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REVIEW

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Progress toward Positive Allosteric Modulators of the Metabotropic Glutamate Receptor Subtype 5 ($mGlu_5$)

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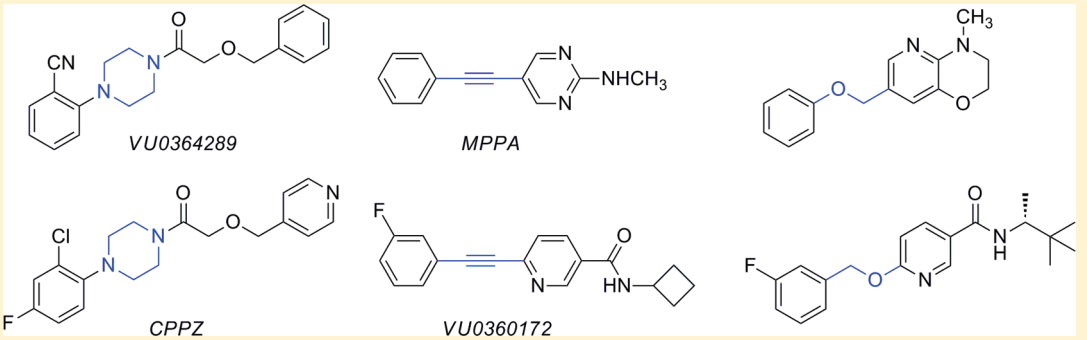
ABSTRACT:



This Review describes recent trends in the development of small molecule $mGlu_5$ positive allosteric modulators (PAMs). A large body of pharmacological, genetic, electrophysiological, and in vivo behavioral evidence has accumulated over the past decade which continues to support the hypothesis and rationale for the activation of the metabotropic glutamate receptor subtype 5 ($mGlu_5$) as a viable and promising target for the development of novel antipsychotics. Until recently, functionally efficacious and potent $mGlu_5$ PAMs have been somewhat structurally limited in scope and slow to emerge. This Review will discuss efforts since late 2008 which have provided novel $mGlu_5$ PAM chemotypes, offering ligands with a diverse range of pharmacological, physicochemical, and DMPK properties that were previously unavailable. In addition, significant biological studies of importance in the past few years using the well established PAMs known as DFB, CPPHA, CDPPB, and ADX-47273 will be discussed.

KEYWORDS: Metabotropic, $mGlu_5$, schizophrenia, allosteric, positive allosteric modulator, DFB, CPPHA, CDPPB, ADX-47273, glutamate

Glutamate is the single most important excitatory neurotransmitter in the mammalian central nervous system (CNS).[1] The concentration of glutamate within the cytoplasm of glutamatergic neurons is several times more than that of any other amino acid, approximately 5−10 mM, and within synaptic vesicles glutamate reserves can be significantly higher. The two major receptor classes that are known to be modulated by glutamate include ionotropic glutamate receptors (iGlus), which are multimeric glutamate-gated cation channels, and metabotropic glutamate receptors (mGlus), which are seven transmembrane heptahelical (7TM) spanning proteins coupled to effector G-proteins.[2] Ionotropic glutamate receptors are responsible for fast acting glutaminergic transmission in the CNS and are mediated by the three subclasses of iGlus: the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) receptors. All three iGlu channels undergo a conformational change and open in response to glutamate binding and induce excitatory post synaptic current, with the NMDA receptor perhaps best characterized and central to many hypotheses of CNS pathologies. A wide range of neurological disorders including chronic pain, schizophrenia, Alzheimer's disease, epilepsy, drug addiction, and fragile X syndrome have been associated with dysfunction of glutamatergic systems.[1,3] In contrast to ionotropic glutamate receptors, metabotropic glutamate receptors bind glutamate to *modulate* either presynaptic neurotransmitter release or postsynaptic excitatory neurotransmission.

Allosteric modulation of metabotropic glutamate receptors as a glutamate-based approach for therapeutic intervention, either via enhancing or inhibiting endogenous agonist responses, is a highly active area of research and drug development.[3−7] Allosteric mechanisms of receptor modulation provide several potential advantages over traditional orthosteric based strategies, including increased receptor subtype selectivity, improved chemical tractability for the targeting the CNS, and, importantly, reduced potential for receptor sensitization. Utilizing an allosteric strategy, modulator ligand does not activate the receptor on its own and therefore the temporal and spatial efficacy of endogenous glutamate is maintained. The full impact of a positive allosteric modulator (PAM) as a means of therapeutic intervention is now beginning to take shape clinically with the recent approval and success of

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the class C GPCR PAM Cinacalcet for renal induced hyperthyroidism and parathyroid cancer.[8] As additional safe and effective PAMs enter the market, the potential to fundamentally change how the entire GPCR-based drug discovery field that has been pursued for over the last half century will undoubtedly take hold.

