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# "Molecular Switches" on mGluR Allosteric Ligands That Modulate Modes of Pharmacology

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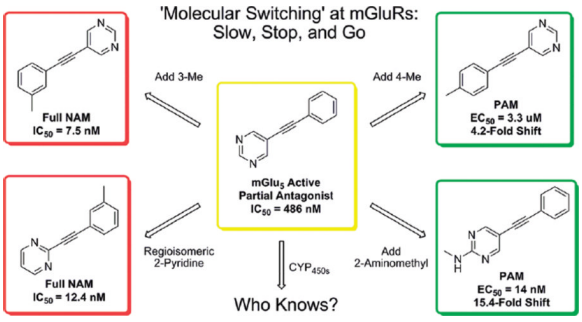
## Abstract




G-Protein-coupled receptors (GPCRs) represent the largest class of drug targets, accounting for more than 40% of marketed drugs; however, discovery efforts for many GPCRs have failed to provide viable drug candidates. Historically, drug discovery efforts have focused on developing ligands that act at the orthosteric site of the endogenous agonist. Recently, efforts have focused on functional assay paradigms and the discovery of ligands that act at allosteric sites to modulate receptor function in either a positive, negative, or neutral manner. Allosteric modulators have numerous advantages over orthosteric ligands, including high subtype selectivity; the ability to mimic physiological conditions; the lack of densensitization, downregulation, and internalization; and reduced side effects. Despite these virtues, challenging issues have now arisen for allosteric modulators of metabotropic glutamate receptors (mGluRs): shallow SAR, ligand-directed trafficking, and the identification of subtle "molecular switches" that modulate the modes of pharmacology. Here, we will discuss the impact of modest structural changes to multiple mGluR allosteric ligands scaffolds that unexpectedly modulate pharmacology and raise concerns over metabolism and the pharmacology of metabolites.

Heterotrimeric G-protein-coupled receptors (GPCRs) represent the largest protein family of cell-surface receptors and are responsible for mediating extracellular to intracellular signaling within a broad range of physiological contexts and organ systems.[1–5] Nearly 1000 GPCRs exist, mediating a host of molecular physiological functions and effector systems by



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serving as receptors for hormones, neurotransmitters, lipids, small molecules, and sensory signals such as light and odors (Figure 1). Moreover, GPCRs represent ~26% of the current market of FDA-approved therapeutic agents, constitute annual revenues in excess of $40 billion, and remain a strong focus of many biomedical research and pharmaceutical drug discovery programs.[1–6]. Historically, all of the FDA-approved drugs bind at the orthosteric site and engender classical agonism (directly stimulating a receptor response), inverse agonism (blocking constitutive activity), or competitive antagonism (blocking the binding of the native agonist) and were discovered by employing radioligand binding assays targeting the orthosteric binding site.[1–6] Despite this success, synthetic ligands exist for only a fraction of the known GPCRs, and many efforts failed to produce highly selective compounds suitable as drug leads because of the highly conserved orthosteric binding site across a family of GPCRs and chemophysical properties of synthetic orthosteric ligands.

Glutamate (L-glutamic acid) **1** is the major excitatory transmitter in the mammalian central nervous system, exerting its effects through both ionotropic and metabotropic glutamate receptors. The mGluRs are family C (also known as family 3) GPCRs, characterized by a seven-transmembrane (7TM) α-helical domain connected via a cysteine-rich region to a large bilobed extracellular "Venus flytrap" amino-terminal domain (Figure 1). While the orthosteric binding domain (ODB) is contained in the amino-terminal domain, currently known allosteric binding sites reside in the 7TM domain. The eight known mGluRs have been classified into three groups on the basis of their structure, preferred signal transduction mechanisms, and pharmacology. Group I receptors ($mGlu_1$ and $mGlu_5$) are coupled to $G_{\alpha q}$, a process that results in an increase in the intracellular level of calcium. Group II receptors ($mGlu_2$ and $mGlu_3$) and group III receptors ($mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$) are coupled to $G_{\alpha i}$, which leads to decreases in cyclic adenosine monophosphate (cAMP) levels. While the group I receptors are predominately located post-synaptically and typically enhance postsynaptic signaling, the group II and III receptors are located presynaptically and typically have inhibitory effects on neurotransmitter release. The identification of small molecule mGluR agonists and antagonists that bind at the orthosteric binding site has greatly improved our understanding of the roles played by these receptors and their corresponding relation to disease; however, the majority of these ligands were designed as analogues of glutamate **1**, and as such, they typically lack the bioavailability and/or central nervous system (CNS) penetration desired in a probe or drug candidate[7–9]. Moreover, because of the highly conserved nature of the glutamate binding site, most orthosteric antagonists lack selectivity among the various mGluRs.

The advent of high-throughput functional assays has allowed scientists to screen for compounds capable of modulating the activity of a receptor through novel, allosteric mechanisms in a convenient manner.[5,10–15] This screening strategy has successfully identified a number of allosteric modulators of cell surface receptors, including ligand-gated ion channels and GPCRs that modify receptor function, and represents a major shift from historical radioligand discovery efforts. Allosteric ligands bind at sites that are topographically distinct from the orthosteric site, which in turn can modulate the potency and affinity or efficacy of the orthosteric ligand, in either a positive or negative direction, by facilitating a global change in receptor conformation that may also enhance or inhibit coupling to the G-protein.[10–15] Therefore, a receptor bound by an allosteric ligand should be viewed as a "new" receptor type with novel behavior. Allosteric ligands can exert a number of unique pharmacological modes of action (Table l): positive allosteric modulation (PAM), negative allosteric modulation (NAM), silent allosteric modulation (SAM), partial antagonism, allosteric agonism, and ago-potentiation (ago-PAM). Allosteric modulators offer numerous advantages, (l) Allosteric binding sites may be under less evolutionary pressure for their conservation, thus allowing high subtype selectivity to be achieved. (2) The effects of an allosteric modulator are saturable; once allosteric sites are occupied, no

additional effects are observed. (3) A positive allosteric modulator will exert its effects only when the endogenous agonist is present, resulting in temporal and spatial activity of the endogenous ligand. (4) The last advantage is improved chemical tractability.[10–15] This new paradigm is quickly gaining momentum and has resulted in the FDA approval of Cinacalet (Sensipar), a positive allosteric modulator (PAM) of the calcium sensing receptor, and Maraviroc (Selzentry), a negative allosteric modulator (NAM) of chemokine receptor 5.[16,17] The first small molecules that were clearly shown to interact allosterically with the mGluRs were CPCCOEt, **2** ($mGlu_1$ selective), and MPEP, **3** ($mGlu_5$ selective), both negative allosteric modulators (NAMs).[18,19] Soon after, positive allosteric modulators (PAMs), compounds that enhance the agonist response with little or no intrinsic agonist activity, were reported for $mGlu_1$ $mGlu_2$, $mGlu_4$, and $mGlu_5$ that also demonstrated unprecedented mGluR subtype selectivity.[10,20–25]

