGlu_5$ NAM, whereas a dichloro analogue (DCB, **31**) was a NAL that blocked the function of both **29** and **30**.[62] After this initial discovery, hundreds of examples of this phenomenon have been

reported, which raised concerns over the in vivo oxidative metabolism engendering molecular switches for hydroxylated ligands.[33–35]

Pharmacologically active metabolites representing mode-switched allosteric modulators produced by simple, common metabolic biotransformations (e.g., hydroxylation of alkyl/aryl moieties, *N*-/*O*-dealkylation) have been observed for certain series of GPCR modulators.[33–35,160] Examples of such phenomena have been particularly pronounced within the biaryl acetylene class of $mGlu_5$ PAMs (Figure 8).[10,35] Although central activation of $mGlu_5$ has been hypothesized to have therapeutic value for schizophrenia and other neurological disorders, a target-mediated adverse effect liability (neurotoxicity and epileptogenesis) was recently discovered.[52–54,65] Although a subset of disclosed $mGlu_5$ PAMs with certain molecular pharmacological properties (e.g., specific affinity/cooperativity profiles and/or signaling bias) have been found to avoid such adverse effects while also retaining efficacy in preclinical models, it is now appreciated that mode-switched active metabolites with distinct PAM pharmacology and/or direct $mGlu_5$ agonist activity can further confound drug discovery and development efforts for this target.[37] For instance, VU0403602 (Figure 8), a $mGlu_5$ PAM with potent but low-efficacy agonist activity, was found to elicit pronounced receptor-mediated adverse effects in the form of seizures when administered systemically to rats; however, these effects were completely abolished by pretreatment with a pan-cytochrome $P_{450}$ inactivator [1-aminobenzotriazole (ABT)], suggesting a role for metabolism in the manifestation of the proconvulsant behavioral effects.[37] Subsequent metabolite identification studies revealed that a principal circulating metabolite (M1) produced by $P_{450}$-mediated monohydroxylation of the VU0403602 cyclobutyl group was a brain-penetrant $mGlu_5$ agonist-PAM exhibiting high agonist efficacy with moderate potency, and its exposure following administration of VU0403602 to rats pretreated with ABT was substantially reduced compared to rats administered the parent compound alone.[37] Systemic administration of chemically synthesized metabolite M1 to rats produced similar adverse behavioral effects, further supporting the hypothesized deleterious role of this metabolite. Additionally, rat brain slice electrophysiology experiments measuring long-term depression (LTD) at the hippocampal SC-CA1 synapse revealed that both parent VU0403602 and metabolite M1 induced LTD and that both ligands also induced epileptiform activity in CA3 neurons of the rat hippocampus.[37] These findings illustrate the importance of thorough identification and characterization of allosteric modulator metabolites, which can carry similar or often unanticipated distinct pharmacology at the receptor target engendered by even subtle single-atom biotransformations. Furthermore, potential species differences in the generation, disposition, and/or pharmacology of modulator metabolite ligands represent an additional layer of complexity and a further barrier to successful drug discovery and development in this area.[1–12] Despite the negative issues with molecular switches,[33–35] they can also be advantageous in other contexts. Once again, the literature is replete with examples in which weak off-target activity at a related GPCR can be exploited to identify a molecular switch that engenders preferential activity at the off-target GPCR while eliminating activity at the original target.[10,35] This beneficial feature enables discovery of in vivo tools and candidates without the need for a new HTS, as recently demonstrated for $mGlu_1$/$mGlu_4$,[156,162] $mGlu_5$/$mGlu_3$,[159,160] and $M_1$/$M_5$.[162–165] However, in a lead-optimization campaign, it is critical to avoid chemical series that exhibit

a strong propensity for molecular switching as this can confound the SARs and the metabolites that are generated in vivo have the potential to switch the mode of pharmacology and/or alter receptor-subtype selectivity.

**2.2.1. Mutations That Give Rise to Molecular Switches—**Allosteric ligands with positive and negative cooperativity with the same orthosteric agonist are known to interact within the same allosteric binding pocket on multiple GPCRs. For example, at $mGlu_5$, multiple structurally diverse scaffolds that have positive cooperativity with glutamate are able to fully displace binding of radiolabeled negative allosteric modulators (Figure 3b). In addition, among allosteric ligands that lack complete selectivity, there are multiple instances in which the direction of cooperativity differs between subtypes: MPEP is a NAM at $mGlu_5$ and a PAM at $mGlu_4$;[166] PHCCC (**20**), an $mGlu_4$ PAM, is a negative modulator at $mGlu_1$.[167,168] The reverse is also observed, where DFB (**29**) and CPPHA, $mGlu_5$ PAMs, negatively modulate responses to glutamate at $mGlu_4$ and $mGlu_8$.[62,169] This suggests that, within shared allosteric pockets, the structural determinants of cooperativity might be different such that ligands stabilize opposing receptor activation states. Moreover, allosteric ligand selectivity can largely be driven by differential cooperativity. Indeed, this was demonstrated for thiochrome interacting with muscarinic receptors (mAChRs), and in fact, it might be more common than is currently appreciated for other classes of mAChR modulators. Specifically, thiochrome has similar affinities for subtypes $M_1$-$M_4$, but it exhibits neutral cooperativity with ACh at $M_1$-$M_3$ and positive cooperativity with ACh at the $M_4$ subtype.[170]

Furthermore, as summarized above, in multiple $mGlu_5$-negative allosteric modulator scaffolds, minor alterations can give rise to ligands with positive or neutral cooperativity, and vice versa. Recently, the subtleties in ligand-receptor interactions that underlie cooperativity were highlighted with the identification of engineered mutations in mGlu receptors that give rise to molecular switches.[33–35] Two early studies found that mutation of a conserved Phe in TM6 could switch allosteric modulator cooperativity. At $mGlu_1$, YM298198 switched from a NAM of glutamate-stimulated $Ca^{2+}$ mobilization to a PAM when F801 was mutated to Ala.[171] Conversely, DFB (**29**), a PAM of quisqualate-stimulated $Ca^{2+}$ mobilization, became a NAM when the equivalent residue, F787, was substituted with Ala in the rat $mGlu_5$ sequence.[172] Subsequent studies applying more rigorous quantitative analyses identified another three conserved residues, Y658, W784, and S808 in rat $mGlu_5$, that gave rise to molecular switches in allosteric ligand pharmacology when mutated. Interestingly, W784A had differential effects on the cooperativity of negative allosteric modulators from different scaffolds, including decreasing the magnitude of negative cooperativity in addition to switching to positive cooperativity with gluta-mate.[173,174] Of note, whereas mutation of this Trp was detrimental to the affinity of both negative and positive allosteric modulators, the cooperativity of PAMs was either unaffected or increased.[174,175] These data suggest that W784 is crucial for stabilization of distinct receptor conformations by negative allosteric modulators. Conversely, Y658 in TM3 and S808 in TM7 converted certain PAMs of glutamate at the wild-type rat $mGlu_5$ receptor to NAMs or neutral ligands.[174,175] Recent $mGlu_5$ cocrystal structures of the 7TM domains with negative allosteric modulators observed Y658, S808 and W784 hydrogen bonding with a water

molecule.[176,177] These data suggest that changes in the water network and/or the stability of water-receptor interactions can also contribute to cooperativity mode switches, instead of, or in addition to, direct ligand-receptor interactions. In the $mGlu_1$ 7TM cocrystal structure with FITM (a negative allosteric modulator), however, no crystallographic waters were present within the allosteric modulator binding pocket.[178] The absence of such interactions within the $mGlu_1$ structure might reflect subtype-dependent differences (despite high homology between $mGlu_1$ and $mGlu_5$) or the absence of water-mediated ligand-receptor interactions within $mGlu_1$ with FITM.

Single-point mutations that give rise to switches in the cooperativity of allosteric ligands are not confined to the metabotropic glutamate receptors. For example, mutations within the orthosteric site of $M_2$ muscarinic receptors can switch LY2033298 from a negative modulator of QNB affinity to a positive modulator.[78] At the $M_1$ muscarinic receptor subtype, a single-point mutation within the allosteric site switches the cooperativity of BQCA with ACh from positive to neutral/NAM in both binding and functional assays.[82] Collectively, these data highlight the sensitivity of allosteric interactions to both the chemical nature of the two ligands under investigation and their respective ligand-receptor interactions.

## 3. ADVANCES IN METABOTROPIC GLUTAMATE RECEPTOR (MGLU) ALLOSTERIC MODULATORS

The metabotropic glutamate receptors (mGlus) are a group of eight GPCRs that bind glutamate, the major excitatory neurotransmitter in the mammalian central nervous system (CNS), and modulate synaptic transmission.[179–181] Characteristic of family C GPCRs, mGlu receptors contain a seven transmembrane (7TM) α-helical domain connected through a cysteine rich-region to a large bilobed extracellular aminoterminal domain, termed the Venus flytrap domain (VFD). The mGlus are further subdivided into three groups according to their homology, signal-transduction mechanisms, and pharmacology.[179–181] Whereas the group I mGlus ($mGlu_1$ and $mGlu_5$) are primarily located postsynaptically in neurons and coupled through $G_q$ to the activation of phospholipase C, the group II mGlus ($mGlu_2$ and $mGlu_3$) and the group III mGlus ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) are primarily located presynaptically and are coupled through $G_{i/o}$ to the inhibition of adenylyl cyclase activity. Orthosteric ligands of the mGlu receptors are typically glutamate analogues with poor physiochemical properties, lack of mGlu subtype selectivity, poor oral bioavailability (requiring prodrugs), and/or poor CNS penetration.[10,179–181] These significant limitations with orthosteric ligands make allosteric modulation, and the desired profiles of allosteric ligands, a particularly attractive approach for mGlu receptors. Again, numerous excellent reviews have covered mGlu receptor allosteric modulators,[1–12,179–181] but the past three years have witnessed significant advances, not yet captured in a review format. Here, we present vignettes covering the latest developments with respect to allosteric modulators of $mGlu_1$, $mGlu_2$, $mGlu_3$, $mGlu_4$, $mGlu_5$, and $mGlu_7$.

### 3.1. Allosteric Modulators of the $mGlu_1$ Receptor

Of the group I mGlu receptors ($mGlu_1$ and $mGlu_5$), $mGlu_5$ is by the far the most understood and validated in numerous CNS disorders with both orthosteric ligands and a wide range of

allosteric NAMs and PAMs.[1–12,23,54,55,90,91,182] For $mGlu_1$, the major focus has been on $mGlu_1$ NAMs, for which several excellent reviews exist.[183,184] In contrast, very little work has been focused on $mGlu_1$ PAMs since the first disclosure in the early 2000s by Roche (Figure 9).[185–188] First-generation PAMs **34–38** were potent but suffered from species differences, poor DMPK profiles, and poor CNS exposures, with **38** as the only $mGlu_1$ PAM tool compound with modest CNS exposure ($K_p$ value of 0.28).[185–188] However, as the only available $mGlu_1$ PAM in vivo tool, it has been employed to preclinically validate $mGlu_1$ in multiple CNS disorders.[189–192] With the new emphasis on genetic basis of disease, two recent, independent studies identified 12 rare, deleterious nonsynonymous single-nucleotide polymorphisms (nsSNPs) in the *GRM1* gene, which encodes $mGlu_1$, in schizophrenic patients; this has renewed interest in $mGlu_1$ PAMs, as these mutations were shown to be loss of function.[193,194] Work from the Vanderbilt group has characterized the mutant $mGlu_1$ receptors and demonstrated that **38** could indeed potentiate their response to glutamate and, in some instances, restore signaling.[162] However, to definitively validate $mGlu_1$ potentiation, improved in vivo tools would be required. In lieu of an HTS, we relied on molecular switches to gain access to novel $mGlu_1$ PAMs.[162] From the earliest days of PHCCC (**20**), pharmacological similarities between the $mGlu_4$ and $mGlu_1$ allosteric sites were known.[167,195] Thus, starting from the $mGlu_4$ PAM **39**, imide manipulation induced a "double molecular switch", involving not only a change in subtype selectivity (from $mGlu_4$ to $mGlu_1$) but also a change in the mode of pharmacology from PAM to NAM, to produce the $mGlu_1$ NAM **40**.[162] Further optimization led to a phthalimide moiety, which, in combination with 6-chloro substitution of the pyridyl amide functionality, modulated the mode of pharmacology to provide the $mGlu_1$ PAM **41**.[162] Subsequent optimization to improve metabolic stability, CNS penetration, and mGlu subtype-selectivity gave rise to $mGlu_1$ PAMs **42** and **43**, which potentiated both human and rat $mGlu_1$ as well as the $mGlu_1$ mutants.[156,196] PAM **42** exhibited an improved DMPK profile ($K_p > 1$, $F_u > 0.04$), but selectivity versus $mGlu_4$ eroded (~35-fold).[156] Application of the fluorine walk strategy led to **43**, a 12.9 nM $mGlu_1$ PAM with >793-fold selectivity versus $mGlu_4$.[196] Excitingly, these new tool compounds enabled the dissection of the adverse effect liability of group I agonists, such as DHPG, toward epileptiform and seizure liability, a consequence noted with $mGlu_5$ ago-PAMs. Interestingly, $mGlu_1$ ago-PAMs/PAMs did not induce epileptiform activity in the CA3 region of the hippocampus or induce seizures in vivo at drug concentrations far above the $mGlu_1$ PAM $EC_{50}$ (>100 times), suggesting that the adverse effect liability of group I agonists, such as DHPG, is mediated solely by agonism at $mGlu_5$.[156] Thus, the genetic data, coupled with the potential for a larger therapeutic window than $mGlu_5$, should garner more attention for $mGlu_1$ in the future.

## 3.2. Allosteric Modulators of the $mGlu_2$ and $mGlu_3$ Receptors

The group II mGlus ($mGlu_2$ and $mGlu_3$) are primarily located presynaptically and are coupled through $G_{i/o}$ to the inhibition of adenylyl cyclase activity.[127,197,198] Of note, $mGlu_3$, but not $mGlu_2$, is also found in glial cells, where its activation plays important roles in glial function and glial-neuronal interactions.[199] Finally, both group II mGlu receptors are widely expressed throughout the CNS, including but not limited to the amygdala, hippocampus, and prefrontal cortex, regions linked to emotional states.[200,201]

Frequently, the arguments undergirding the rationale for the development of allosteric modulators of GPCRs can be traced to historical studies conducted with small-molecule orthosteric ligands. Such is the case with the group II mGlu receptors, where research with both orthosteric $mGlu_{2/3}$ agonists and $mGlu_{2/3}$ antagonists helped establish the potential for these receptors as drug targets for the treatment of a variety of CNS disorders. In both cases, a handful of highly functionalized glutamate analogues served as workhorse tools for in vivo preclinical studies (Figure 10). Bicyclo[3.1.0]hexane LY354740 (**45**, eglumegad)[202] and its closely related ether analogue LY379268 (**46**),[203] both discovered at Eli Lilly, are prototypical $mGlu_{2/3}$ orthosteric agonist tools and have been used preclinically to establish potential therapeutic applications for $mGlu_{2/3}$ activation in anxiety,[202,204–207] addiction,[206,208–210] and certain types of neuroprotection.[211–214] Moreover, LY354740 (**45**) and its corresponding *N*-acyl L-alanine-derived prodrug (LY544344)[215,216] advanced into multiple clinical trials in patients for the treatment of anxiety disorders; although results were mixed, the drug was well-tolerated.[217–221] In addition, much of the preclinical research with these tools has been directed toward the establishment of the potential utility of an $mGlu_{2/3}$ agonist in novel treatments for schizophrenia.[222–225] Furthermore, a prodrug of the sulfone $mGlu_{2/3}$ agonist LY404039 (**47**, pomaglumetad)[226] known as LY2140023 (**48**, pomaglumetad methionil)[227] (Figure 10) advanced into multiple clinical trials in schizophrenic patients with initially encouraging results.[228] Unfortunately, subsequent clinical trials with LY2140023 were either inconclusive or failed to differentiate from placebo,[229–234] and further development of the compound was halted in 2012.[235]

Concomitant to the development of these $mGlu_{2/3}$ agonist tools, studies with orthosteric $mGlu_{2/3}$ antagonists were establishing a potential therapeutic role for $mGlu_{2/3}$ inhibition in the treatment of a number of CNS disorders as well.[236] Again, two highly functionalized glutamate analogues, LY341495 (**49**)[237] and MGS0039 (**50**)[238] (Figure 10), were employed for the vast majority of this preclinical work. Whereas MGS0039 (**50**) is an analogue of $mGlu_{2/3}$ agonist LY354740 (**45**), LY341495 (**49**) is quite structurally distinct. These tools have been used to help establish antagonism of $mGlu_{2/3}$ as a novel target for the treatment of obsessive-compulsive disorder (OCD),[239,240] anxiety,[241] cognition,[242] and Alzheimer's disease.[243–245] Additionally, much work describing the antidepressant effects of these compounds has been published,[238,239,241,246–251] including studies designed to model treatment-resistant depression (TRD)[252] and anhedonia.[253] With such therapeutic promise for both $mGlu_{2/3}$ activation and inhibition, it was clear that selective ligands for the individual group II receptors were required to further understand the role of each in these various indications. The design of allosteric modulators, both positive and negative, offers an attractive mechanism for achieving such goals.