The mGlu receptors, for which eight receptor subtypes are known, belong to the GPCR family C class of receptors and contain a large extracellular amino-terminal agonist binding domain which is linked to the 7TM via a cysteine-rich region. Glutamate and other orthosteric agonists and competitive antagonists bind within the extracellular *N*-terminal region while allosteric modulators have been shown to bind within the 7TM domain.[9] The mGlu subtypes belong to one of three groups based upon their structure, preferred effector coupling partners, and pharmacology.[10] Group I receptors, which include $mGlu_1$ and $mGlu_5$, couple to $G_{\alpha q}$ and its associated effector mechanisms and result in increases in intracellular calcium as a secondary messenger. Group II receptors, $mGlu_2$ and $mGlu_3$, and Group III, which includes $mGlu_4$ and $mGlu_{6-8}$ receptors, couple with G_i/G_o G proteins and decrease cyclic AMP synthesis. Group I mGlus are predominantly expressed postsynaptically in the CNS, whereas group II and III receptors are located primarily presynaptically where they control neurotransmitter release.[10−12] Group I selective orthosteric agonists[13−16] and later PAMs[17] of the Group I mGlu receptors, and in particular $mGlu_5$, demonstrated a unique ability to potentiate NMDA receptor currents in vitro in a highly specific manner. These studies in conjunction with the emerging "NMDA receptor hypofunction" hypothesis as a concept to account for the major symptom clusters of schizophrenia came to light in the mid-1990s[18−22] and provided the groundwork for a highly testable and novel hypothesis for this complex disorder which led the way to multiple strategies to enhance glutamatergic transmission.[23] The first successful and promising application of this approach through a mechanism also aimed at enhancing NMDA receptor function was recently validated clinically in a small patient population using the GlyT1 inhibitor RG1678, demonstrating for the first time statistically significant effects in treating the negative symptoms of the disease.[24] In the case of $mGlu_5$, the rationale and interest in pursuing $mGlu_5$ as a novel target for the treatment of schizophrenia continues to show great promise and has been bolstered recently by several pro-cognitive studies reported using novel chemotypes (vide infra). Elegant reviews which outline the detailed genetic and pharmacological evolution of the supporting rationale for the role $mGlu_5$ as it relates to NMDA mediated circuitry and potential therapeutic benefit in schizophrenia are noted.[5,10,25,26] This Review provides a discussion of recent progress since mid-2008 in the field of positive allosteric modulation of the $mGlu_5$ receptor and the development of new selective $mGlu_5$ PAMs and their potential utility in the treatment of schizophrenia. An additional aim is to discuss new data that has emerged for first generation PAMs which continue to provide new insights for the field.

I. SCHIZOPHRENIA AND FIRST GENERATION $MGLU_5$ PAMS

Schizophrenia is a complex disorder characterized by a combination of positive (thought disorder, hallucinations, delusions, paranoia) and negative (social withdrawal, apathy, anhedonia) symptoms along with significant cognitive deficits (perception, attention, learning, short and long-term memory, executive function). Due to poor efficacy,[27] poor patient compliance,[28] and cardiac risk,[29] researchers have long sought alternative hypotheses and improved therapeutic approaches to treat this disorder. The first outward symptoms of schizophrenia often initiate in early adulthood, requiring lifelong daily maintenance therapy at an annual cost to the United States of $68 billion (both direct and indirect, 2002 data), a figure which is second to Alzheimer's disease and nearly the same as stroke and congestive heart failure combined.[30] Current treatments have been in existence since the 1950s and include both typical and atypical antipsychotics. These agents rely on D_2 antagonism as the major biological mechanism of action and have been elegantly shown to correlate with clinical efficacy in treating the positive symptoms of the disorder.[31−33] Although these drugs were championed at the time as the greatest advance in psychiatric care resulting in worldwide deinstitutionalization, nearly six decades later the biomedical community still lacks fundamental advances in antipsychotic treatments to more fully address the negative symptoms and cognitive impairments. Tremendous advances in the current understanding of the neural circuits important for normal affect, sensory processing, and cognition have shifted the paradigm from hyperdopaminergia to NMDA receptor hypofunction as a potential primary causative component of the etiology underling schizophrenia.[34,35]