Despite these advantages, a new phenomenon is emerging whereby very subtle structural changes, i.e., a “molecular switch”, to an mGluR allosteric ligand can modulate either the mode of pharmacology, i.e., from PAM to NAM, or selectivity for a given subtype. This, in addition to the often shallow structure—activity relationships (SAR) and ligand-biased signaling behavior, further complicates allosteric ligand optimization and raises concerns over the pharmacology of putative metabolites. Here, we will briefly review the data to date, which focus on the mGluRs, but molecular switches have now also been observed for other GPCRs, such as muscarinics (mAChRs), as well as both allosteric kinase and phospholipase allosteric ligands. [26–31]

## mGluRs and Chemical Switches That Modulate Modes of Pharmacology

The paradigm of targeting allosteric modulation has proven to be critically important for drug discovery efforts within the metabotropic glutamate receptor (mGluR or $mGlu_{1–8}$) family of class C GPCRs.[10–25] From the first discovery of an $mGlu_5$ PAM, subtle modifications, i.e., molecular switches, were found to exert significant effects on the new receptor’s conformation, resulting in modulation of the mode of pharmacology. In 2003, O’Brien reported on 3,3′-difluorobenzaldazine (DFB, **4**) as the first $mGlu_5$ PAM ($EC_{50}$ = 2.6 μM), but replacement of the fluoro groups led to interesting, and quite unexpected, results (Figure 2).[32] A 3,3′-dimethoxy conger (DOMeB, **5**) proved to be an $mGlu_5$ NAM ($IC_{50}$ = 3.0 μM) of comparable potency, while a 3,3′-dichloro analogue (DCB, **6**) was the first $mGlu_5$ SAM, or pharmacologically silent or neutral ligand, that blocked both the PAM and NAM activity of DFB and DOMeB, respectively. Interestingly, radioligand binding assays confirmed that this series of $mGlu_5$ allosteric ligands was competitive with the well-characterized MPEP allosteric binding site.[32] Soon after, two new, structurally distinct series of $mGlu_5$ PAMs, represented by CPPHA, **7** (a non-MPEP site PAM), and CDPPB, **8** (an MPEP site PAM), that did not display mode switching were reported; all proved to be PAMs or inactive, with shallow SAR.[33–36] Thus, was the dynamic switching of modes of pharmacology unique to the benzaldazine series and not a major concern for allosteric ligands? The issue was about to become far more complex.

In 2005, Rodriguez and co-workers reported on the first $mGlu_5$ partial antagonists, allosteric ligands that fully occupy the MPEP allosteric site but induce an only partial blockade of signaling.[37] Here, they described M-5MPEP (**9**) and Br-5MPEPy (**10**), both 50% partial antagonists, as well as a second SAM, 5MPEP (**11**) (Figure 3). A functional HTS identified another partial antagonist, **12** ($IC_{50}$ = 486 nM; 71% partial antagonism), from a 5-(phenylethynyl)pyrimidine scaffold.[38] SAR around this scaffold identified key molecular switches that uniformly modulated the partial antagonist **12** into either a NAM **13** with the addition of a 3-methyl substituent to the distal phenyl ring or a PAM **14** with the addition of a 4-methyl substituent. Further optimization efforts identified additional molecular switches

leading to a centrally active NAM **15** ($IC_{50}$ = 12 nM), based on a regioisomeric pyrimidine switch, and PAM **16** ($EC_{50}$ = 14 nM; 128% Glu Max, 15-fold shift), based on a 2-aminomethyl switch. Both **15** and **16** displayed robust in vivo efficacy in punish responding and amphetamine-induced hyperlocomotion, respectively, confirming the switch in mode observed in vitro was mirrored in vivo.[38,39] These data caused concern, as in vivo metabolism of **12** could result in hydroxylation at either the 3- or 4-position of the distal phenyl ring. Could major circulating metabolites of $mGlu_5$ allosteric ligands possess opposing modes of pharmacology?

At the same time, the structure of ADX-47273 (**17**), another MPEP site $mGlu_5$ PAM, that also possessed strong intrinsic agonist activity was disclosed and is therefore more correctly coined an ago-PAM.[40] Analogues within this series possessed a dynamic range of allosteric agonism, requiring fold-shift assays to be conducted at low (l μM) concentrations.[41,42] Here too, we discovered a number of molecular switches. Via replacement of the 4-fluorophenyl moiety of **17** with a moderately basic 2-pyridyl moiety as in **18**, the intrinsic agonism was abolished affording a pure PAM of comparable potency ($EC_{50}$ = 390 nM; 113% Glu Max, 25-fold shift). Replacing the benzamide in 18 with a cyclo-butyl amide **19** delivered a weak $mGlu_5$ NAM ($IC_{50}$ = 8.7 μM). Further optimization of **19** identified a number of molecular switches based on stereochemistry, ring size, and simple aryl substitution (i.e., fluoro vs methyl) that afforded compounds with NAM (**20**), PAM (**21**), partial antagonist (**22**), and ago-PAM (**23**) activity.[41,42] This type of subtle switch in mode is a true nightmare for the medicinal chemist trying to develop SAR and drive a program toward a clinical candidate for a single mode of pharmacological manipulation of a molecular target.