Research related to the design of selective positive allosteric modulators (PAMs) of $mGlu_2$ as potential alternatives to $mGlu_{2/3}$ orthosteric agonists has been ongoing for some time.[254,255] The rationale for selective activation of $mGlu_2$ was buoyed by studies with knockout mice that implicated activation of that receptor in driving the antipsychotic efficacy of the Eli Lilly orthosteric $mGlu_{2/3}$ agonists.[256,257] The prototypical $mGlu_2$ PAM preclinical tools are two structurally unrelated compounds (Figure 11): a tertiary sulfonamide known as LY487379 (**51**)[258,259] and a 2-cyclopentyl indanone known as BINA

(**19**).[260] Studies with these $mGlu_2$ PAMs have since recapitulated much of the preclinical efficacy observed with $mGlu_{2/3}$ agonists and sparked significant interest in this class of compounds.[261–265] In fact, despite recent questions regarding the future prospects for $mGlu_{2/3}$ orthosteric agonists,[265] newly published and promising research efforts with $mGlu_2$ PAMs have continued to emerge in recent years (2012-present) and are summarized below.

A collaborative effort between scientists at Sanford-Burnham, UC San Diego, and Vanderbilt has continued to investigate new analogues of BINA (**19**) in search of $mGlu_2$ PAMs with improved potency and pharmacokinetic (PK) properties (Figure 12). One effort centered on the preparation and evaluation of a number of isoindolinone analogues such as **52** (Y = $CH_2$) and benzisothiazolone analogues such as **53** (Y = S).[266] Functional $mGlu_2$ activity, passive membrane permeability, rat plasma stability, and rat liver microsomal stability were assessed to evaluate the new compounds. Five optimized compounds were evaluated in rat PK studies, and all exhibited generally poor CNS penetration (brain/plasma $K_p \leq 0.13$); however, compound **52** was a low-clearance compound with good bioavailability and attained brain levels consistent with its $mGlu_2$ PAM functional potency following oral dosing. Oral administration of **52** significantly reduced nicotine self-administration in rats. A second optimization effort from this group departed more dramatically from the BINA (**19**) chemotype; still, key structural features, such as the aryl carboxylic acid and lipophilic ketone, were maintained (**54**, Figure 12).[268] These compounds employ a 1,4-diaryloxybutane core, a feature previously employed by Merck for the design of selective $mGlu_2$ PAMs.[267] Interestingly, the strategy used in this effort was not solely focused on $mGlu_2$, instead pursuing the design of $mGlu_{2/3}$ PAMs from within this scaffold. Optimization of functional potency and in vitro DMPK properties identified nine compounds for rat PK studies. Even though brain distribution was low ($K_p \leq 0.03$), compound **54** reached brain levels in excess of both its $mGlu_2$ and $mGlu_3$ functional potency. Subsequent evaluation of **54** in a rat model of cocaine dependence showed that it dose-dependently decreased both cocaine- and food-maintained responding, in contrast to the prior studies with the selective $mGlu_2$ PAM BINA (**19**), in which only decreased cocaine-maintained responding was altered.[268] These studies provide additional evidence that activation of $mGlu_2$ might selectively modulate responding for drugs as opposed to natural rewards.

Researchers at Taisho Pharmaceuticals recently published the detailed characterization of the $mGlu_2$ PAM TASP0433864 (**55**),[269] a compound that is closely related to a series of $mGlu_2$ PAMs previously reported by Merck and exemplified by compound 56 (Figure 13).[270] A host of in vitro pharmacology and ex vivo electrophysiology experiments established TASP0433864 (**55**) as a generally selective $mGlu_2$ PAM; however, inhibitions of radioligand binding to $5\text{-}HT_{2B}$ and MAO-B by TASP0433864 (**55**) were within approximately 15 and 2.5 times, respectively, its $mGlu_2$ functional activity. TASP0433864 (**55**) reduced brain metabolic activity elicited by the *N*-methyl-D-aspartate (NMDA) antagonist memantine in the mouse prefrontal cortex (PFC), and quantitative electroencephalogram (EEG) studies in rats demonstrated that TASP0433864 (**55**) dose-dependently attenuated the increases in γ-band oscillation (GBO) induced by the NMDA antagonists MK-801 and ketamine. Because the pathophysiology of schizophrenia is thought to involve cortical hyper-glutamatergic

transmission caused by NMDA receptor hypofunction, these studies might indicate the potential of an $mGlu_2$ PAM such as TASP0433864 (**55**) to modulate that signaling pathway in schizophrenic patients. Finally, the antipsychotic effects of TASP0433864 (**55**) were established in vivo through its ability to inhibit ketamine-induced hyperlocomotion in mice and methamphetamine-induced hyperlocomotion in rats. Satellite PK studies in rats demonstrated that drug in plasma, brain, and CSF reached levels in excess of the functional $mGlu_2$ potency at efficacious doses.[269]

Janssen previously reported on its successful use of a computational strategy utilizing the three-dimensional shape and electrostatic similarity of multiple known $mGlu_2$ PAM chemotypes for the discovery of a new imidazopyridine $mGlu_2$ PAM scaffold, exemplified by compound **57**.[271] Recently, the same group described further optimization work within this scaffold that culminated in the discovery of compound **58** (Figure 14).[272] The objective in this work was to improve upon poor oral PK observed with **57**, which was attributed in part to its high lipophilicity. New analogues were prepared with diversity at the $C_7$ position and either a chloro or cyano group at the $C_8$ position of the imidazopyridine ring. Compounds **58–60** were among the new analogues that demonstrated both good $mGlu_2$ PAM potency, stability in rat and human liver microsomes, and superior plasma exposure in rats relative to lead 57 following oral dosing; however, **58** demonstrated the best balance of properties. Compound **58** was evaluated in a sleep-wake EEG model, and oral administration of **58** significantly suppressed REM sleep without clear effects on the other sleep-wake stages; these effects are consistent with other $mGlu_2$ activators from distinct chemotypes. A subsequent publication detailed continued optimization within this series in the context of a 4-phenylpiperidine substituent at the $C_7$ position (Figure 5) and described the discovery of JNJ-42153605 (**61**).[273] In this case, a triazolopyridine core was used as a less lipophilic alternative to the imidazopyridine. Critical to the success of this effort was the identification of the trifluoromethyl group at the $C_8$ position as an optimal substituent for $mGlu_2$ PAM activity. An extensive pharmacology, DMPK, and safety profile shows JNJ-42153605 (**61**) to be a highly optimized compound. As was the case with **15**, JNJ-42153605 (**61**) produced the expected phenotype in the rat sleep-wake EEG model. Moreover, the antipsychotic effect of JNJ-42153605 (**61**) was demonstrated by its ability to reverse PCP-induced hyperlocomotion. Additional behavioral studies more fully evaluating the antipsychotic properties of this compound were also recently reported.[274] In conditioned-avoidance experiments, JNJ-42153605 (**61**) demonstrated an ability to inhibit avoidance at doses that do not impair the escape response on par with $mGlu_{2/3}$ agonist LY404039 (**47**, pomaglumetad) and $D_2$ receptor antagonists. This study constitutes the first published example of efficacy with an $mGlu_2$ PAM in this established antipsychotic model. Finally, efforts to incorporate a radiolabel into this scaffold for the purposes of positron emission tomography (PET) imaging studies culminated in the discovery of [$^{11}$C]-labeled compound **62**, which appeared to bind specifically and reversibly to $mGlu_2$ receptors in vivo.[275]

A collaborative effort between Janssen and Addex Pharmaceuticals has resulted in a number of recent advances in $mGlu_2$ PAM research. Compound **63** is a weak $mGlu_2$ PAM that was identified as a hit from an HTS of the Addex library (Figure 15).[276] Computational modeling of the three-dimensional shape and overlay of **63** with other known $mGlu_2$ PAMs

helped inform an optimization strategy. SARs were generated around potency, stability, and hERG activity with significant chemical diversity examined around the substituents attached to the phenyl ring at the $C_4$ position of the pyridone ring. Careful attenuation of the basicity of the 4-pyridyl nitrogen shown in compound **64** proved key for overcoming hERG activity. Compound **64** was among the most attractive analogues and demonstrated superior brain levels following subcutaneous dosing in mice relative to other comparators. Again, efficacy in the aforementioned rat sleep-wake EEG model was employed to demonstrate activation of $mGlu_2$ in the CNS by **64**. A second compound from this series known as JNJ-40068782 (**65**), containing a 4-phenylpiperidine at the $C_4$ position, was also recently described.[25] Importantly, also disclosed was a radiolabeled version of JNJ-40068782 (**65**) containing a tritium at the $C_4$ position of the phenyl ring appended to the piperidine ring. This radiolabeled compound was successfully employed in both in vitro and in vivo studies. Interestingly, $mGlu_2$ PAMs from distinct chemotypes displaced [$^3$H]-JNJ-40068782 from cortical $mGlu_2$ receptors, indicating a potential common binding site. JNJ-40068782 (**65**) was found to be active in the rat sleep-wake EEG model and reversed PCP-induced hyperlocomotion.

In 2012, Addex Pharmaceuticals and their Janssen partners released top-line data from an exploratory phase IIa clinical study in patients with schizophrenia with a compound known as ADX71149 that met the primary objectives of safety and tolerability. Also, the drug demonstrated a positive effect as adjunctive treatment to antipsychotics in patients with residual negative symptoms.[277] At the time, the structure of the compound was not disclosed; however, recently, that information was released to the public.[278] ADX71149 is also known as JNJ-40411813 (**11**, Figure 15) and is a member of the pyridone scaffold highlighted above and a close structural analogue of JNJ-40068782 (**65**). The exchange of the 3-cyano group in JNJ-40068782 (**65**) for the 3-chloro group in JNJ-40411813 (**11**) was key for enhancing CNS penetration. The *n*-butyl group on the pyridone nitrogen of JNJ-40411813 (**11**) was chosen as it provided the best balance of properties, including hERG inhibition profile and efficacy following oral dosing in the rat sleep-wake EEG model. Additionally, a pair of back-to-back publications provided further detailed descriptions of its pharmacological and PK properties[279] and preclinical evaluation in antipsychotic models.[280] Not surprisingly, JNJ-40411813 (**11**) has a generally attractive preclinical profile, including efficacy similar to that of $mGlu_{2/3}$ agonist LY404039 (**47**) in multiple antipsychotic animal models. JNJ-40411813 (**11**) was also recently examined in a phase II proof-of-concept study in patients with major depressive disorder (MDD) with significant anxiety symptoms.[281] Although efficacy signals were met on some measures, the signal on the primary outcome measure was not significant, and the overall data did not support continued development of the compound in anxious depression.[278]

AstraZeneca has developed an $mGlu_2$ PAM known as AZD8529 (**13**) that has also advanced into phase II clinical trials (Figure 16).[282] AZD8529 (**13**) is an isoindolinone $mGlu_2$ PAM with a 1,2,4-oxadiazole at the $C_5$ position.[283] Detailed preclinical information concerning the compound is limited in the literature; however, a recent report describes its efficacy in nonhuman primate models of nicotine reinforcement and relapse.[284] Moreover, a phase II study (NCT02401022) for smoking cessation in female smokers is currently recruiting

participants.[285] There is also a publication providing details on the process chemistry optimization of the synthesis of AZD8529 (**13**) that describes the development of an intramolecular Diels-Alder reaction for the rapid synthesis of the key indolinone intermediate **57**.[286] Even though AZD8529 (**13**) was active in seven preclinical antipsychotic and two anxiolytic models, in a phase II study in patients with symptomatic schizophrenia, the compound failed to distinguish from placebo. It should be noted that this trial was conducted at a single dose and lacked a method for directly measuring target engagement (e.g., PET).[282]

The development of allosteric antagonists of the group II mGlus has been a fruitful area of research as well.[236,255] Two related benzodiazepine analogues developed at Roche, **26** (RO4491533)[287] and 59 (RO4432717)[288,289] (Figure 17), are useful $mGlu_{2/3}$ NAM in vivo tools and have demonstrated efficacy in rodent models of depression[290] and cognition.[288,290–292] One $mGlu_{2/3}$ NAM, decoglurant (**60**, RO4995819),[293] has advanced into human clinical trials, including a phase II trial in patients with major depressive disorder (MDD) resistant to ongoing treatment with antidepressants (NCT01457677).[294] Interestingly, decoglurant contains a 1,2-disubstituted alkyne, a feature also found in multiple $mGlu_5$ NAM clinical compounds.[295] Although research directed toward the design of novel allosteric antagonists of the group II mGlus has not been as extensive as that described above for $mGlu_2$ PAMs, some recent (2012–present) studies have been reported and are summarized below.

Domain Therapuetics recently disclosed details regarding a pyrazolo[1,5-*a*]quinazolin-5-one scaffold as a chemotype for the design of $mGlu_{2/3}$ NAMs (Figure 18).[296] A proprietary FRET assay was used to screen a small compound collection and identified hit **61** as a relatively weak $mGlu_{2/3}$ NAM. Substitution of the $C_8$ position was noted as key for improving potency, and modification of the N-acyl group to an endocyclic amide improved metabolic stability. Compound **62** is a potent $mGlu_{2/3}$ NAM with oral bioavailability and CNS exposure ($K_p$ = 0.27), and it was selected for study in a rodent memory deficit model. Results showed that oral administration of compound **62** dose-dependently improved spatial working memory in mice challenged with scopolamine. Following an earlier disclosure of this general chemotype by Domain,[297] scientists at Vanderbilt independently investigated the SARs within this series.[298] Several analogues were prepared and tested in functional assays of $mGlu_2$ and $mGlu_3$ with a diversity of aryl and heteroaryl groups at the $C_2$ and $C_8$ positions. Among the active compounds were $mGlu_{2/3}$ NAMs that were either equipotent at each receptor or $mGlu_3$-preferring. Compound **63** was the most potent compound and exhibited approximately 3-fold preference for $mGlu_3$.

The Vanderbilt group has also made substantial advances in the design of selective $mGlu_3$ NAMs (Figure 19). Their initial efforts began with an observation that compounds from within a series of 1,2-diphenylethyne $mGlu_5$ positive allosteric modulators (PAMs)[36] sometimes displayed weak $mGlu_3$ NAM coactivity but no $mGlu_2$ activity. Initial optimization began from the simple amide cross-screening hit **64** and progressed to VU0463597 (**65**, ML289).[159] The methoxy group at the $C_4$ position of the distal phenyl ring proved unique in conferring $mGlu_3$ potency and selectivity versus $mGlu_5$, that is, a molecular switch. Further optimization within this scaffold led to the second-generation

analogue VU0469942 (**66**, ML337).[160] This new compound is devoid of both $mGlu_2$ and $mGlu_5$ activity and can be used at high doses as an in vivo tool in mice; however, lower CNS penetration and higher protein binding in rats prevent its utility in that species. In fact, the Vanderbilt group recently published electrophysiology and in vivo work in a fear extinction model in mice with VU0469942 (**66**, ML337).[299] These studies implicated $mGlu_3$ as a major regulator in PFC function and demonstrated the practical utility of a selective $mGlu_3$ NAM tool.