The discovery of the first selective $mGlu_5$ PAMs as one mechanism to begin to test the NMDA receptor hypofunction hypothesis were first described in 2003 by researchers at Merck beginning with a benzaldazine class of PAMs represented by 3,3′-difluorobenzaldazine (DFB, **1**, Figure 1).[36] Through radioligand binding assays, this class of modulators was confirmed to interact at a single well characterized negative allosteric modulator binding site in a competitive manner, known as the MPEP-site.[36] Interestingly, from the very outset, these novel allosteric modulators displayed an unexpected and subtle capacity to modulate calcium mobilization with all three modalities of pharmacology, including positive and negative (DMeOB, **2**) allosteric modulation as well as neutral or silent (DCB, **3**) modulation through the use of simple halogen and alkoxy group substitution. This phenomena, referred to as a "molecular switch", is the result of a subtle molecular modification leading to gross changes in receptor pharmacology-response via allosteric modulation.[37] "Molecular switches" have been observed across several other $mGlu_5$ chemotypes (vide infra) and can also be found within allosteric modulator scaffolds for entirely different classes of GPCRs.[38−40]

DFB is not particularly potent or brain penetrant, and the Merck team quickly moved to alternate lead scaffolds; however, in 2006, intracerebroventricular (i.c.v.) administration of DFB was reported in models of cognition and was shown in a spatial alternation task to enhance consolidation of memory upon administration immediately after learning, serving as the first $mGlu_5$ PAM to demonstrate pro-cognitive effects in vivo.[41] Additional i.c.v. studies reported in 2008 using DFB demonstrated reversal of ketamine-induced hyperlocomotion and improvements in ketamine-induced cognitive impairments in a novel object recognition test.[42] Following DFB, *N*-[5-chloro-2-[(1,3-dioxoisoindolin-2-yl)methyl]phenyl]-2-hydroxybenzamide (CPPHA, **4**) was disclosed as a much more potent $mGlu_5$ PAM.[17] The CPPHA benzamide series of PAMs was characterized as having a shallow structure−activity relationship (SAR), poor physicochemical properties, and limited activity at the rat $mGlu_5$ receptor and was therefore not utilized in vivo. Interestingly, unlike other $mGlu_5$ PAMs characterized to date, CPPHA

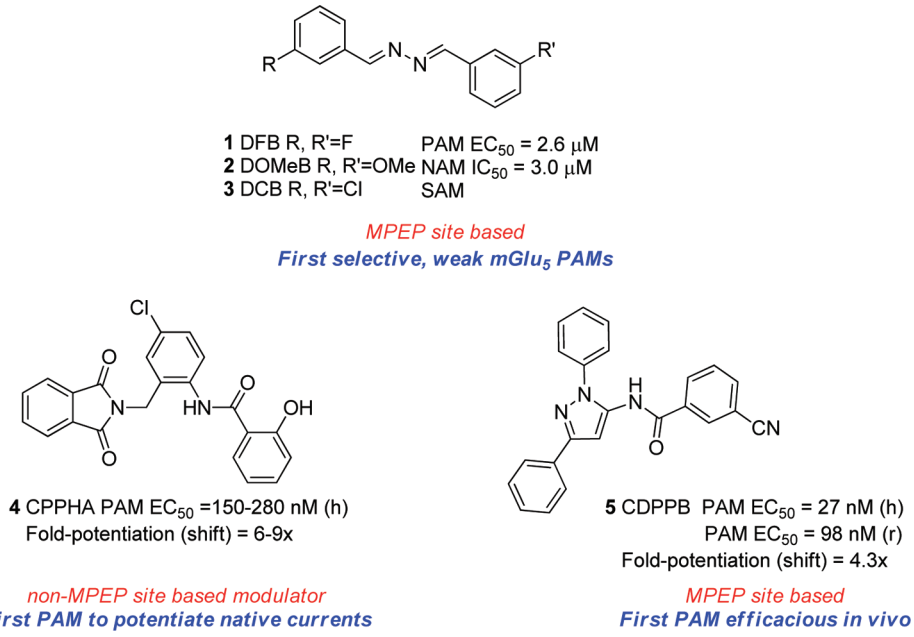


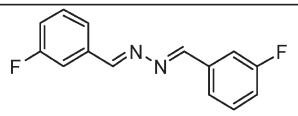
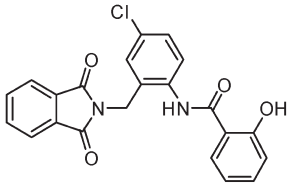
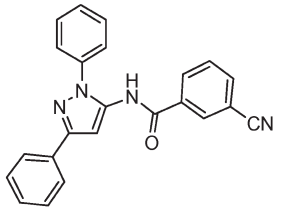
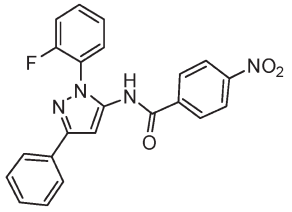
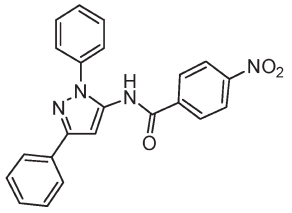
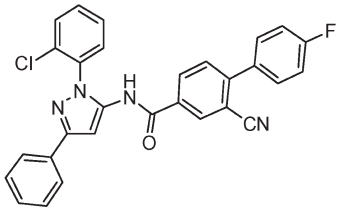
Figure 1. First-generation $mGlu_5$ PAMs: DFB (**1**), CPPHA (**4**) and CDPPB (**5**). Within DFB series "molecular switches" were identified to afford a NAM (DOMeB, **2**) and a SAM (DCB, **3**). DFB (**1**−**3**) and CDPPB series (**5**) share the same NAM binding site characterized by MPEP, whereas CPPHA (**4**) binds at a distinct, non-MPEP site.