These data provided further impetus for the discovery and characterization of additional, novel $mGlu_5$ allosteric ligands. A functional high-throughput screening campaign, employing the triple-add protocol, identified a number of novel chemotypes as both $mGlu_5$ PAMs and NAMs.[43] In particular, an $mGlu_5$ PAM hit, exemplified by a simple benzamide scaffold **24** ($EC_{50}$ = 350 nM; 84% Glu Max), seemed worthy of follow-up (Figure 5).[44] An iterative parallel synthesis effort produced 22 analogues and optimized $mGlu_5$ PAM activity to afford **25** ($EC_{50}$ = 33 nM; 92% Glu Max), differing in only a single 4-fluoro moiety. Importantly, radioligand binding and mutagenesis studies indicated that **25** did not bind to either the MPEP site or the CPPHA site; thus, **25** and its analogues bind at a yet uncharacterized, third allosteric site on $mGlu_5$. None of the analogues exhibited NAM activity, but experiments to identify a SAM were productive delivering **26** (100 nM vs **25**), the first SAM at a non-MPEP site. Here, the 2,4-difluoro congener **25** is a potent PAM, while the regioisomeric 2,6-difIuoro derivative **26** is a potent SAM, once again, a very subtle molecular switch, and one that would be impossible to predict.[44]

This same HTS afforded a number of novel $mGlu_5$ NAM chemotypes, and several of these were found to possess molecular switches that converted them into weak PAMs.[45–47] Of these, NAM **27** ($IC_{50}$ = 990 nM) proved to be unique in terms of chemotype (a heteroaryl piperazinyl amide) as well as mode switching (Figure 6). Extensive SAR identified the *N*-heteroaryl moiety as a conserved domain engendering NAM activity. For example, thiazole analogue **28** exhibited improved NAM activity and, despite representing a non-MPEP chemotype, exhibited [$^3$H]methoxyPEPy with a $K_i$ equivalent to its functional activity ($IC_{50}$ = 540 nM; $K_i$ = 440 nM), suggesting this series binds at the MPEP allosteric site. Analogue libraries exploring replacements for the adamantyl group proved to be more interesting and defined this position as a molecular switch leading to PAM activity. Here, a benzyloxy acetyl amide congener **29** proved to be a modest PAM ($EC_{50}$ = 5.4 μM; 86% Glu Max). Subsequent libraries held this portion of the molecule constant and surveyed divesre aryl and heteroaryl pierazine motifs. While submicromolar PAMs resulted, PAM **30** ($EC_{50}$ = 1.6μM; 78% Glu Max), with a balance of properties (potency, physiochemical, and DMPK), proved

to be translational. PAM **30** was selective for mGlu5, possessed 10% free fraction in rat and excellent permeability and solubility, and reversed amphetamine-induced hyperlocomotion in rats without sedation (in a nontoxic vehicle).[47] This was a major step forward in the $mGlu_5$ PAM field, as the preexisting PAMs required DMSO in the vehicle formulations for in vivo work because of the poor physicochemical properties. Again, it is interesting that such an important $mGlu_5$ PAM tool compound evolved, and did so very quickly, from a NAM lead.

## mGluRs and Chemical Switches That Modulate Subtype Selectivity

Thus far, the focus has been on molecular switches that modulate the mode of pharmacology; however, an equally important finding centers on molecular switches that alter mGluR subtype selectivity. So far, this phenomenon is limited to the (−)-PHCCC scaffold (**31**), the prototypical $mGlu_4$ PAM, within the mGluRs;[48,49] however, this is also well-known for mAChR allosteric ligands.[26–28] While an important proof-of-concept tool for studying $mGlu_4$, (−)-PHCCC **31** also possesses $mGlu_4$, NAM activity. A multidimensional optimization effort found extremely shallow SAR but did identify a molecular switch that abolished the $mGlu_1$ NAM activity ($IC_{50}$ = 2.1 μM). Replacement of the phenyl amide in **31** with a moderately basic 2-pyridyl amide provided **32** (VU03595216), the switch that engendered complete selectivity for $mGlu_4$ (>30 μM vs $mGlu_1$, $mGlu_2$, $mGlu_3$, $mGlu_5$, $mGlu_6$, $mGlu_7$, and $mGlu_8$) as well as enhanced potency and efficacy ($EC_{50}$ = 380 nM; 121% Glu Max, 20-fold shift).[48]

Recently, Schann and co-workers reported on the application of an $mGlu_2$ FRET-based binding assay to selectively identify $mGlu_2$ allosteric modulators.[49] This effort identified **33**, a close, 4-fluorophenyl analogue of (−)-PHCCC **31**, with an $mGlu_2$ $K_i$ of 6.6μM, but devoid of functional activity at $mGlu_2$ and $mGlu_3$. Three additional analogues (**34**–**36**) explored alternative functionalization at the 4-position, and all demonstrated improved binding affinities with $K_i$ values of 1,0.6, and 0.8 μM, respectively. In functional assays, **34**–**36** proved to be potent $mGlu_2$ NAMs ($IC_{50}$ values of 800 nM to 1.5 μM) and weak $mGlu_3$ PAMs ($EC_{50}$ values of 9–13 μM), a unique and unprecedented pharmacological profile with opposing modes of mGluR group III functional activity. Importantly, **33** possessed no functional activity on its own but was able to block the activities of **34**–**36**; therefore, **33** is the first $mGlu_2$ SAM.[49] This screening paradigm represents another novel approach to the identification of allosteric ligands with diverse modes of pharmacology and subtype selectivity and further highlights the subtleties at play in the chemical optimization of allosteric ligands.

## Biochemical Characterization of the $mGlu_5$ MPEP Allosteric Binding Site

The subtle changes to an allosteric ligand that engender unique receptor conformations of $mGlu_5$ and result in switches in the mode of pharmacology call for an understanding of the allosteric binding pocket and receptor–ligand interactions. Unfortunately, little is known about the allosteric binding pockets of $mGlu_5$, as no X-ray crystal structures for class C GPCRs exist. To date, only the MPEP (**1**) site has received any attention in this regard. In 2000, Kuhn and co-workers elucidated the MPEP binding site in the transmembrane TM-spanning region. Mutagenesis work with human $mGlu_5$ identified P655, S568, and A810 as critical residues for high-affinity MPEP binding. A homology model was constructed on the basis of the X-ray crystal structure of bovine rhodopsin, and MPEP was predicted to bind in a pocket formed by residues in TM III (P655, S658, and Y659) and TM VII (S809 and A810).[50] Shortly thereafter, Malherbe and co-workers at Hoffman-La Roche performed a related study with rat $mGlu_5$.[51] Using a combination of site-directed mutagenesis, [$^3$H]MPEP binding, a functional $Ca^{2+}$ mobilization assay, and rhodopsin-based homology

modeling, they identified eight residues (P654, Y658, L743, T780, W784, F787, Y791, and A809) that are required for MPEP binding to rat $mGlu_5$ receptors. Of these key amino acids, four mutations, Y658, W784, F787, and A809, resulted in a complete loss of [$^3$H]MPEP binding. In addition, these mutations also blocked the MPEP-mediated inhibition of orthosteric agonist (quisqualate)-induced intracellular $Ca^{2+}$ mobilization. On the basis of these experimental findings, and utilizing the X-ray crystal of bovine rhodopsin, a homology model was developed that predicted a possible binding mode of MPEP. The homology model suggests that by several interactions with a network of the aromatic residues, including Y658 in the transmembrane 3 (TM3) helix and W798, F787, and Y791 in the TM6 helix, MPEP inhibits the conformational movement of the TM6 helix relative to the TM3 helix.[51,52] Despite these notable advances with the MPEP allosteric binding pocket, a mechanistic and/or con-formational rational for how such subtle variations to a ligand can result in dramatic conformational changes to engender the opposing mode of pharmacology is unclear. Moreover, the other two $mGlu_5$ allosteric sites remain completely uncharacterized.