Seeking to identify still improved $mGlu_3$ NAMs, we continued our effort toward this end.[300] The goals in this instance were two-fold. First, the desire was to move beyond the 1,2-diarylethyne scaffold because this motif is prone to bioactivation and formation of reactive metabolites that can lead to toxicity.[301,302] Second, the new $mGlu_3$ NAM required pharmacology and PK properties that enabled its use in both rats and mice. Once again, mining an internal collection of $mGlu_5$ PAMs proved fruitful by identifying compound **67** (Figure 20), which is essentially equipotent as an $mGlu_5$ PAM and an $mGlu_3$ NAM. Importantly, as was the case with the aforementioned 1,2-diphenylethyne compounds, an inherent selectivity versus $mGlu_2$ was also found with these analogues. Extensive SAR development led to the identification of an optimized compound known as VU0650786 (**68**, Figure 20). Installation of the 5-chloro substituent on the western pyridyl ring was key for engendering good PK properties. Variation of the eastern aryl ring identified the 2-fluoropyridin-3-yl ring as optimal for $mGlu_3$ versus $mGlu_5$ selectivity and enhanced PK properties. VU0650786 (**68**) demonstrated efficacy in a mouse marble burying model and a forced swim test in rats, anxiolytic and antidepressant models, respectively, where efficacy had previously been noted with orthosteric $mGlu_{2/3}$ antagonists.[239,240]

Whereas highly selective and optimized tools now exist to study the effects of $mGlu_2$ PAMs and $mGlu_3$ NAMs in animal models of CNS disorders, selective $mGlu_2$ NAMs and $mGlu_3$ PAMs have remained elusive. Such compounds would add tremendous value and are almost certainly being pursued in multiple laboratories. It is worth noting that one new selective $mGlu_2$ NAM was just recently documented in the primary literature.[299] To better understand the results of this study with VU0469942 (**66**, ML337) through the complementary use of an $mGlu_2$ NAM, we synthesized and characterized MRK-8–29 (**70**, Figure 21), a compound discovered at Merck and reported to be an $mGlu_2$ NAM in the patent literature.[236,303] MRK-8–29 (**70**) is a potent $mGlu_2$ NAM with excellent selectivity versus $mGlu_3$ in functional assays for those receptors. The generic features of this chemotype can be seen in Markush structure **69** (Figure 21). Based on data from within the patent application, a primary carboxamide was preferred to a cyano group at the $C_2$ position ($R^Q$). A number of aryl and heteroaryl groups (A) were tolerated at the $C_4$ position. The $C_7$ position was tolerant of a wide array of functional groups, including linkers (L) of varying lengths and atom compositions and terminal groups ($R^1$) that included heteroaryl rings and tertiary amines.[299,303] The disclosure of MRK-8–29 (**38**) led to the speculation that the structural similarity between **70** and the $M_1$ PAM BQCA (**71**)[304] was striking and that a scaffold-hopping exercise might afford a novel $mGlu_2$ NAM chemotype. The exercise did yield a potent ($IC_{50}$ = 207 nM) and highly selective (>30 μM vs other mGlus) $mGlu_2$ NAM tool compound, VU6001192 (**72**), validating that an established $M_1$ PAM chemotype serves as a

viable alternative for new analogue design.[305] With the development of new, subtype-selective group II NAMs, the field will soon understand the physiological roles and therapeutic potential of the individual subtypes.

### 3.3. Allosteric Modulators of the $mGlu_4$ Receptor

The metabotropic glutamate receptor 4 ($mGlu_4$) is a member of the group III mGlu receptor family (along with $mGlu_{6–8}$).[129,180,181] The group III mGlu receptors are predominantly expressed presynaptically and act as both auto-and heteroreceptors in the regulation of neurotransmitter release.[129,180,181] Although this group has received less attention than the group I and II mGlu receptors, because of the implication of $mGlu_4$ in a number of therapeutic areas, this receptor has received growing research interest over the past eight years, with interest predominantly centered on the role of $mGlu_4$ in Parkinson's disease (PD).[136–138,144,145] PD is caused by the degeneration of dopaminergic neurons in the substantia nigra that project to nuclei of the basal ganglia (BG). With the introduction of the direct and indirect functional models of the BG, researchers identified $mGlu_4$ as a potential druggable target within the BG to bring balance to the indirect pathway. Subsequent gene-profiling studies found $mGlu_4$ mRNA in the striatum and in presynaptic terminals at the globus pallius external (GPe), which is overactive in PD.[136–138,144,145]

Because of the difficulty in identifying subtype-selective orthosteric ligands, much of the research has been focused on identifying PAMs of $mGlu_4$.[1–12,136–138,144,145] A number of selective PAMs have been identified and shown to be active in preclinical models of PD. The first $mGlu_4$ PAM that was profiled was (−)-PHCCC (**20**) (Figure 22); however, this compound is a relatively weak $mGlu_4$ PAM and is not selective. Nevertheless, (−)-PHCCC (**19**) has been shown to be active in a number of models of PD including those modeling neuroprotection;[167] however, these studies were after either intracerebroventricular (icv) injection, or systemically in a 50% dimethyl sulfoxide vehicle because of its poor pharmacokinetic (PK) profile and limited brain exposure. Next, another $mGlu_4$ PAM (**23**, VU0155041) was reported to be active in the haloperidol-induced catalepsy model of PD;[144] however, this compound too suffered from poor brain exposure and was administered by icv injection.[148] Additionally, VU0155041 (**23**) was shown to be neuroprotective in the 6-hydroxydopaine (6-OHDA) rat model.[306] A subsequent report of an $mGlu_4$ PAM with systemic exposure in a nontoxic vehicle focused on the compound VU0364770 (**73**, ML292).[307] VU0364770 (**73**) was found to be active in a number of PD models when administered alone, including reversal of haloperidol-induced catalepsy and forelimb asymmetry-induced by 6-OHDA lesions in the median forebrain bundle.[146] In addition, when dosed in combination with an inactive dose of L-DOPA, reversal of forelimb asymmetry was potentiated,[146] suggesting that $mGlu_4$ PAMs might provide L-DOPA-sparing activity in the clinic. Two additional reports from Lundbeck (**24**, Lu AF21934)[136] and Addex (**74**, ADX88178)[149] further support the use of $mGlu_4$ PAMs as possible therapeutic interventions for PD through the modulation of the indirect pathway of the BG. Both Lu AF21934 and ADX88178 were shown to be active in the 6-OHDA model; however, they were only active in combination with L-DOPA. In addition to PD, Lu AF21934 and ADX88178 have been shown to be active in animal models of anxiety[308,309] and psychosis.[309,310]

### 3.4. Allosteric Modulators of the $mGlu_5$ Receptor

$mGlu_5$ is by the far the most advanced of all of the mGlu receptors in terms of allosteric ligand tool compound and drug discovery, defining the field in terms of PAM, NAM, and NAL ligands, as well as the core concepts of allosteric pharmacology and chemical optimization.[1–12,23,24,90,91] As mentioned previously, multiple $mGlu_5$ NAMs are in the clinic, and many excellent reviews are available.[1–12,23,24,90,91] Therefore, this vignette will cover new advances in the past year concerning partial NAMs, signal bias, and the first reports of $mGlu_5$ PAMs approved for investigational-new-drug- (IND−) enabling studies.

Complete blockade or inverse agonist activity by some full $mGlu_5$ NAM chemotypes, such as MPEP (**75**) and MTEP (**76**), demonstrated adverse effects, including psychotomimetic-like effects in animals and psychosis in humans (with related acetylene-based NAMs), suggesting a narrow therapeutic window.[90,91] In response to this potential issue, we identified $mGlu_5$ allosteric ligands with a new mode of pharmacology: partial antagonism.[27] These allosteric ligands display weak negative cooperativity. Based on this, concentrations of these compounds that fully occupy the allosteric site, in this case, the MPEP site, only partially block receptor signaling, in essence allowing varying degrees of agonist activity.[27] Development of “partial” $mGlu_5$ NAMs, characterized by their submaximal but saturable levels of blockade (and negative cooperativity), might represent a novel, more general approach to broaden the therapeutic window. However, this is not a consistent mode of pharmacology conserved within a given chemotype; rather, the degree of partial antagonism varies greatly.[33–35,311] To understand potential therapeutic versus adverse effects in preclinical behavioral assays, the activities of the partial $mGlu_5$ NAMs M-5MPEP (**77**), Br-5MPEPy (**78**), and VU0477573 (**79**), in comparison with the full $mGlu_5$ NAM MTEP (**76**), were examined across models of addiction and psychotomimetic-like activity (Figure 23).[312,313] M-5MPEP (**77**), Br-5MPEPy (**78**), and MTEP (**76**) all dose-dependently both decreased cocaine self-administration and attenuated the discriminative stimulus effects of cocaine. Moreover, the partial NAMs M-5MPEP (**77**) and Br-5MPEPy (**78**) demonstrated antidepressant-like and anxiolytic-like activity, corresponding with increasing in vivo $mGlu_5$ occupancy. PCP-induced hyperlocomotion, as well as the discriminative-stimulus effects of PCP, was potentiated by MTEP (**76**), but not by M-5MPEP (**77**) and Br-5MPEPy (**78**).[312] More recently, VU0477573 was reported as another partial NAM within this series that has higher affinity than the earlier partial NAMs, an excellent PK profile, and efficacy in rodent models of anxiolytic activity.[313] Thus, data are accumulating that demonstrate that efficacy with partial $mGlu_5$ NAM activity is comparable to that observed with full NAM activity but with a broader therapeutic index.

Recent advances are shedding light on the potential importance of differences in allosteric agonist activity and signal bias in determining adverse effects of $mGlu_5$ PAMs.[1–12,90] Certain $mGlu_5$ PAMs engender epileptiform activity, seizures, and neurotoxicity as evidenced by fluorojade staining.[52–55,65] Many PAM chemotypes drift in and out of ago-PAM activity, wherein the ligand activates $mGlu_5$ on the absence of glutamate.[90] In a recent study, VU0403602 (**81**), an $mGlu_5$ pure PAM derived from VU0360172 (**80**) optimized to eliminate allosteric agonist activity, has robust in vivo efficacy and does not induce adverse effects at doses that yield high brain concentrations (Figure 24). In sharp contrast, both in

vitro mutagenesis and in vivo pharmacology studies demonstrated that VU0422465 (**82**) is a potent ago-PAM that induces epileptiform activity and behavioral convulsions in rodents.[37] Thus, drug development efforts must avoid ago-PAM activity at $mGlu_5$ in both the parent and, as described earlier, the principle circulating oxidative metabolites.

In addition, multiple examples of signal bias induced by $mGlu_5$ PAMs were recently uncovered.[52–55] Within the CPPHA (**83**) series of $mGlu_5$ PAMs (a non-MPEP site ligand), the closely related analogue *N*-(4-chloro-2-((4-fluoro-1,3-dioxoisoindolin-2-yl)methyl)phenyl)picolinamide (NCFP, **84**) was found to be pharmacologically similar in all respects (Figure 24), except that it did not potentiate the induction of LTD and LTP in the hippocampus (i.e., synaptic plasticity), suggesting that NCFP (**84**) stabilizes a unique activated conformation of $mGlu_5$.[52] This finding is even more striking upon consideration that a single fluorine atom modulated the signal bias. With the adverse effect liability of $mGlu_5$ PAMs, thought to be mediated by the NMDA receptor, one approach to avoid the liability would be to identify PAMs that display signal bias away from potentiation of NMDA receptor activation.[53] In 2015, an industrial-academic collaboration between Janssen Research and Development and the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) identified a PAM with this profile.[54,55] VU0409551 (**85**) is a potent, selective, and orally bioavailable $mGlu_5$ PAM that displays robust antipsychotic and cognition-enhancing efficacy in the absence (stimulus bias) of direct potentiation of NMDA receptor modulation (Figure 24). This unique signal bias broadened the therapeutic window, enabling endorsement as the first disclosed $mGlu_5$ PAM clinical candidate for which IND-enabling studies were initiated.[54,55]

Finally, Eisai recently disclosed their novel $mGlu_5$ PAM safety assessment candidate **86**, wherein their strategy to avoid adverse effect liability was a low maximal glutamate fold-shift (i.e., low cooperativity).[314] This is consistent with a strategy proposed by Merck to avoid adverse effect liability of $mGlu_5$ PAMs by optimizing compounds with relatively low cooperativity. These exciting advances highlight multiple strategies to overcome target-related adverse events and the unique approaches and pharmacology possible with allosteric ligands.

### 3.5. Allosteric Modulators of the $mGlu_7$ Receptor

Although most of the work surrounding the group III mGlu receptors has been concentrated on $mGlu_4$, a receptor with growing implications for therapeutic relevance in Parkinson's disease and other disorders such as meduloblastoma, autism, and multiple sclerosis, both NAM and PAMs of the metabotropic glutamate receptor 7 ($mGlu_7$) were reported recently in the literature. $mGlu_7$ is thought to be a therapeutic target for various CNS disorders; polymorphisms in the *GRM7* gene have been linked to autism, depression, bipolar disorder, attention deficit hyperactivity disorder (ADHD), and schizophrenia.[315–329] The first reported allosteric agonist of $mGlu_7$, AMN082 (**87**), demonstrates agonist activity in vitro and was reported to be active in models of stress-related CNS disorders (Figure 25).[330] However, more recent reports suggest that the in vivo activity might involve mechanisms in addition to $mGlu_7$.[331] Although selective PAMs of $mGlu_7$ have yet to be reported, two recent compounds have been disclosed as pan-group III PAMs, namely, VU0422288 (**88**) and

VU0155094 (**89**).[332] These compounds, much like pan-PAMs of the muscarinic receptor families, have proven to be valuable tool compounds for beginning to validate the role of $mGlu_7$ in various biological and pathological processes.[51] For example, these compounds have been studied by electrophysiological experiments at Schaffer collateral-CA1 synapses in the hippocampus. Among the group III mGlu receptors, these synapses appear to express $mGlu_7$ only in adult animals, and activation or potentiation of $mGlu_7$ produces robust effects in modulation of synaptic transmission by a presynaptic mechanism.[332–335] These studies provide valuable proof-of-concept data that $mGlu_7$ activity can be modulated by a PAM, thus providing key indications for future therapeutic development. In addition to PAMs, there have been several reports of antagonists/NAMs of the $mGlu_7$ receptor. A recent report details the pharmacology of the $mGlu_7$ antagonist XAP044 (**90**), which acts not through the seven transmembrane region but rather through the extracellular Venus flytrap-like domain, normally reserved for orthosteric binding.[336] XAP044 (**90**) was shown to be CNS-penetrant and to exhibit adaptogenic (antistress), antidepressant, and anxiolytic-like efficacy in rodent models.[336] The isoxazolopyridone allosteric antagonist MMPIP (**91**) is selective for $mGlu_7$, exhibits a favorable in vivo pharmacokinetic profile, and is CNS-penetrant.[337,338] In addition, a radiolabeled version of the compound ([$^{11}$C]MMPIP) has been reported, and although high radioactive signals were detected in in vitro autoradiography in the thalamus, medulla oblongata, and striatum, no specific uptake relative to $mGlu_7$ was found in the examined brain regions.[339] MMPIP (**91**) also shows interesting pharmacology in vitro and does not antagonize all responses mediated by $mGlu_7$.[340] Addex Therapeutics recently reported a potent and selective $mGlu_7$ NAM, ADX71743 (**92**).[341] ADX71743 (**92**) was shown to be inactive against other subtypes of the mGlu receptor family and showed anxiolytic-like efficacy in a mouse model. ADX71743 (**92**) was also used, along with a group III receptor agonist, to elucidate the role of $mGlu_7$ in modulating transmission in hippocampal area CA1 in adult mice.[335,342] The results of this study suggest that $mGlu_7$ serves as a heteroreceptor at inhibitory synapses in area CA1 and that the effect of activation of $mGlu_7$ by stimulation of glutamatergic afferents is disinhibition and not reduced excitatory transmission.[335]

## 4. LATE-BREAKING DISCOVERIES

Here, in the final section, we capture hot, late-breaking discoveries in the realm of GPCR allosteric modulators, beyond the mGlu receptors discussed in depth. Three vignettes are covered that include endogenous GPCR allosteric modulators, $GABA_B$ NAMs, and proton-sensing GPR4 NAMs.

Recently, attention has focused on the possibility that allosteric sites on GPCRs, targeted by exogenous synthetic ligands, can also be modulated by endogenous allosteric ligands.[56] These endogenous ligands consist of G proteins, ions, lipids, amino acids, peptides, and a diverse array of accessory proteins.[56] First, G proteins themselves have been shown to alter GPCR conformation in an allosteric manner that is capable of modulating either binding or signaling of both orthosteric agonists and antagonists.[56] Ions, such as sodium and magnesium, have been shown to functionally modulate GPCRs, with the first report appearing in 1973 that $Na^+$ is a NAM of opioid agonist binding.[342] Since that time, mutagenesis studies have found a conserved aspartate residue in the second transmembrane

domain of class A GPCRs as critical for allosteric action of $Na^+$,[56,343] and new examples continue to be described. Third, lipids such as cholesterol have been shown to induce conformational changes in GPCRs, by modulation of lipid membrane or lipid raft compositions. In addition, endocannabinoids, lipoxin A4, pregnenolone and oleamide display allosteric modulation of a variety of GPCRs.[56] Amino acids, notably aromatic amino acids (L-Phe, L-Trp, and L-Tyr), act as endogenous allosteric modulators of specific GPCRs, such as the CaSR and $GABA_B$, and both small and large peptides have also been found to function as discrete GPCR allosteric modulators.[56] This area is in its infancy, and we expect that additional endogenous allosteric modulators will emerge as focused efforts search them out.

In 2014, Nan and co-workers reported the discovery of the first negative allosteric modulator (NAM) of $GABA_B$ receptors 93 (Figure 26), derived from a scaffold-hopping exercise based on the $GABA_B$ PAM CGP7930 (**94**).[344] Whereas the literature is replete with $GABA_B$ PAMs, until now, NAMs remained elusive. NAM **93** decreased $GABA_B$-induced IP3 production ($IC_{50}$ = 37.5 μM), displayed no effect on other class C GPCRs, and did not bind to the $GABA_B$ orthosteric binding site.[344] This new tool will enable further exploration of $GABA_B$ function and therapeutic potential.

Last year, Okajima and co-workers reported the identification and characterization of a series of imidazopryridine analogues, exemplified by **95** (Figure 27), that proved to be the first negative allosteric modulators of proton-sensing GPR4 in extracellular acidification-induced responses.[345] Moreover, **95** inhibited acidic-pH-stimulated cAMP accumulation, GPR4 internalization, and mRNA expression in inflammatory genes and was highly selective among proton-sensing GPCRs. In contrast to the GPR4 orthosteric antagonist psychosine (**96**), which loses efficacy in a histidine to phenylalanine mutation in the orthosteric site, the NAM **95** retains the ability to inhibit acidic-pH-induced activity.[345] This new tool compound, with a distinct, more drug-like chemotype than its orthosteric congener, will be invaluable in unraveling the complex pharmacology of proton-sensing GPR4.

## 5. CONCLUSIONS

A decade of intense research and development has elucidated both benefits and challenges of allosteric modulation of GPCRs, as well as the many caveats to successful optimization. Highly subtype-selective allosteric modulators now exist for a wide array of GPCRs with a diverse range of modes of efficacy beyond what is possible with traditional orthosteric ligands. Strategies and tactics have emerged to address steep SARs, molecular switches, signal bias, and differential effects on heterodimeric versus homodimeric complexes. The speed and frequency of crystal structures of all families of GPCRs (A, B, and C), alone and in complex with orthosteric and allosteric ligands, will offer new insights for ligand design and receptor theory. What new challenges and discoveries will be made in the next decade? Will surgical activation of discrete signaling pathways be commonplace? How many GPCR allosteric modulators will enter the market as therapeutics and become standards of care? One thing is certain: GPCR allosteric modulators have fueled a renaissance in GPCR pharmacology and small-molecule design and discovery.

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## Biographies

Dr. Lindsley is a Professor of Pharmacology and Chemistry at Vanderbilt University, holding the William K. Warren, Jr., Chair in Medicine and Director of Medicinal Chemistry for the Vanderbilt Center for Neuroscience Drug Discovery. He received his Ph.D. degree from the University of California, Santa Barbara, in 1996 (Lipshutz research group) and pursued postdoctoral studies at Harvard University (Shair research group) as an Institute of Chemistry & Cell Biology Fellow. Dr. Lindsley was a Senior Research Fellow and Group Leader in the Medicinal Chemistry Department at Merck and Company and then moved to Vanderbilt University as the cofounding director of the Vanderbilt Center for Neuroscience Drug Discovery, where he has advanced multiple drug candidates into development for neurological and psychiatric indications. Dr. Lindsley is the founding Editor-in-Chief of ACS Chemical Neuroscience and was the lone Associate Editor for *Current Topics in Medicinal Chemistry*. He has received numerous awards for his translational research including the John J. Abel Award from ASPET (2014), and the 2013 Philip S. Portoghese Medicinal Chemistry Lecture Award from the American Chemical Society, among others. His current interests lie in the area of allosteric modulation of GPCRs for the treatment of schizophrenia, addiction, depression, Parkinson's disease, Alzheimer's disease, and other neuropsychiatric disorders. Dr. Lindsley is the author of over 300 peer-reviewed manuscripts, review articles, and book chapters. In addition, he is the inventor on over 60 issued U.S. patents.

Kyle A. Emmitte received his Ph.D. in Organic Chemistry in 2001 from the University of North Carolina at Chapel Hill under the direction of Professor Michael T. Crimmins. He subsequently joined the Oncology Medicinal Chemistry group at GlaxoSmithKline in Research Triangle Park, North Carolina, where he made key contributions to the discovery of the PLK1-inhibitor GSK461364 and co-led the team that discovered the IGF-1R inhibitor GSK1904529. In 2008, he joined Vanderbilt University Medical Center as Research Assistant Professor and the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) as Associate Director of Medicinal Chemistry. During his time with the VCNDD, he led the teams that discovered an $mGlu_5$ NAM preclinical candidate as well as a number of allosteric modulator tool compounds, including an optimized and highly selective $mGlu_3$ NAM in vivo tool. In 2015, he joined the University of North Texas Health Science Center as Associate Professor in the Department of Pharmaceutical Sciences in the UNT System College of Pharmacy.

Corey R. Hopkins received his B.S. in Chemistry from Indiana University and his Ph.D. in Organic Chemistry from the University of Pittsburgh in 2002 under the direction of Professor Peter Wipf. After his doctoral studies, he joined the medicinal chemistry department at Aventis Pharmaceuticals where he worked on a number of CNS-related therapeutic targets (multiple sclerosis, depression) and inflammation-related targets (asthma, rheumatoid arthritis). In 2008, he joined the faculty as an Assistant Professor in the Departments of Pharmacology and Chemistry at the Vanderbilt University Medical Center, Nashville, TN, where he is also the Associate Director of Medicinal Chemistry for Vanderbilt's Center for Neuroscience Drug Discovery. His current interests lie in the area of allosteric modulation of GPCRs; kinase inhibitors as they apply to rare and neglected diseases; and ion channels for vector-borne diseases, such as malaria. Dr. Hopkins is the author of over 70 peer-reviewed manuscripts, review articles, and book chapters. In addition, he is the coinventor on over 25 patents.

Thomas Bridges is a Research Assistant Professor in the Department of Pharmacology at Vanderbilt University and a drug metabolism and pharmacokinetics (DMPK) scientist within the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD). After obtaining a B.S. in Biology from Wheaton College, he obtained his Ph.D. in Pharmacology in the Lindsley and Conn laboratories of the VCNDD before completing a postdoctoral fellowship in the Daniels laboratory, also of the VCNDD. He has since served as a DMPK Drug Discovery Scientist-I and as the primary DMPK representative to multiple interdisciplinary drug discovery project teams within the VCNDD (as well as program coleader), including those in active partnerships with pharmaceutical companies. Consequently, his research background and interests range from small-molecule medicinal chemistry and molecular pharmacology to preclinical pharmacokinetics and pharmacometrics.

Karen J. Gregory is an early career researcher (Ph.D. 2009) with a strong expertise in the molecular pharmacology of GPCRs. She has published 20 journal articles and 10 reviews/book chapters on allosteric modulation and stimulus bias of neurotransmitter GPCRs. Her research efforts are primarily directed toward the structural and molecular pharmacology of metabotropic glutamate receptors, with a particular focus on allosteric modulators and biased pharmacology. Research in her laboratory is currently supported by National Health & Medical Research Council (Australia) Project Grant 1084775 and CJ Martin Postdoctoral Fellowship 1013709.

Colleen M. Niswender, Ph.D., is an Associate Professor of Pharmacology and the Director of Molecular Pharmacology for the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) at Vanderbilt University. She obtained her doctorate in pharmacology in 1996 at Vanderbilt and then pursued postdoctoral studies in pharmacology at the University of Washington. She has worked with the VCNDD since 2004 and has extensive experience with receptor biology, cell signaling, assay development, high-throughput screening, lead optimization, and concepts of allosteric modulation. She is the author of over 140 publications, has contributed to numerous patents, and is currently involved in the progression of several lead candidates into studies required for clinical trials. She is highly interested in the basic biology and therapeutic potential of metabotropic glutamate and

muscarinic receptors in autism spectrum disorders, Parkinson's disease, schizophrenia, and other pathophysiological states that affect neurological and psychiatric health.

P. Jeffrey Conn is the Lee E. Limbird Professor of Pharmacology at Vanderbilt University and Director of the Vanderbilt Center for Neuroscience Drug Discovery. He received his Ph.D. degree from Vanderbilt in 1986 and pursued postdoctoral studies at Yale University before joining the faculty at Emory University in 1988. Dr. Conn served as head of the Department of Neuroscience at Merck and Company (West Point, PA) from 2000 to 2003 and then moved to Vanderbilt University as the founding director of the Vanderbilt Center for Neuroscience Drug Discovery, where he has advanced multiple drug candidates into development for neurological and psychiatric indications. Dr. Conn served as Editor in Chief of *Molecular Pharmacology*, on editorial boards of multiple other journals, and on Scientific Advisory Boards of multiple foundations and companies. He has received numerous awards for his translational research. Dr. Conn's research is focused on understanding the pathophysiology changes that contribute to serious brain disorders, including Parkinson's disease, schizophrenia, and depression, and using this understanding to develop novel therapeutic strategies for the treatment of these devastating disorders.

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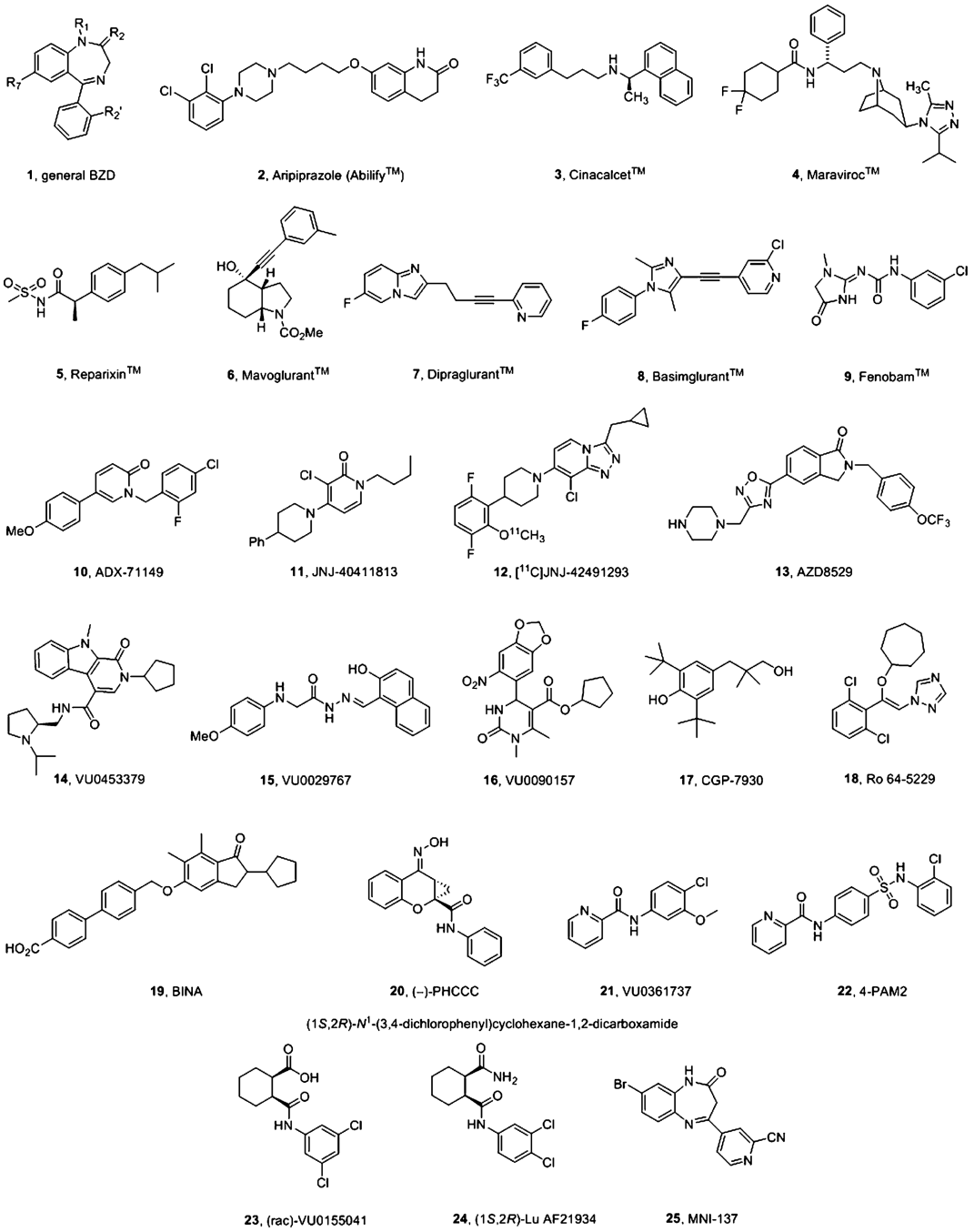



**Figure 1.**
Structures of compounds **1–25** discussed in the Introduction.

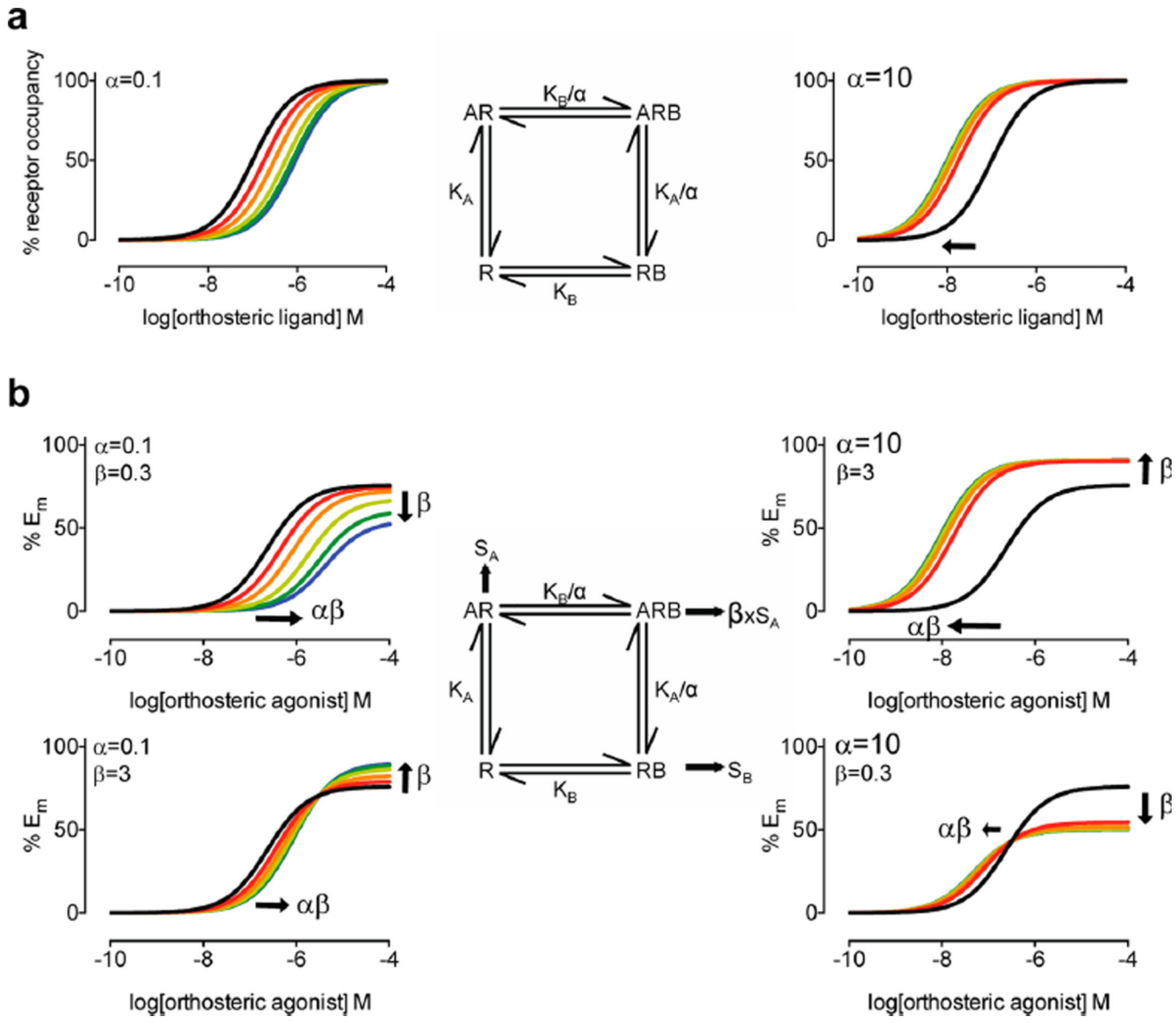



**Figure 2.**

Allosteric interactions can manifest as altered affinity and/or efficacy. (a) Simulations of the effect of an allosteric modulator on receptor occupancy by an orthosteric ligand, as described by the allosteric ternary complex model (center). In the absence of an allosteric ligand (black curve), relative receptor occupancy is determined by the concentration of orthosteric ligand (A) and the equilibrium dissociation constant ($K_A$), which is the concentration of A that occupies 50% of receptors. Increasing concentrations (red, $K_B$; orange, $3K_B$; yellow, $10K_B$; green, $30K_B$; blue, $100K_B$) of a negative allosteric modulator (left, α = 0.1) or a positive allosteric modulator (right, α = 10) alter the apparent affinity of the orthosteric ligand 10fold. (b) Simulations of allosteric interactions in a functional assay, as described by the operational model of allosterism (center). Top left, an allosteric ligand that negatively modulates both affinity and efficacy. Top right, an allosteric modulator that

potentiates both affinity and efficacy. Bottom left and right, allosteric ligands with opposing effects on affinity versus efficacy.