## FUTURE DIRECTIONS

From the beginning, the optimization of allosteric ligands proved challenging. Now, in addition to shallow SAR and ligand-based signaling complexities,[53] the emergence of subtle molecular switches that can engender the opposing mode of pharmacology and/or modulate subtype selectivity within a receptor family requires a careful, thoughtful approach in lead optimization. For instance, even the primary screen that drives the discovery effort should be run in “triple-add” mode to identify, in a single assay, PAMs, NAMs, and agonists to capture mode switching. Inactive compounds in a primary assay, for instance, for $mGlu_5$, should not be discarded. Inactive $mGlu_5$ allosteric ligands may be SAMs or may possess functional activity at another mGluR. With tool compounds desperately needed for $mGlu_6$, $mGlu_7$, and $mGlu_8$, potent ligands may have already been synthesized for other mGluR programs, but rarely are full mGluR selectivity panels run for compounds inactive at the primary target. On the basis of the data to date, vast treasures of allosteric ligands are out there, waiting to be discovered.

Finally, beyond the basic optimization challenges, significant effort must be focused on understanding the pharmacology of major circulating metabolites prior to advancing allosteric compounds into the clinic. Simple hydroxylated intermediates may afford new allosteric modulators with the opposing mode of pharmacology or alter subtype selectivity. Either scenario could spell disaster for a clinical candidate and afford various adverse events. Thus, characterization, synthesis, and evaluation (across all family members and in a triple-add screening paradigm) of the major metabolites of allosteric ligands are prudent. Hard data to this point are currently being sought in several laboratories.

## SUMMARY

Drug discovery efforts have historically focused on developing ligands that act at the orthosteric site of the endogenous agonist. Recently, efforts have focused on functional assay paradigms and the discovery of ligands that act at allosteric sites to modulate receptor function in either a positive, negative, or neutral manner. Allosteric modulators have numerous advantages over orthosteric ligands, including high subtype selectivity; the ability to mimic physiological conditions; the lack of densensitization, downregulation, and internalization; and reduced side effects. Despite these virtues, challenging issues have now arisen for allosteric modulators of metabotropic glutamate receptors (mGluRs): shallow SAR, ligand-directed trafficking, and the identification of subtle molecular switches that modulate the modes of pharmacology and/or modulate subtype selectivity. The molecular

switches are often very subtle, i.e., a fluorine for a chlorine atom or replacing a chlorine with a methyl group, represent the types of modifications usually interchangeable in orthosteric ligands, and raise new concerns over metabolism and the pharmacology of metabolites. While still a highly successful and tractable approach for novel therapeutics, allosteric modulation possesses several caveats and requires careful thought and execution.

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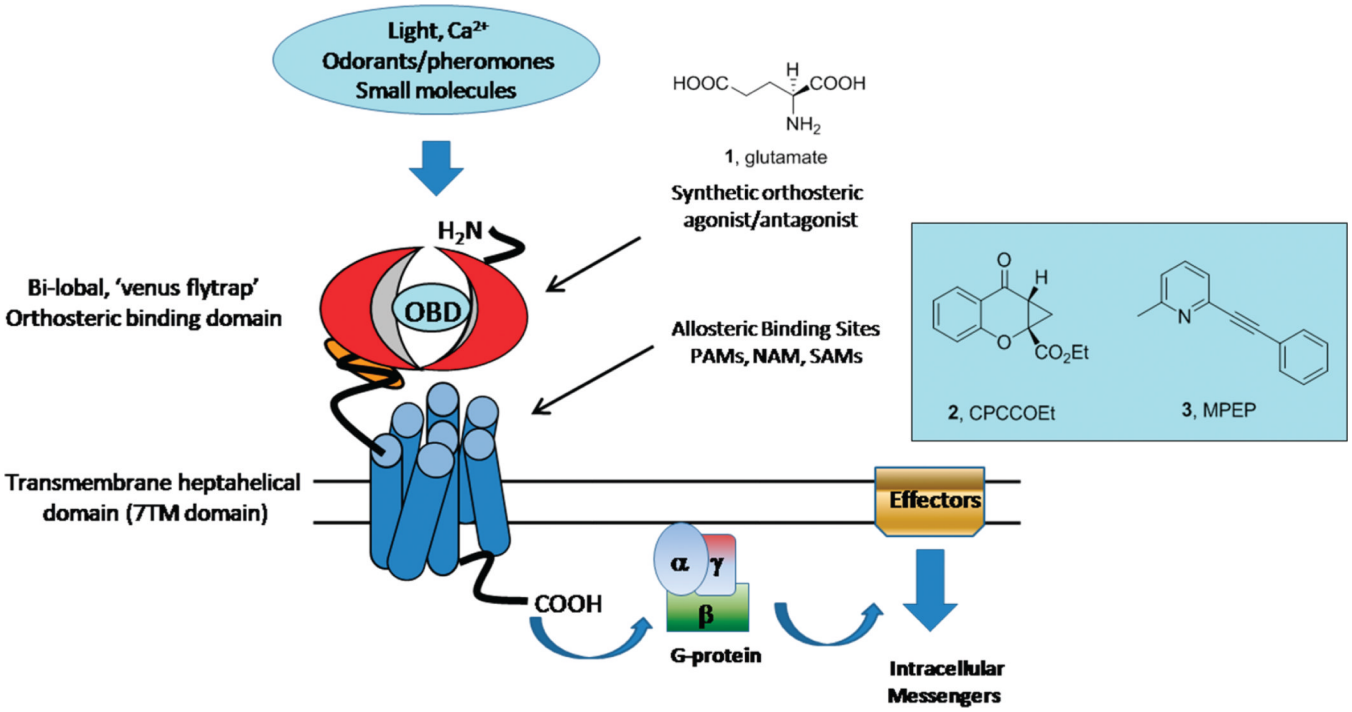