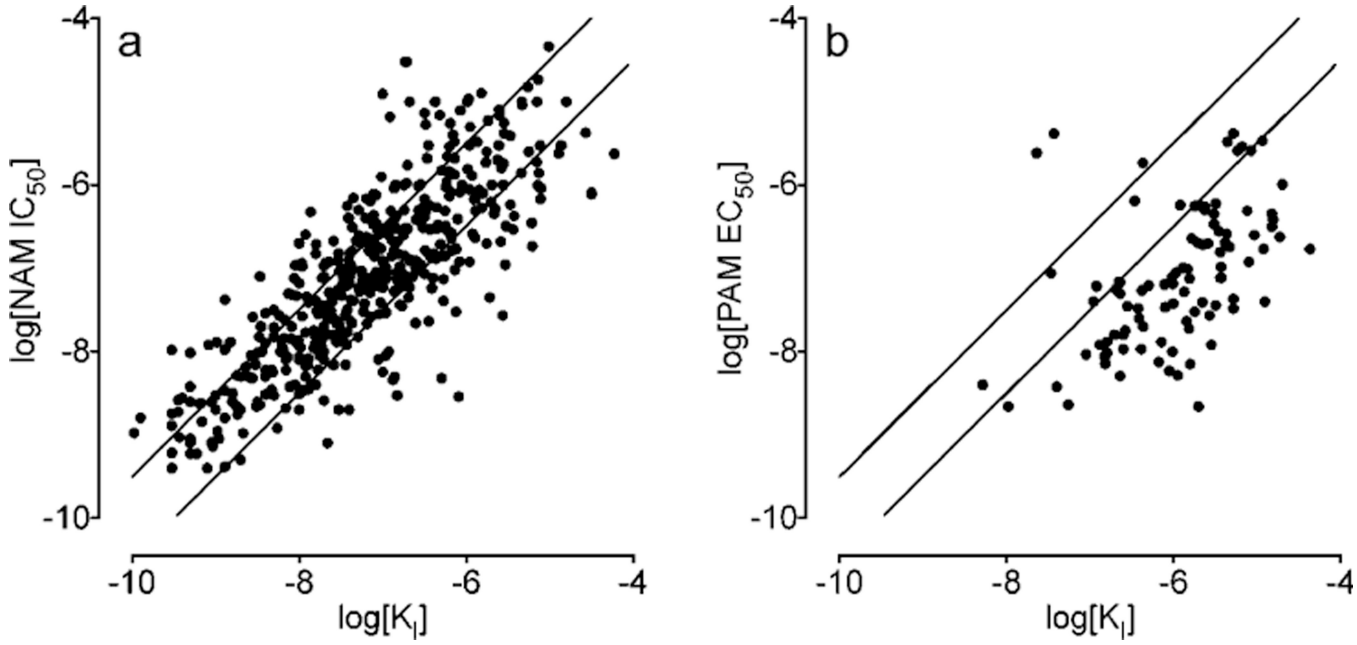



**Figure 3.**

Comparison of $mGlu_5$ modulator potency and affinity estimates. Potency values were pooled for (a) inhibition or (b) potentiation of orthosteric agonist activity (glutamate or quisqualate) in multiple paradigms including recombinant cell lines expressing either human or rat $mGlu_5$, or primary cultures, and using intracellular $Ca^{2+}$ mobilization and inositol phosphate accumulation. Affinity estimates were pooled from inhibition binding studies using radiolabeled allosteric ligands using membranes or whole cells from recombinant cells lines expressing either human or rat $mGlu_5$, primary cultures, or tissue homogenates.[64]

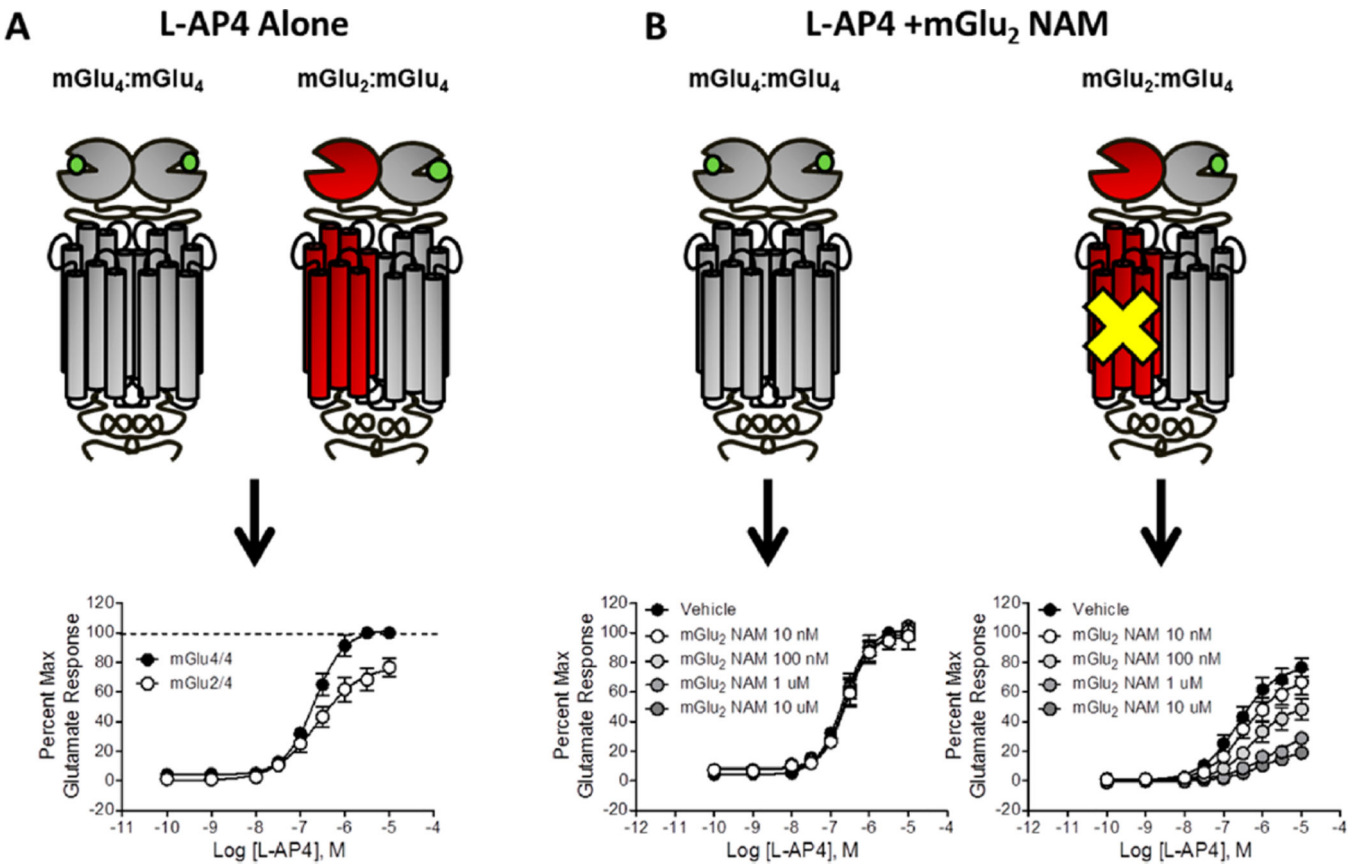



**Figure 4.**

Heteromerization of $mGlu_4$ and $mGlu_2$ permits an $mGlu_2$ NAM to block $mGlu_4$ agonist-mediated responses. (A) When $mGlu_4$ (gray) is expressed alone, both protomers respond to L-AP4 ($mGlu_4$-selective agonist, green circles), which induces responses with predicted potency and full efficacy (lower panel, black circles in graph). When $mGlu_2$ (maroon) is coexpressed with $mGlu_4$, the L-AP4 response is more shallow, and the response is approximately 75% (white circles) that of L-AP4 when $mGlu_4$ is expressed alone. (B) Incubation of increasing concentrations of an $mGlu_2$ NAM with $mGlu_4$ homomers results in no blockade of response. Incubation of NAM (yellow X) with $mGlu_{2/4}$ heteromers results in a concentration-dependent, noncompetitive blockage of L-AP4 responses, indicating transactivation between the two protomer subunits. Graphs are simulated based on data presented in ref 134.

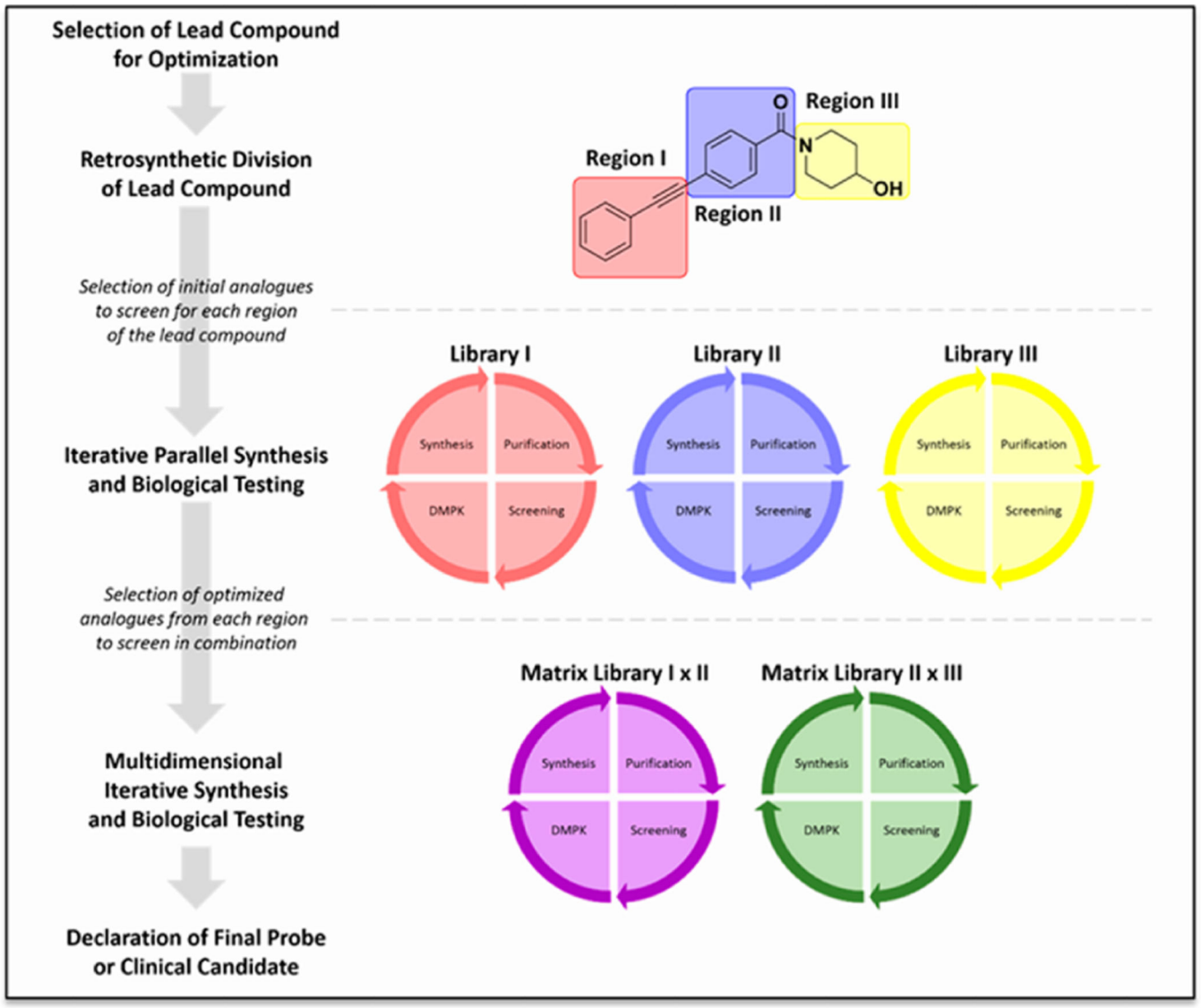



**Figure 5.**

Iterative, multidimensional parallel synthesis approach, coupled with matrix libraries for the chemical lead optimization of GPCR allosteric modulators.

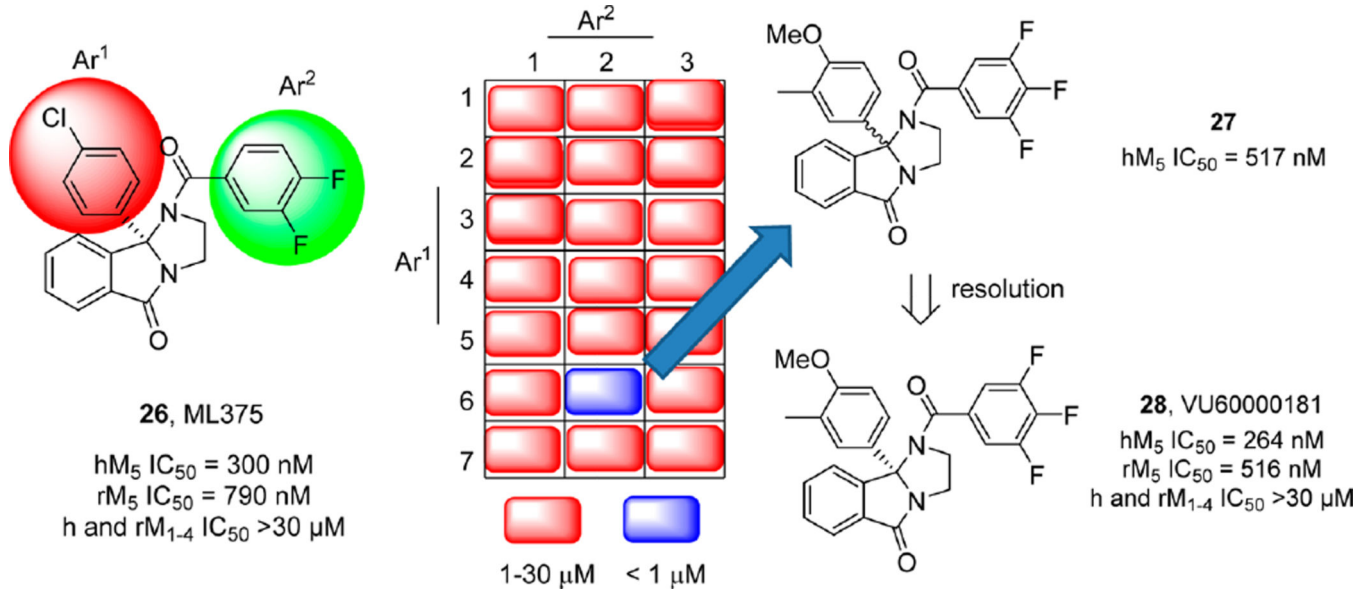



**Figure 6.**

Matrix library strategy for the chemical lead optimization of a series of $M_5$ negative allosteric modulators with steep SARs.

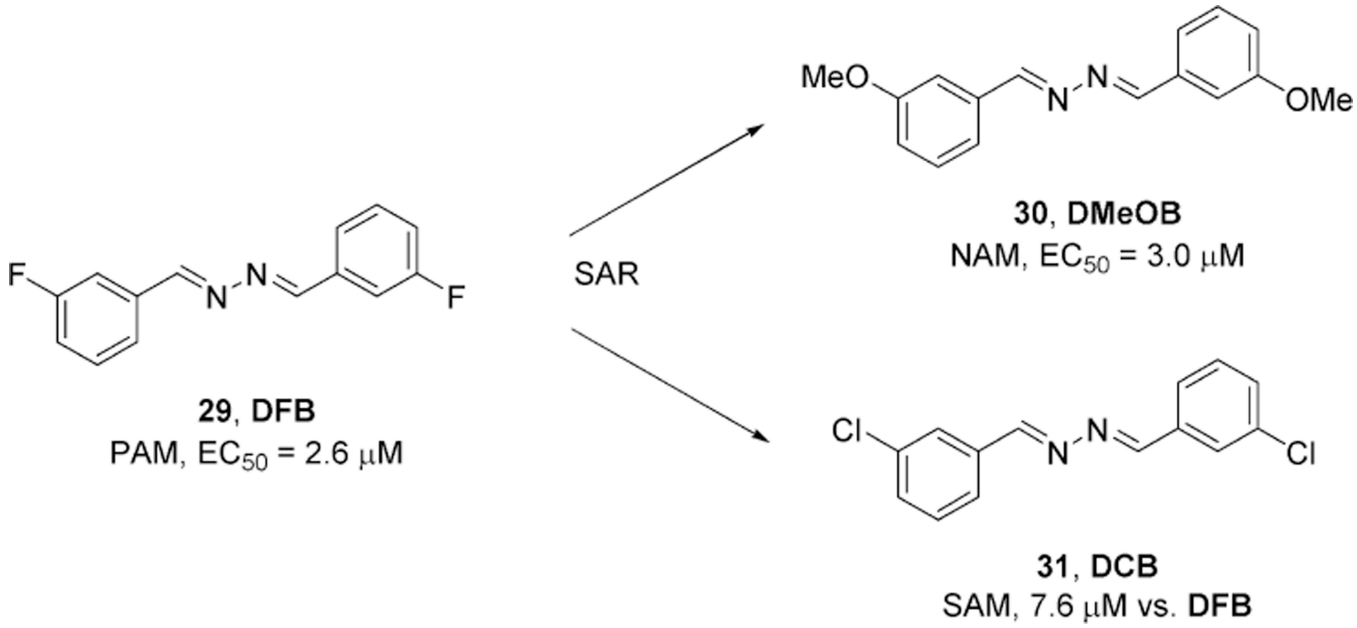



**Figure 7.**

Molecular switches were apparent in the first reported series of $mGlu_5$ PAMs, wherein small modifications afforded PAMs, NAMs, and NALs.

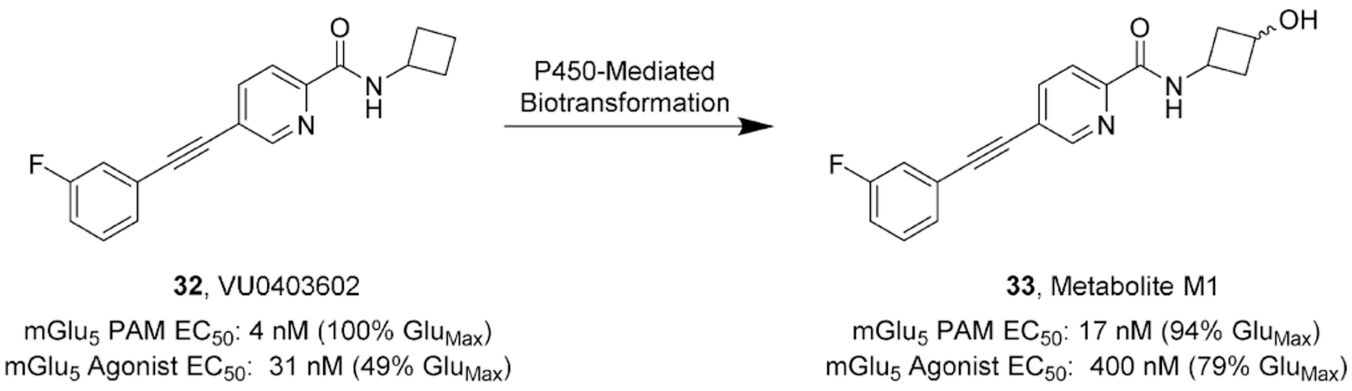



**Figure 8.**

Biotransformation of a potent $mGlu_5$ agonist-PAM, VU0403602 (**32**), through cytochrome P450-mediated metabolism to a major circulating and brain-penetrant active metabolite (M1, **33**) displaying similar PAM pharmacology with higher efficacy and lower potency intrinsic agonist activity in rat. Values represent means of at least three independent determinations in fluorometric calcium mobilization assays using rat $mGlu_5$-expressing HEK cells.

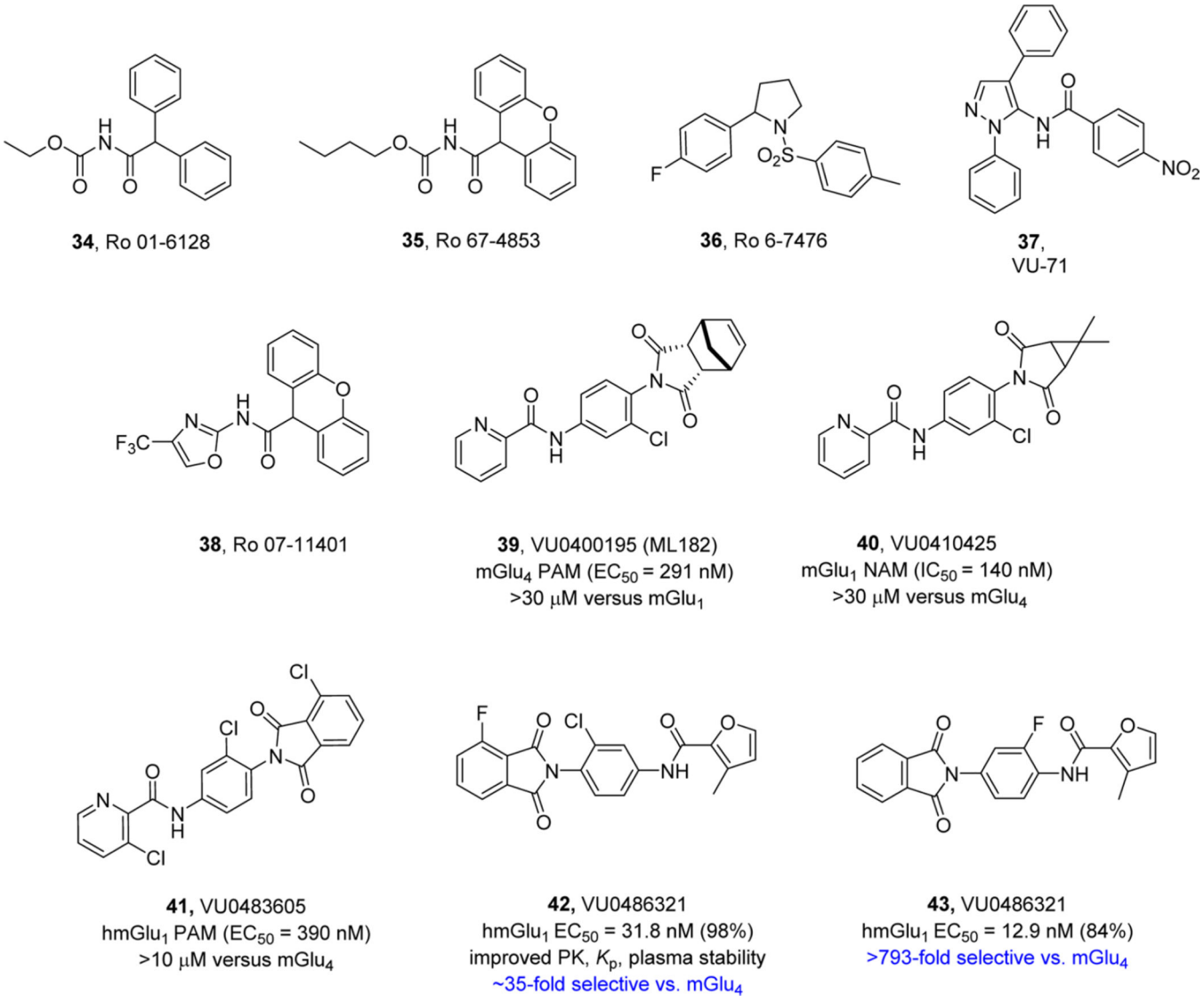



**Figure 9.**

Historical (**34–38**) and recent (**39–43**) mGlu1 PAMs, the latter of which were developed by exploiting molecular switches.

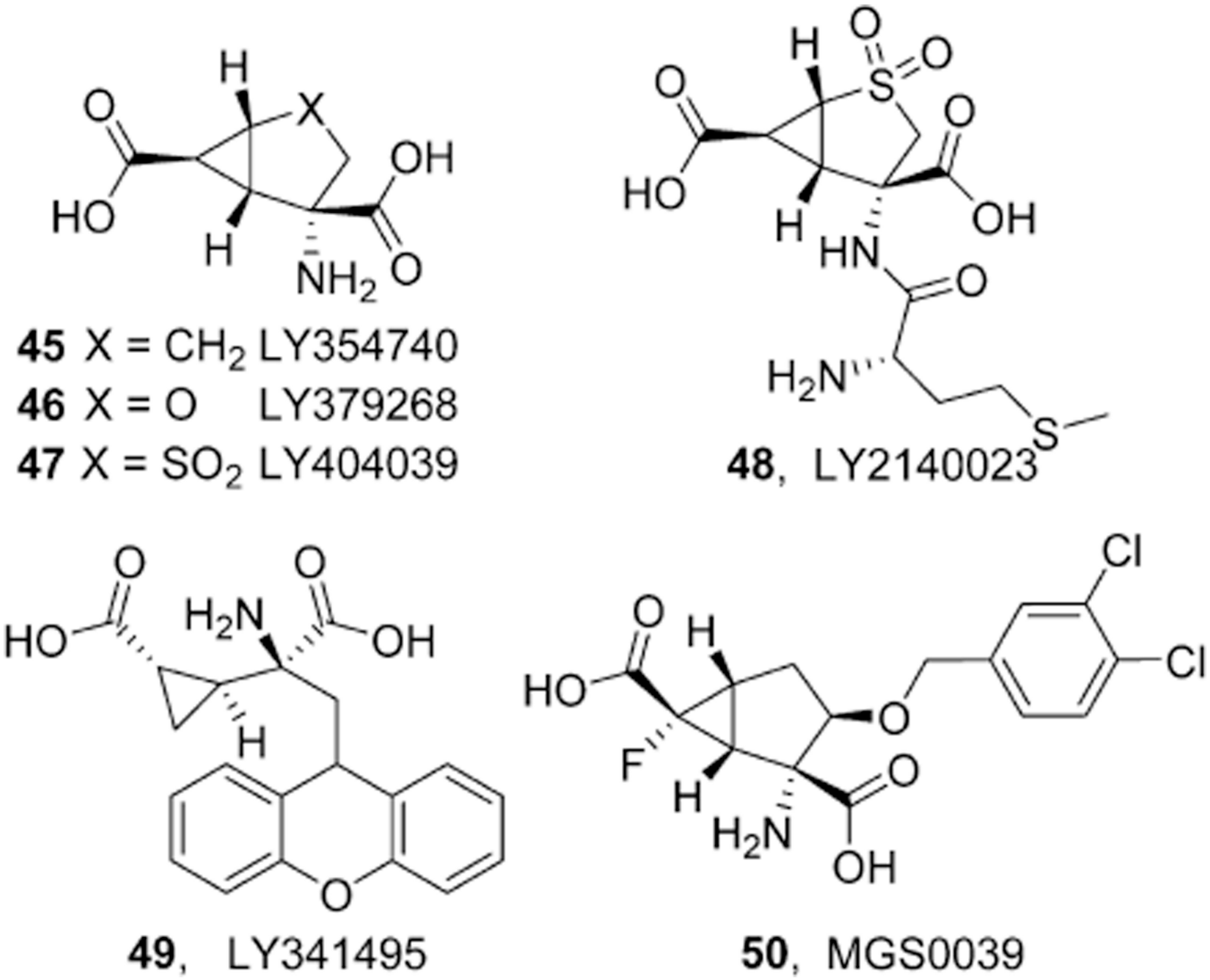



**Figure 10.**

$mGlu_{2/3}$ orthosteric agonists **45** (LY354640), **46** (LY379268), and **47** (LY404039); orally available clinical prodrug **48** (LY2140023); and $mGlu_{2/3}$ orthosteric antagonists **49** (LY341495) and **50** (MGS0039).

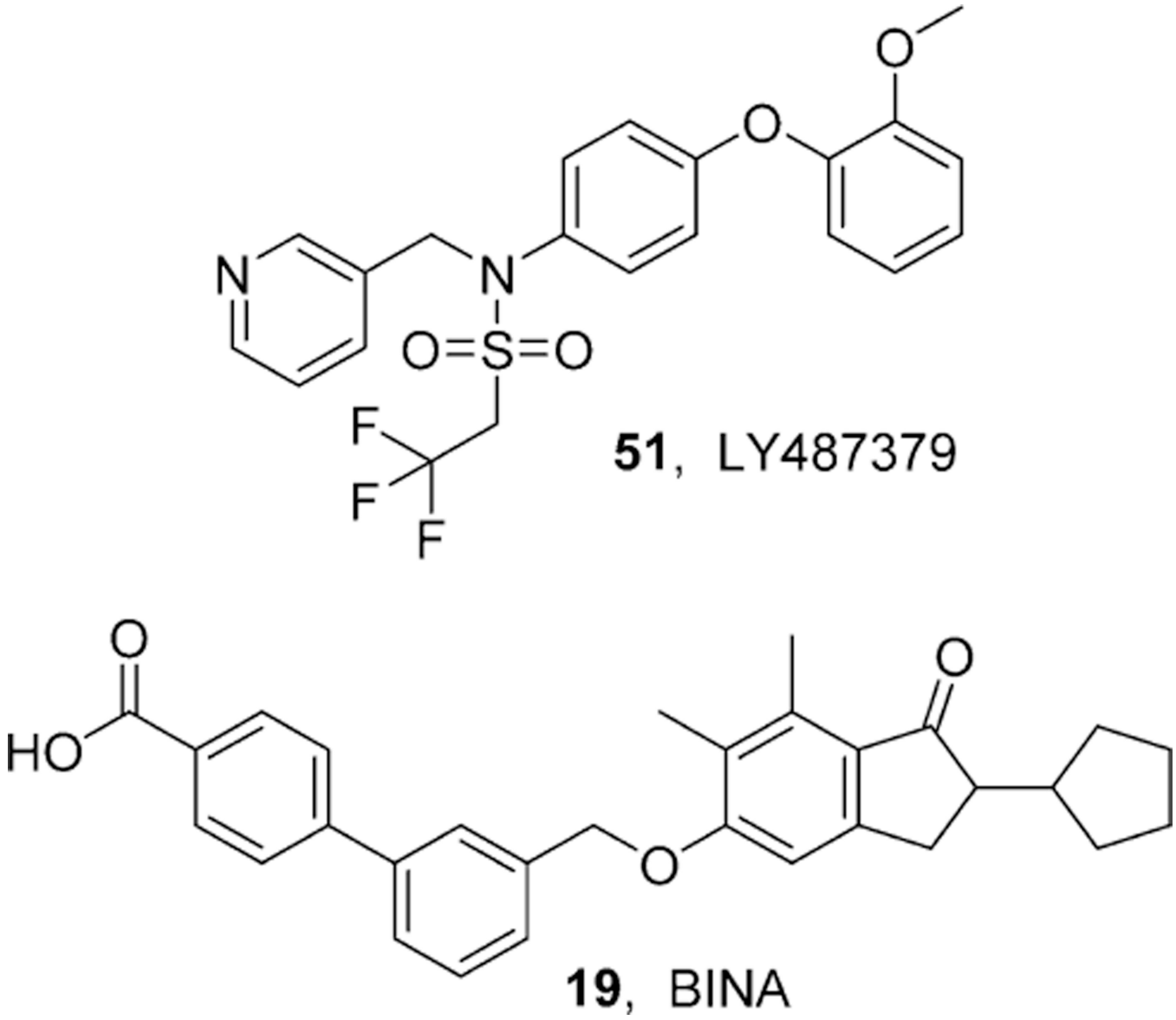



**Figure 11.**
Prototypical $mGlu_2$ PAM tools **51** (LY487379) and **19** (BINA).

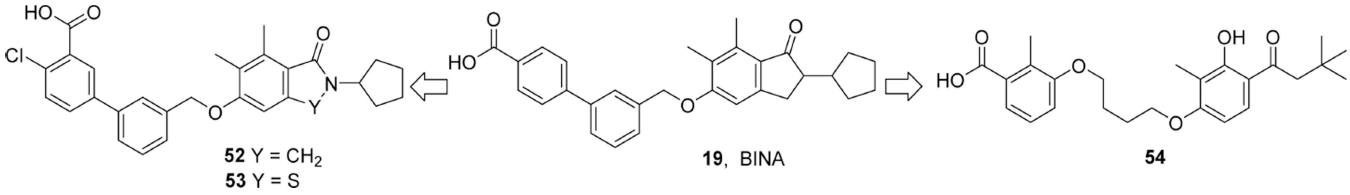



**Figure 12.**
$mGlu_2$ PAMs **52, 53** and **19** (BINA) and $mGlu_{2/3}$ PAM **54**.

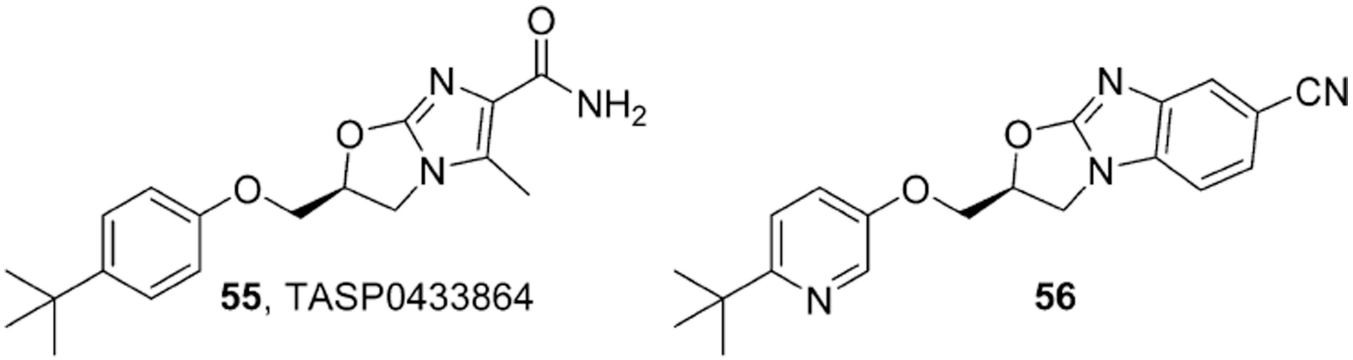



**Figure 13.**
2,3-Dihydroimidazo[2,1-*b*]oxazole-based $mGlu_2$ PAMs **55** (TASP0433864) and **56**.

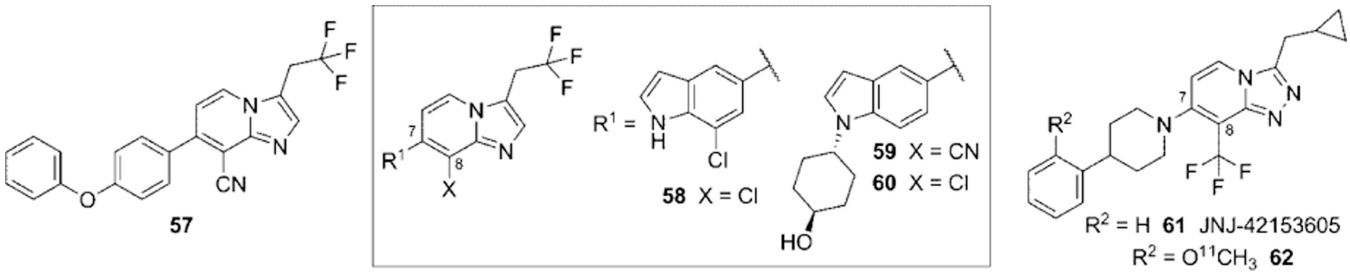



**Figure 14.**

Imidazopyridine $mGlu_2$ PAMs **57–62**, triazolopyridine $mGlu_2$ PAM **61** (JNJ-42153605), and [$^{11}$C]-labeled triazolopyridine $mGlu_2$ PAM **62**.