**Figure 1.**

Cartoon structure of a class C (family 3) metabotropic glutamate receptor (mGluR) G-protein-coupled receptor (GPCR). mGluRs all have a common core composed of seven-transmembrane helices (the 7TM domain comprised of TM I—TM VII) with a large, bilobal Venus flytrap extracellular N-terminal domain and an intracellular C-terminal domain. The GPCR receives an extracellular stimulus (light, calcium, odorants, pheromones, small molecules, and proteins) that induces a conformational change in the receptor that either facilitates or inhibits the coupling of the receptor to a G-protein, comprised of α-, β-, and γ-subunits. The G-protein, in turn, interacts with a diverse group of effectors that control intracellular messengers. All orthosteric ligands bind in the bilobal orthosteric binding domain (OBD) and are analogues of glutamate **1**. Allosteric ligands bind exclusively in the 7TM domain, far removed from the OBD, and are represented by novel, non-glutamate chemotypes such as CPCOOEt, **2** ($mGlu_1$ NAM), and MPEP, **3** ($mGlu_5$ NAM).

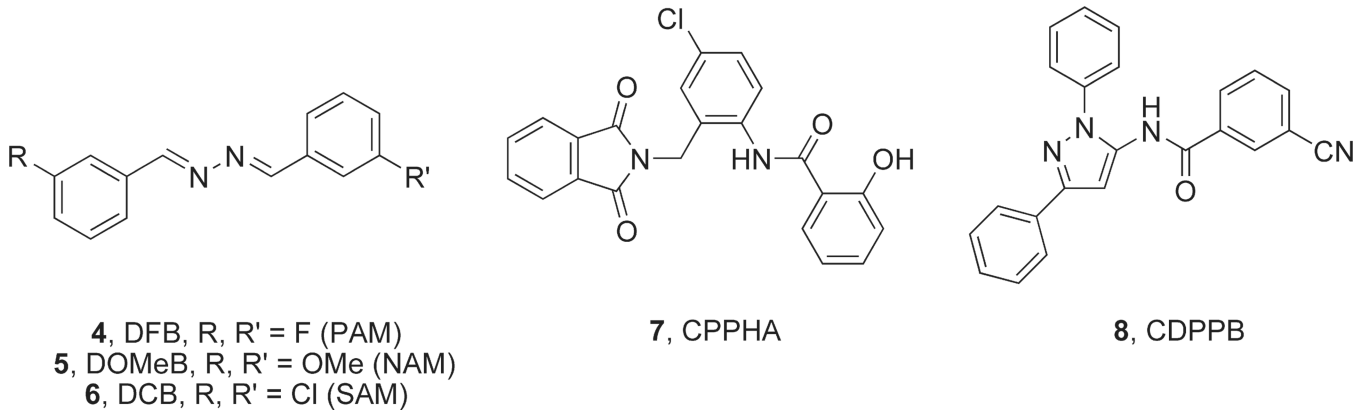



**Figure 2.**

Structures of the first $mGlu_5$ PAMs: DFB (**4**), CPPHA (**7**), and CDPPB (**8**). With vary subtle substitutions within the DFB series, the first molecular switches that modulated the mode of pharmacology to afford a NAM (DOMeB, **5**) and a SAM (DCB, **6**) were identified. Both the DFB (**4–6**) and CDPPB series (**8**) bind at the MPEP (**3**) allosteric site, whereas CPPHA (**7**) binds at a second, non-MPEP site.