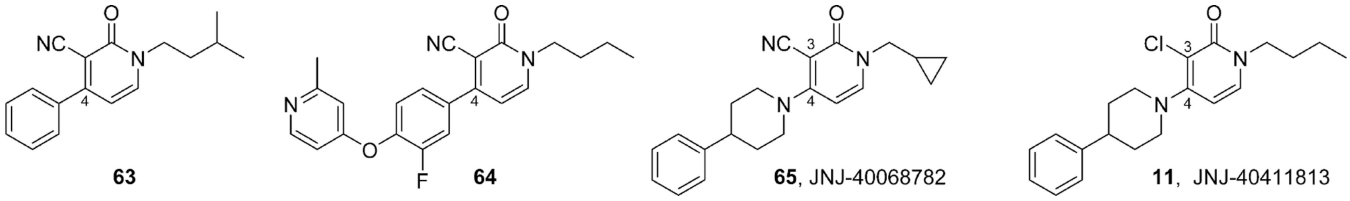



**Figure 15.**

Pyridone $mGlu_2$ PAMs: HTS hit **63**, in vivo tools **64** and **65** (JNJ-40068782), and clinical compound **11** (JNJ-40411813).

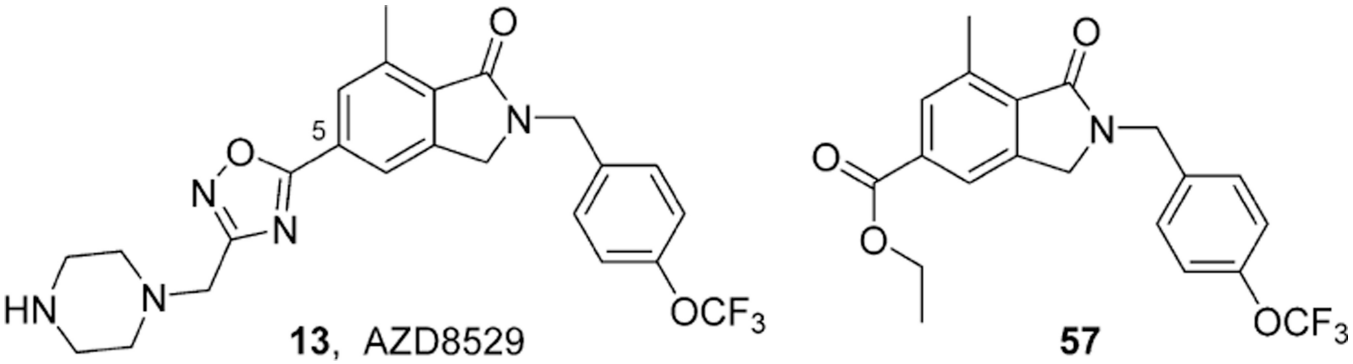



**Figure 16.**
$mGlu_2$ PAM clinical compound **11** (AZD8529) and its synthetic precursor **57**.

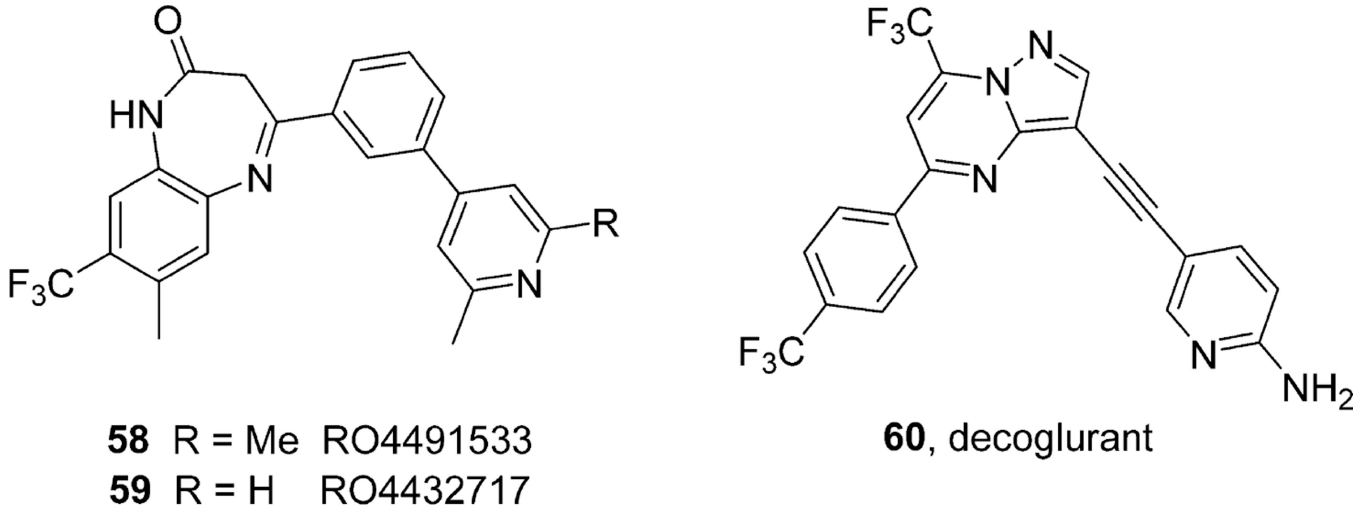



**Figure 17.**

$mGlu_{2/3}$ NAM tools **58** (RO4491533) and **59** (RO4432717) and clinical compound **60** (decoglurant).

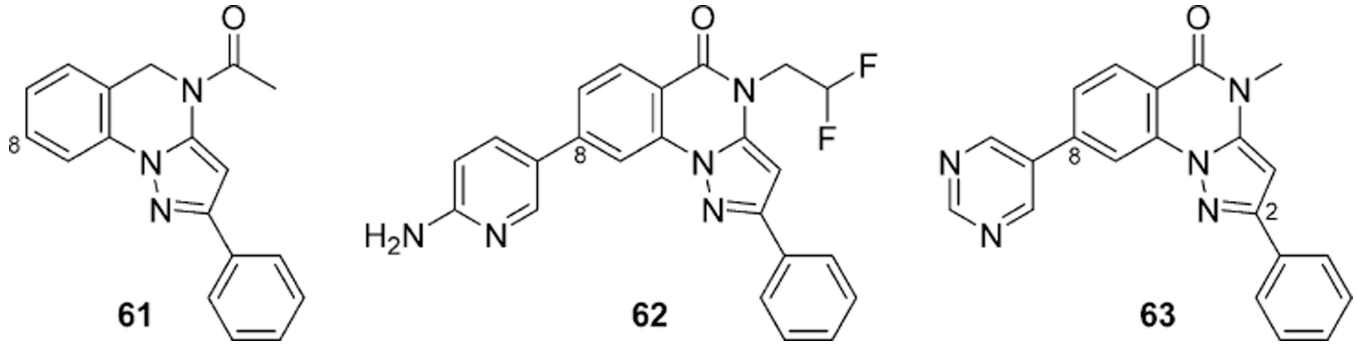



**Figure 18.**
Screening hit **61** and pyrazolo[1,5-*a*]quinazolin-5-one $mGlu_{2/3}$ NAMs **62** and **63**.

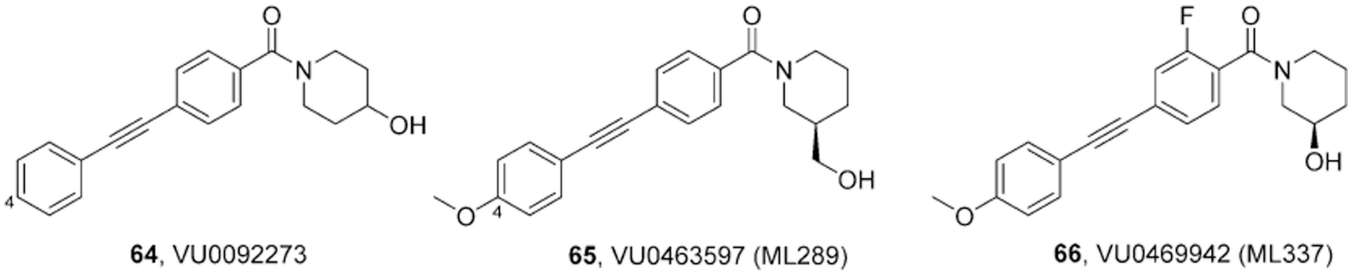
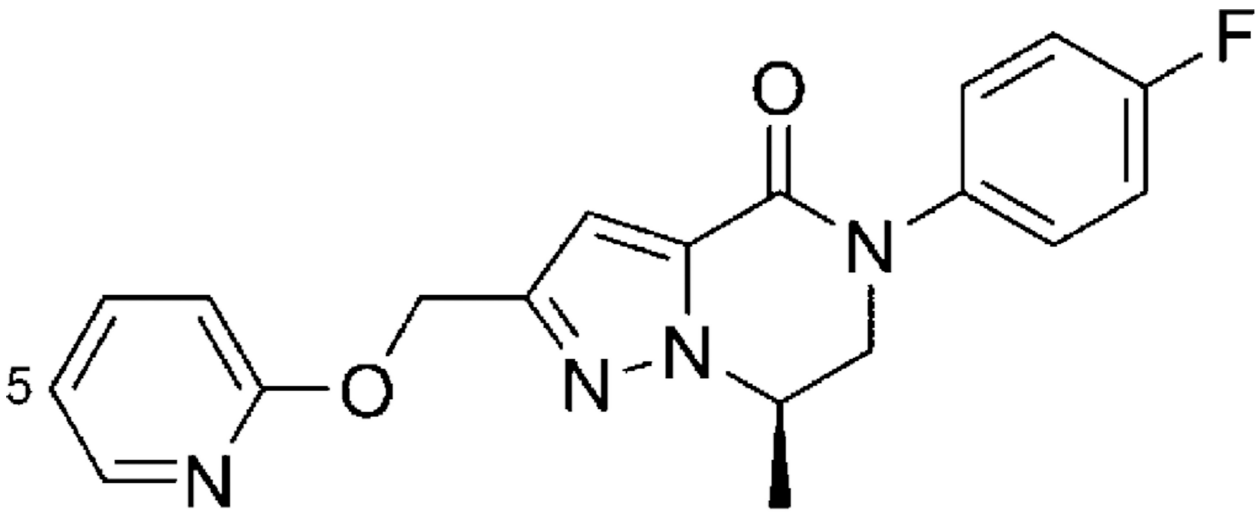
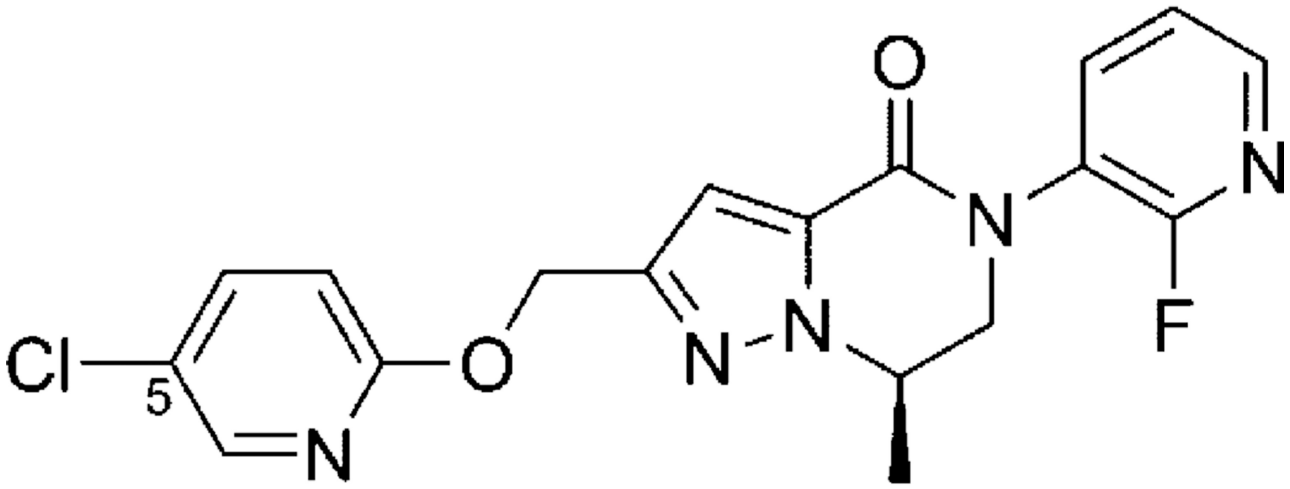



**Figure 19.**

Cross-screening hit **64** and 1,2-diphenylethyne $mGlu_3$ NAMs **65** (VU0463597, ML289) and **66** (VU0469942, ML337).

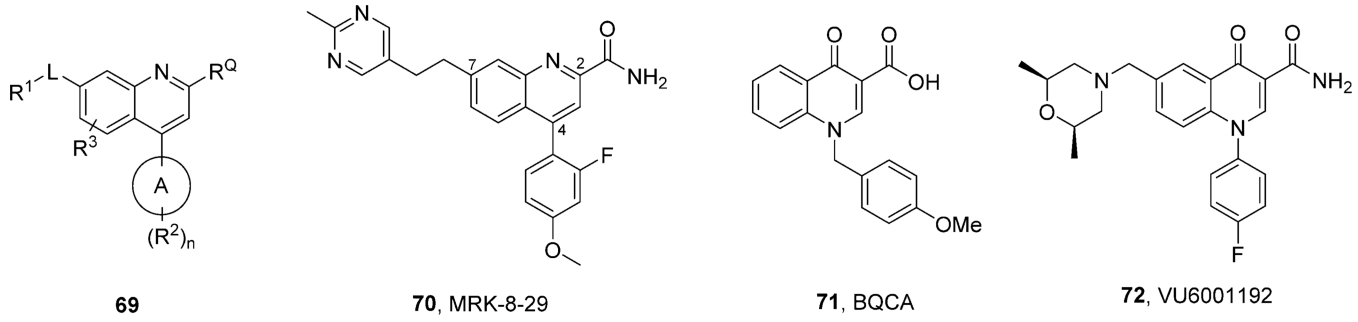



67




68, VU0650786

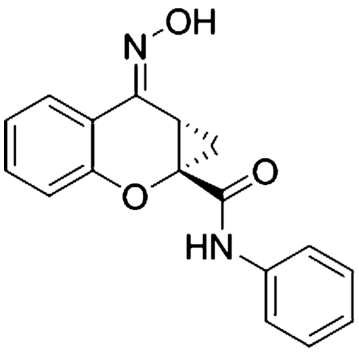
**Figure 20.**
Cross-screening hit **67** and optimized mGlu3 NAM in vivo tool **68** (VU0650786).



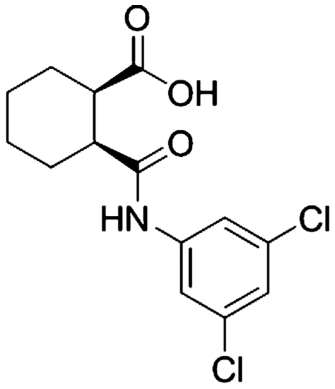

**69** **70**, MRK-8-29 **71**, BQCA **72**, VU6001192

**Figure 21.**

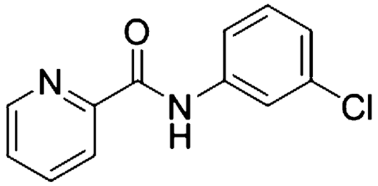
Markush structures of **69** and $mGlu_2$ NAM **70** (MRK-8–29). The similarity between **70** and the prototypical $M_1$ PAM BQCA (**71**) led to a scaffold-hopping exercise that identified the novel $mGlu_2$ NAM VU6001192 (**72**).



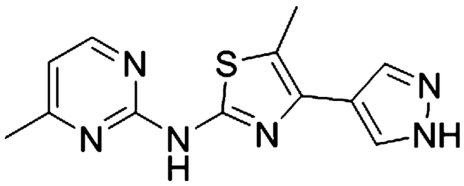

**20**, (−)-PHCCC



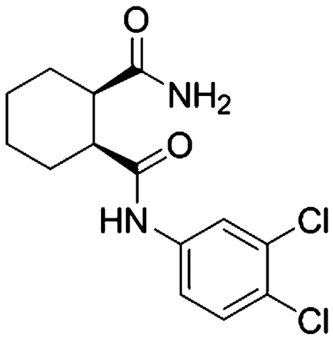

**23**, (rac)-VU0155041




**73**, VU0364770 (ML292)




**74**, ADX88178




**24**, (1*S*,2*R*)-Lu AF21934

**Figure 22.**

Structures of $mGlu_4$ PAMs with reported in vivo activity in preclinical models of Parkinson's disease.

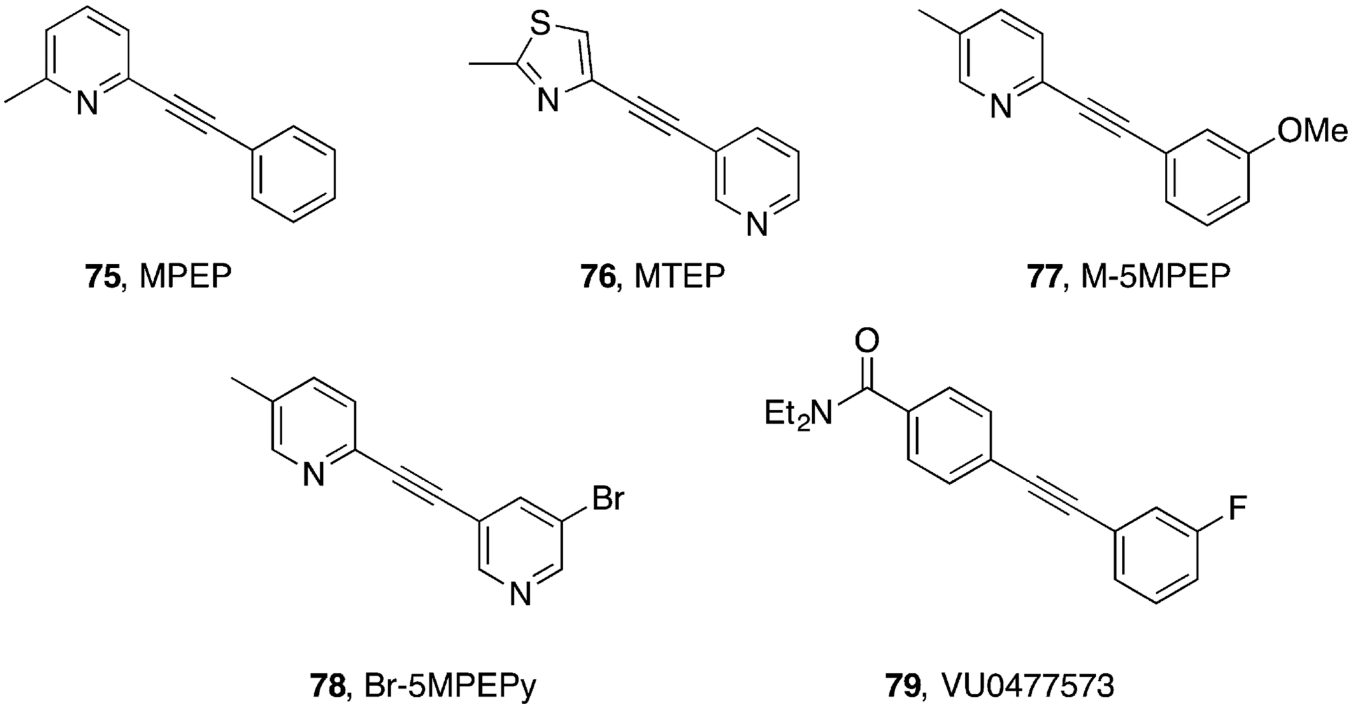



**Figure 23.**

Structures of $mGlu_5$ full (**75** and **76**) and partial (**77–79**) NAMs with reported in vivo activity in preclinical models of drug abuse and depression.

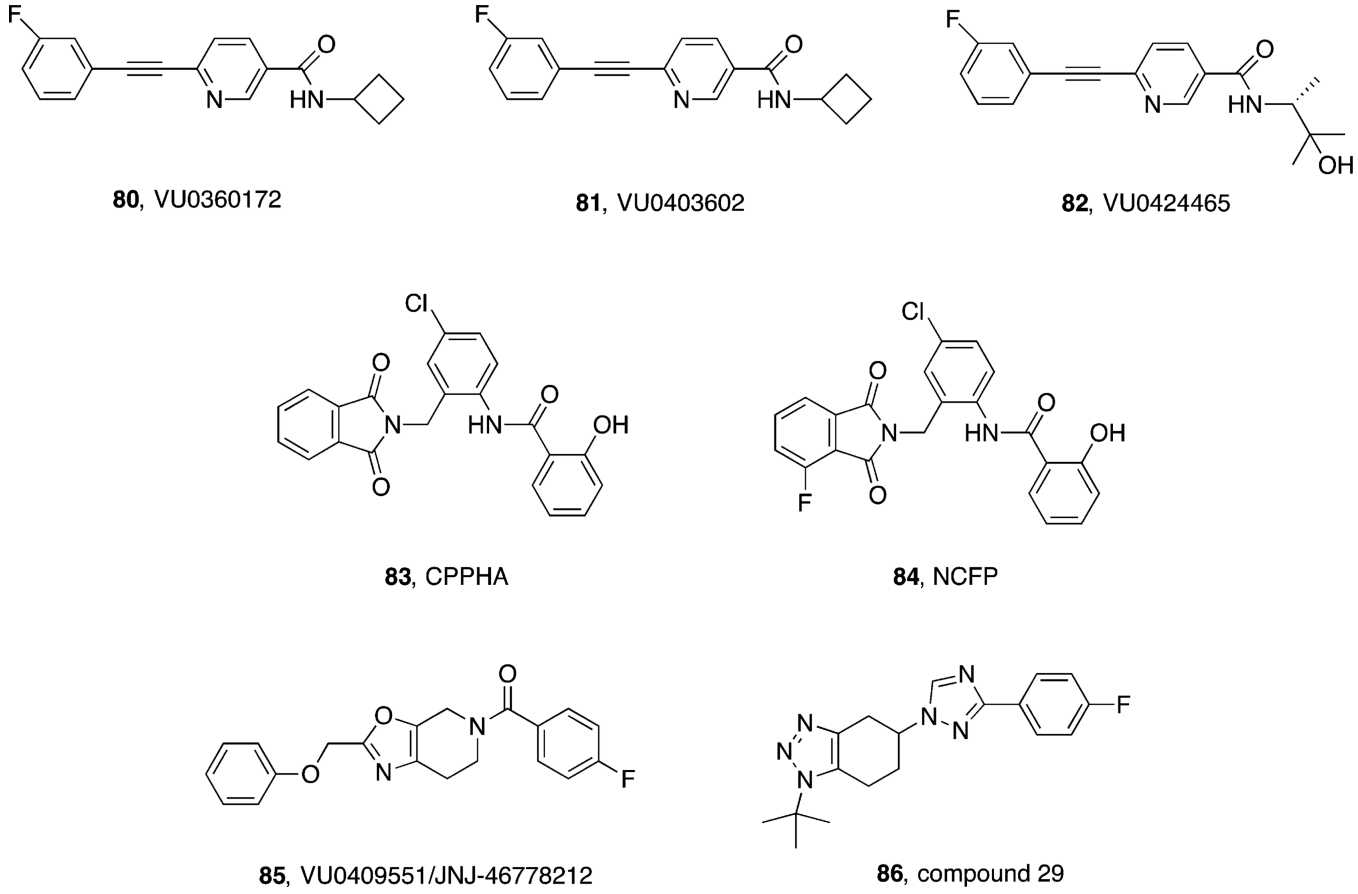



**Figure 24.**

Structures **80–86** of $mGlu_5$ PAMs and ago-PAMs displaying signal bias, including two (**85** and **86**) that have advanced to safety assessment.

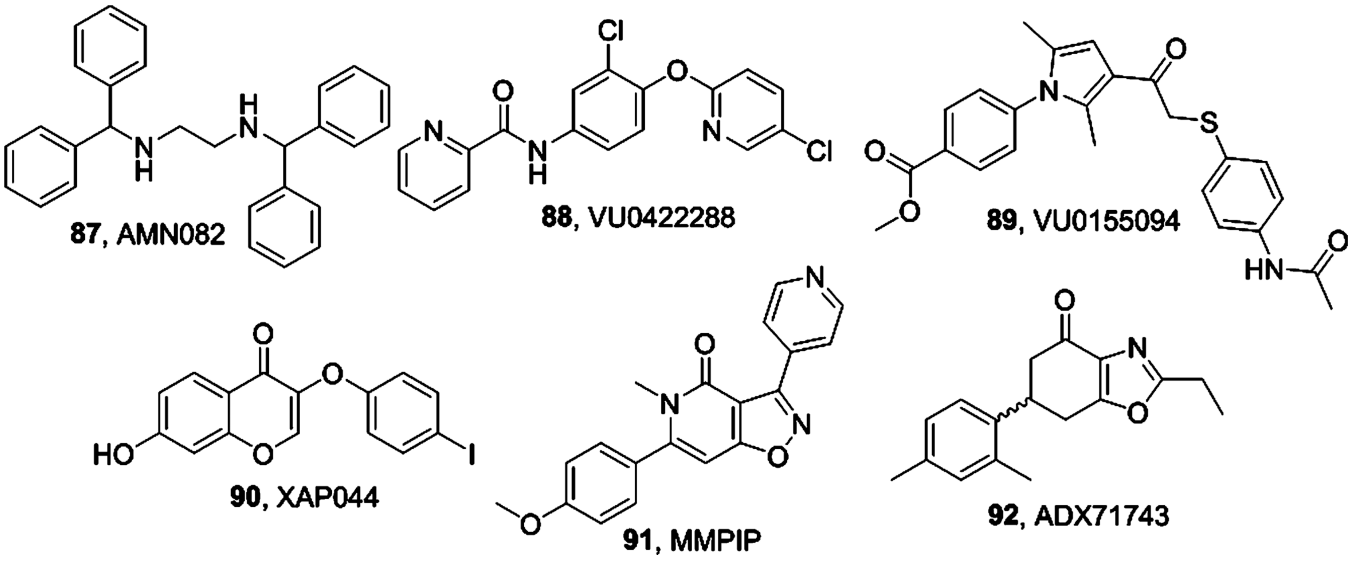
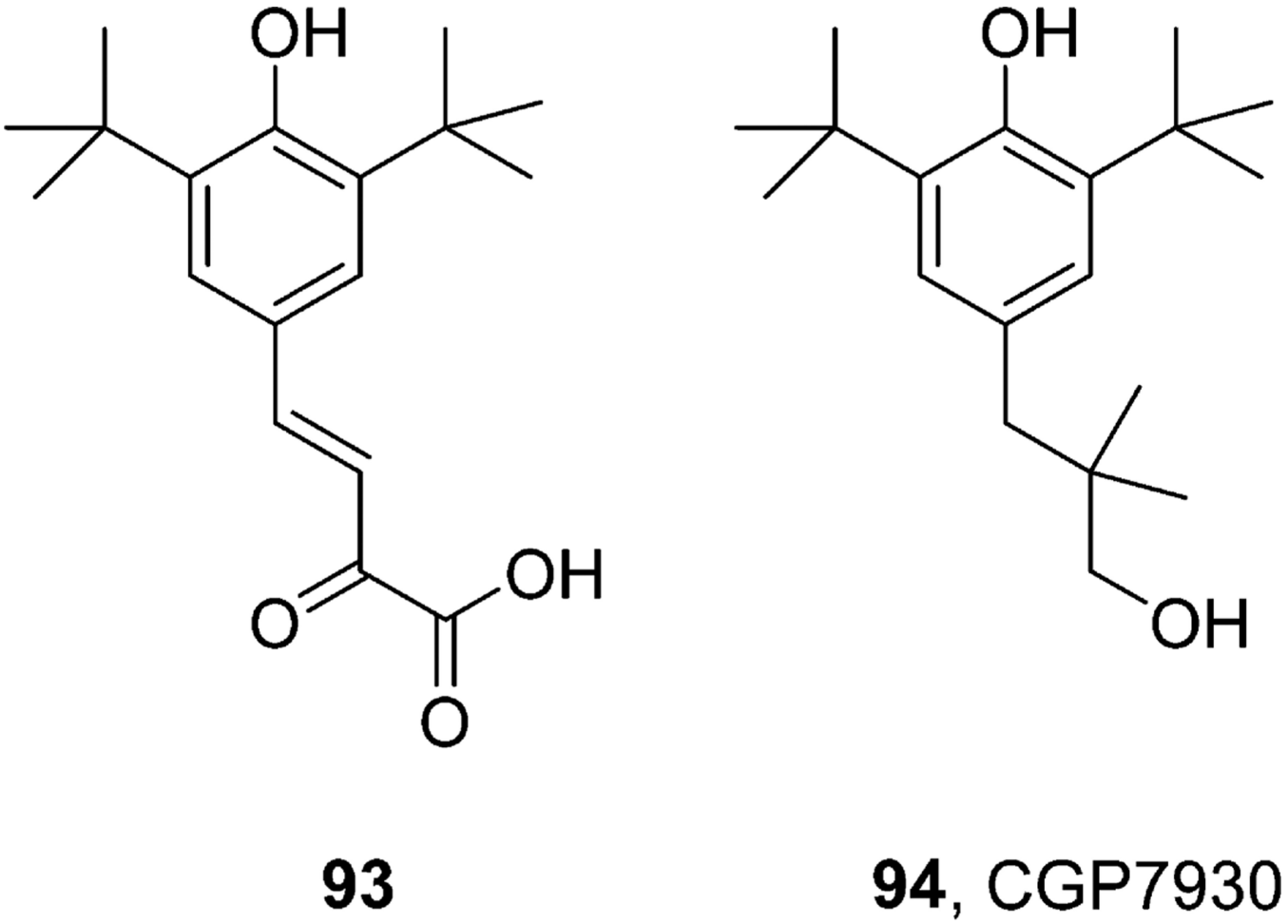



**Figure 25.**

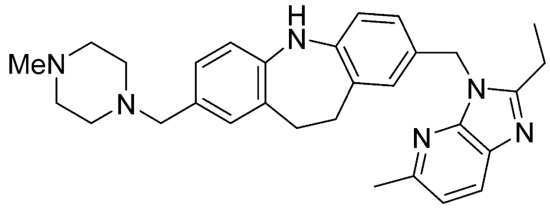
Structures of $mGlu_7$ allosteric agonist (**87**, AMN082), pan-group III PAMs (**88**, VU0422288; **89**, VU0155094), and antagonist/NAMs (**90**, XAP044; **91** MMPIP; and **92** ADX71743).



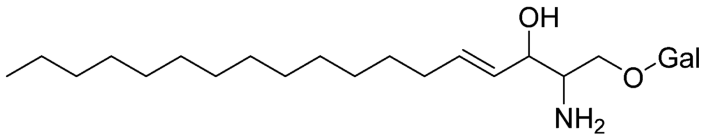

**93** **94**, CGP7930

**Figure 26.**

Structures of the first $GABA_B$ NAM (**93**) and the $GABA_B$ PAM CGP7930 (**94**), from which **93** was derived by scaffold hopping.




95




96

**Figure 27.**

Structures of the first GPR4 NAM (**95**) and the orhtosteric antagonist psychosine (**96**). Gal is galactosyl.