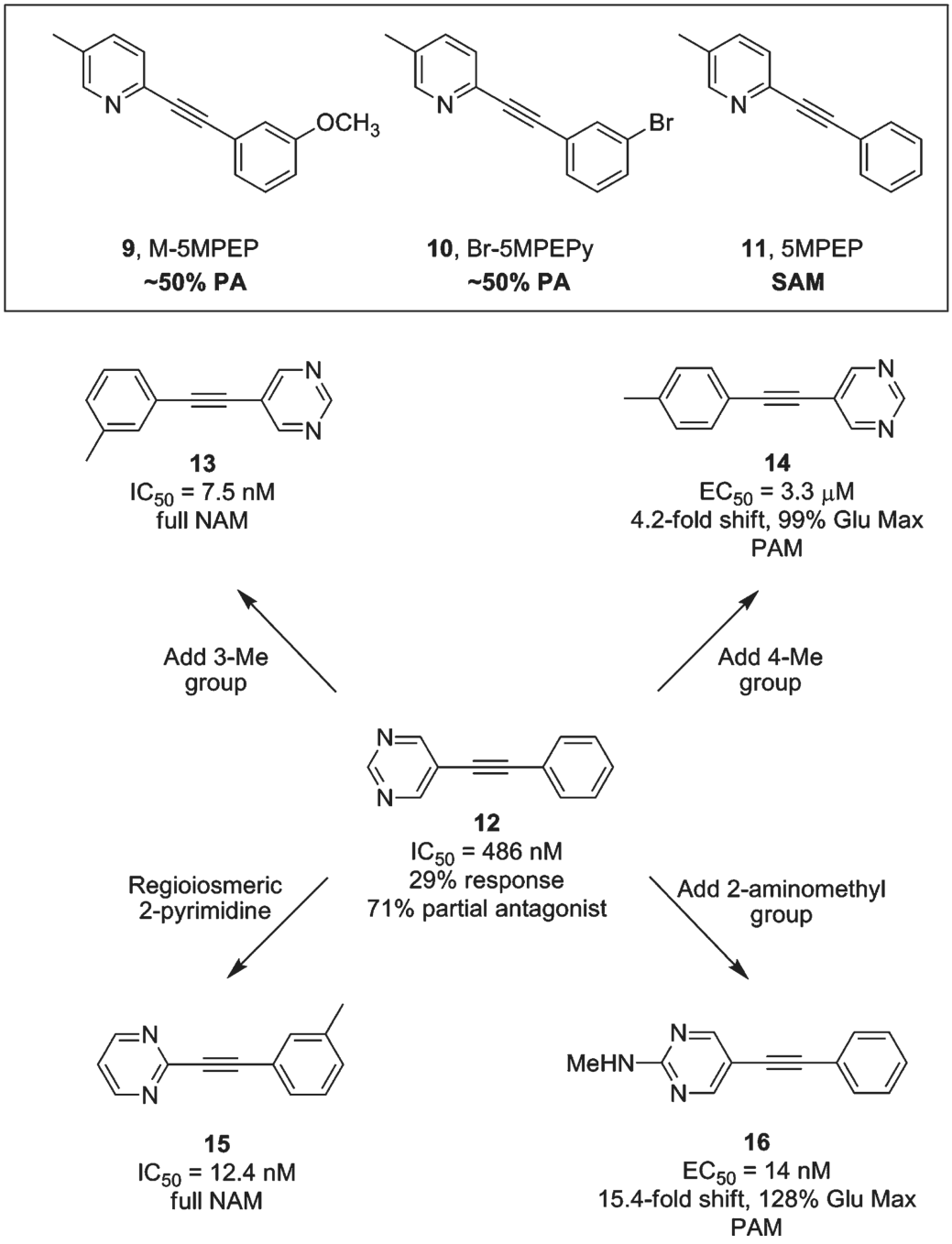



**Figure 3.**

Structures (inset) of the first $mGlu_5$ partial antagonists **9** and **10**, and a molecular switch, the removal of the 3'-phenyl substituent, within this scaffold that afforded 5MPEP (**11**), a SAM. Optimization of partial antagonist **12** resulted in the identification of either simple methyl substitution (either 3' or 4') to afford both a full NAM **13** and a PAM **14**. Further optimization provided NAM **15** and PAM **16**, highly potent ligands that exhibited NAM and PAM activity, respectively, in vivo.

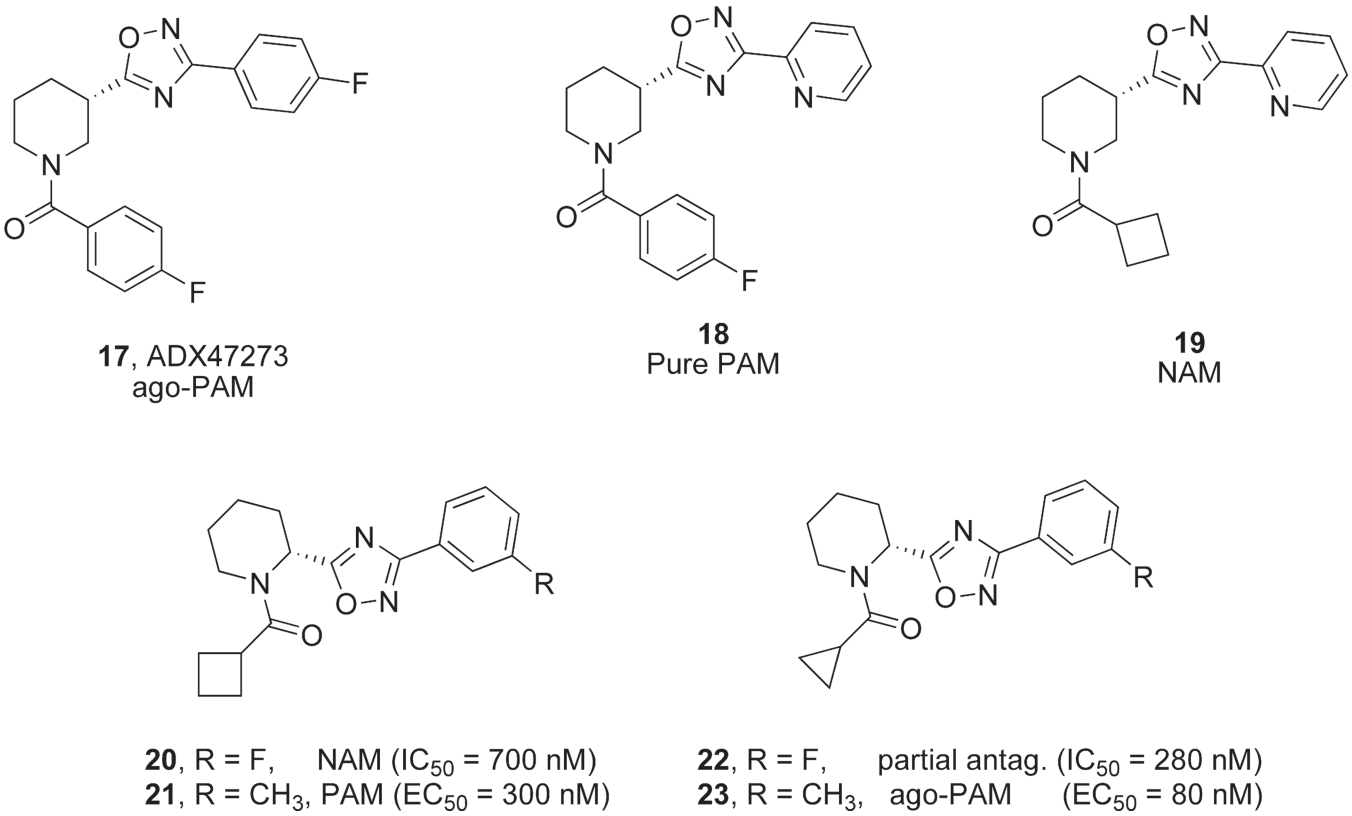



**Figure 4.**

Structure of ago-PAM ADX47273 (**17**) and analogues with subtle molecular switches that modulate modes of pharmacology to provide $mGlu_5$ allosteric ligands with the full breadth of potential pharmacology: pure PAM (**18**), weak NAM (**19**), full NAM (**20**), potent PAM (**21**), partial antagonist (**22**), and potent ago-PAM (**23**).

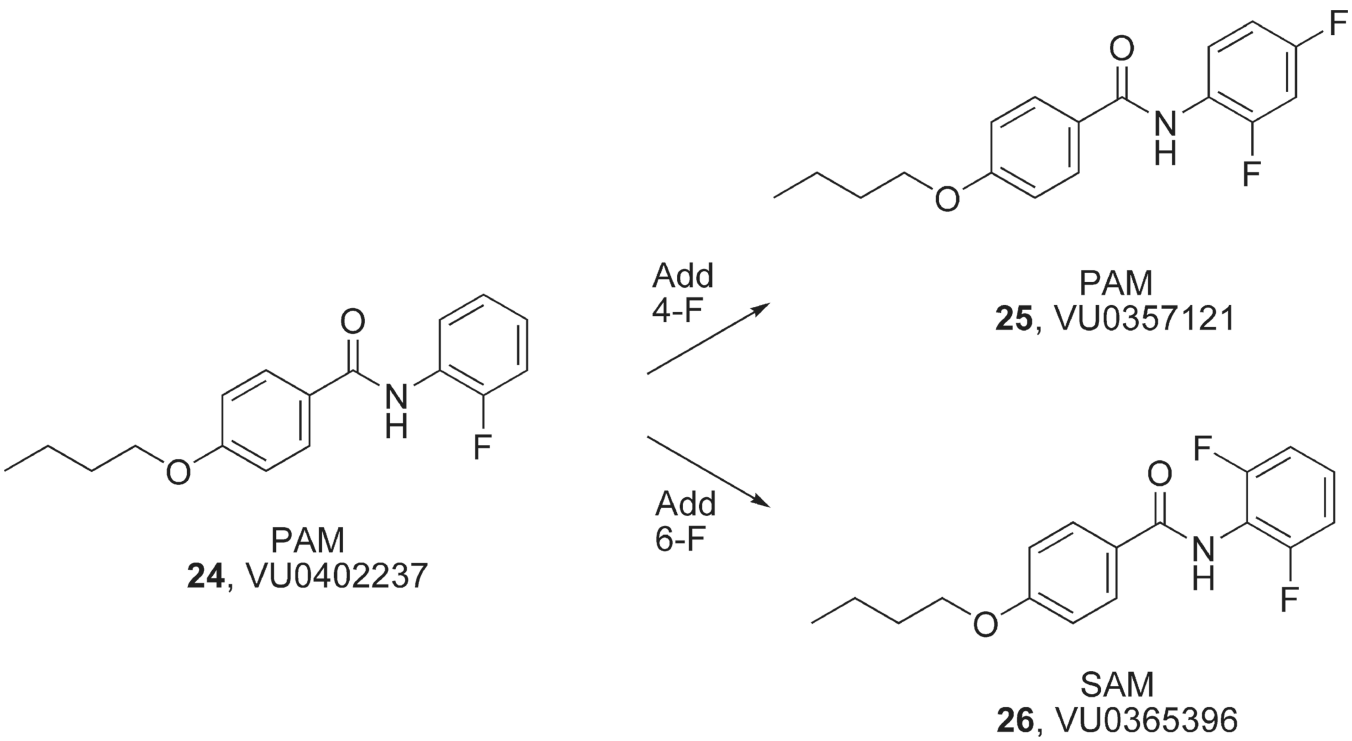



**Figure 5.**

Novel series of $mGlu_5$ PAMs that bind an as yet fully characterized third allosteric site, distinct from the MPEP and CPPHA sites. Optimization of PAM hit **24** led to a potent PAM **25** by the addition of a fluorine atom to the 4-position of the benzamide moiety. Unexpectedly, addition of a fluorine atom to the 6-position of **24** provided **26**, a potent SAM.

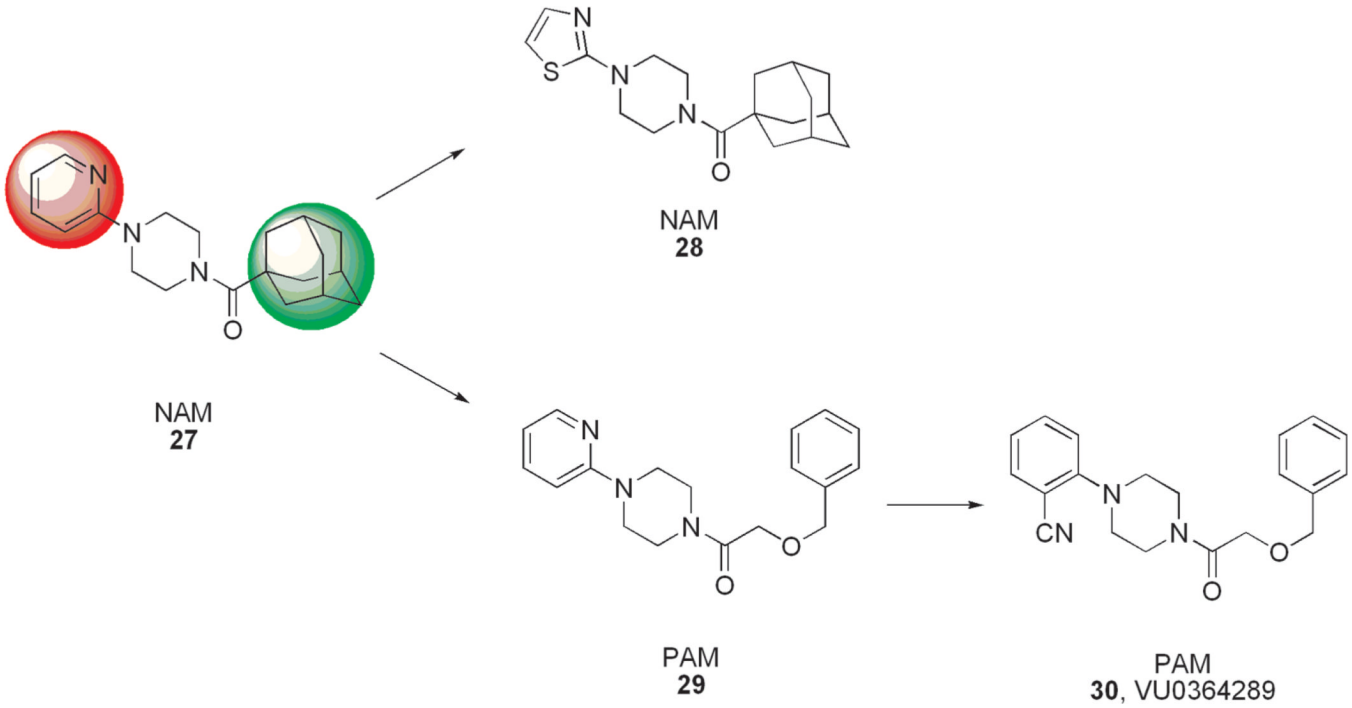
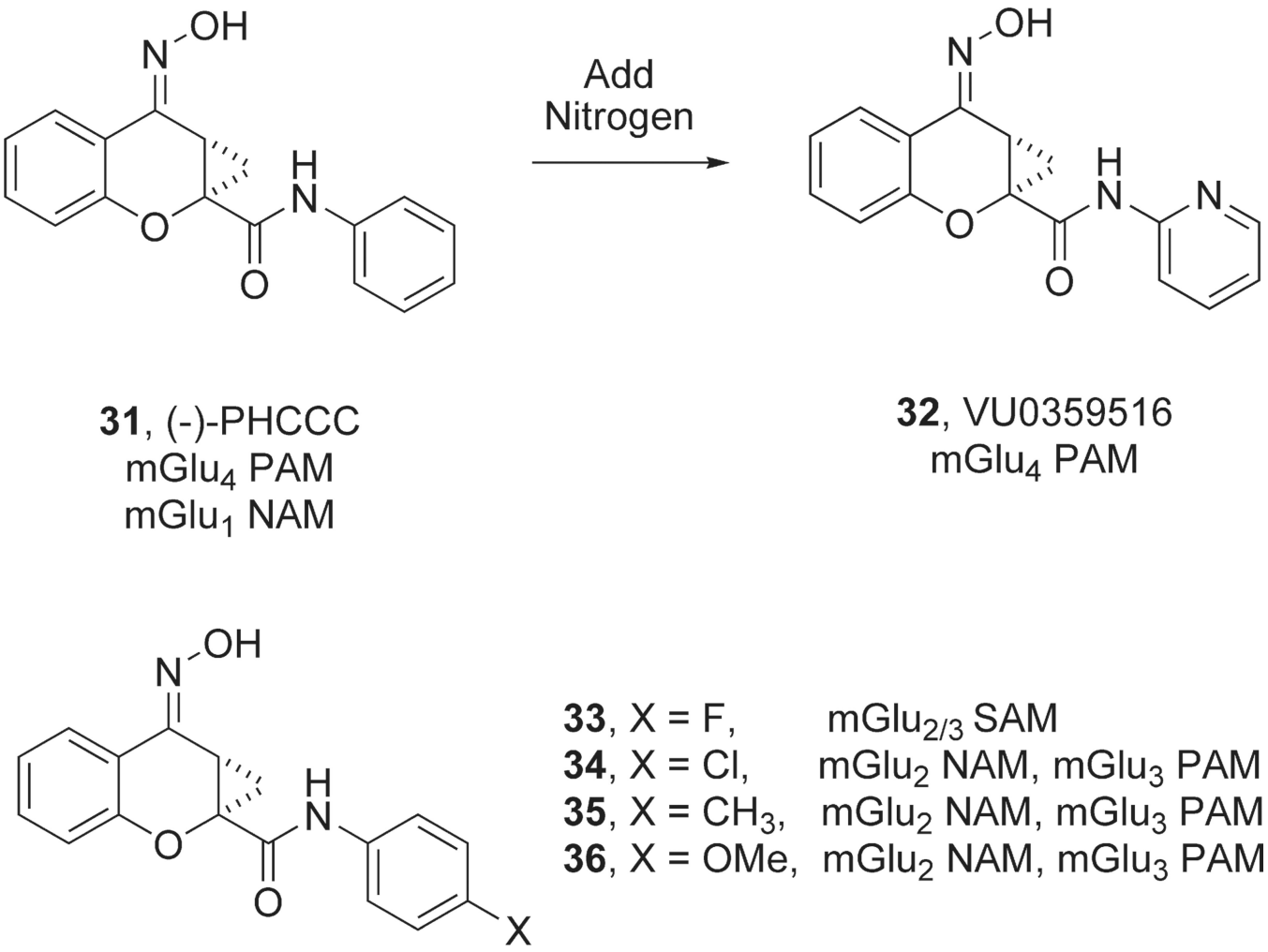



**Figure 6.**

HTS $mGlu_5$ NAM hit **27** displayed bidirectional SAR. Optimization of the heteroaryl moiety afforded additional, potent $mGlu_5$ NAMs, such as **28**. Optimization of the amide moiety identified the benzyloxy acetyl amide, as in **29**, as a molecular switch engendering $mGlu_5$ PAM activity. Further chemistry led to **30**, a potent, centrally active, and important new $mGlu_5$ PAM tool compound.







**33**, X = F, $mGlu_{2/3}$ SAM
**34**, X = Cl, $mGlu_2$ NAM, $mGlu_3$ PAM
**35**, X = $CH_3$, $mGlu_2$ NAM, $mGlu_3$ PAM
**36**, X = OMe, $mGlu_2$ NAM, $mGlu_3$ PAM

**Figure 7.**

Identification of molecular switches that alter mGluR subtype selectivity. The prototypical $mGlu_4$ PAM (−)-PHCCC **31** is a dual $mGlu_4$ PAM/$mGlu_1$ NAM. The addition of a moderately basic nitrogen atom to provide **32** abolishes $mGlu_1$ NAM activity, providing a highly selective and 10-fold more potent $mGlu_4$ PAM. A FRET assay identified **33**, an $mGlu_{2/3}$ SAM. Subtle substitution of the 4-fluoro moiety provided allosteric ligands **34**–**36** with unprecedented dual $mGlu_2$ NAM/$mGlu_3$ PAM activity.

## Table 1

Terms and Definitions

| term | definition (based on IUPHAR) |
|---|---|
| orthosteric site | binding site of the receptor's endogenous agonist |
| allosteric site | ligand binding site topographically distinct from the orthosteric binding site |
| positive allosteric modulator (PAM) | A ligand that can strengthen the action of an orthosteric ligand by binding at an allosteric site. The PAM may enhance the affinity and/or efficacy of the orthosteric ligand without any intrinsic activity. The PAM may also enhance coupling to the G-protein. |
| negative allosteric modulator (NAM) | A ligand that can weaken the action of an orthosteric ligand by binding at an allosteric site. The NAM may also inhibit coupling to the G-protein. Also known as a noncompetitive antagonist. |
| silent allosteric modulator (SAM) | ligand that fully occupies an allosteric site but exerts no pharmacological function on its own but can block the allosteric activity of both PAMs and NAMs; also termed neutral or pharmacologically silent ligands |
| partial antagonist (PA) | NAM that fully occupies the allosteric binding site but induces only a partial blockade of receptor signaling |
| allosteric agonist (AA) | ligand capable of receptor activation alone, in the absence of the orthosteric ligand, by binding at an allosteric site and engendering an active conformation of the receptor |
| ago-potentiator (ago-PAM) | An allosteric ligand that functions as both a PAM and an allosteric against. However, the agonism is usually observed at concentrations higher than that for the PAM activity. |