appears to elicit receptor activation through a novel allosteric site that does not appear to directly involve the MPEP site.[17,26,43,44] Radioligand tools, either as negative allosteric modulators (NAMs), PAMs, or silent allosteric modulators (SAMs), are currently unavailable for the CPPHA site, and the potential impact of an appropriate in vivo tool compound from the CPPHA series is still lacking and thus leaves an important remaining question regarding potential in vivo efficacy for PAMs which modulate their activity outside the MPEP site. Interestingly, examination of DFB and CPPHA in independent signaling mechanisms (ERK1/2 phosphorylation and calcium mobilization) demonstrated that differential PAM signaling is possible, supporting the notion that PAMs which engage distinct allosteric binding sites and presumably unique modulator-receptor conformations can differentially facilitate downstream signaling responses.

The pyrazol-5-yl-benzamide series, which was characterized by 3-cyano-*N*-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB, **5**) and shown to have a significant MPEP site interaction became a significant and lasting tool compound for the field.[26,45,46] In contrast to DFB, CDPPB was shown to be the first systemically available $mGlu_5$ PAM, thus allowing for behavioral assessment in antipsychotic models, including reversal of amphetamine induced hyperlocomotion and reversal of deficits in prepulse inhibition, both of which have translational validity in patients with schizophrenia eliciting positive symptoms and cognitive deficits in sensory motor gating, respectively. CDPPB was shown to be efficacious in both of these models at moderate subcutaneous (s.c.) doses between 10 and 30 mg/kg. Further SAR studies were conducted as a means to improve the potency and physicochemical properties of CDPPB with the goal to alleviate the need for the use of toxic vehicle formulations previously required for systemic dosing of CDPPB.[47,48] Two subsequent compounds from the pyrazol-5-yl-benzamide series partially succeeded in fulfilling this goal with 10-fold improvements in potency and MPEP site affinity. These included 4-nitro-*N*-(1-(2-fluorophenyl)-3-phenyl-1H-pyrazol-5-yl)benzamide (VU-1545, **6**, Table 1)[47] and the related des-fluoro derivative VU-29 (**7**, Table 1).[48] Although neither VU-1545 nor VU-29 proved to have utility in vivo due to poor physicochemical properties inherent to the scaffold (cLogP > 4.5), a number of important in vitro relationships were established with these advanced CDPPB analogues, including confirmation for MPEP site interaction as a requirement for functional activity demonstrating a consistent 15−20-fold cooperativity driven leftward shift in potency relative to affinity. Importantly, CDPPB and VU-29 were shown to potentiate normal $mGlu_5$ responses in native subthalamic nucleus (STN) brain slices where $mGlu_5$ is expressed and shown to contribute to neuronal depolarization.[48] In contrast to $mGlu_5$ responses in the STN in brain regions where $mGlu_5$ is expressed but functionally incapable of depolarization, including the substantia nigra pars reticulata (SNr neurons), VU-29 and related $mGlu_5$ potentiators did not enhance agonist-induced depolarizations. VU-29 has also been shown to enhance both hippocampal LTD and LTP synaptic plasticity, which are critical forms of synaptic plasticity important for learning and memory formation.[49] Furthermore, these studies demonstrated that VU-29 maintained the endogenous balance of these two forms of synaptic plasticity.

Numerous in vivo studies using CDPPB have recently surfaced which continue to add evidence and support for the potential to treat CNS disorders associated with aberrant NMDA receptor function, including the cognitive impairments and negative symptoms of schizophrenia. Modulation of $mGlu_5$ with CDPPB has been shown to enhance spatial learning (Morris water maze),[49] reverse MK-801 induced deficits in behavioral[50] and cognitive flexibility,[51] negative learning,[52] and sucrose preference,[53] an animal model reported to be useful in measuring hedonic experiences which are a component of the negative symptoms found in patients with schizophrenia. Another interesting aspect of $mGlu_5$ activation recently uncovered using CDPPB is the ability of potentiation of $mGlu_5$ to facilitate

Table 1. First Generation $mGlu_5$ PAMs and Derivatives[a]

Compound	Structure	$rmGlu_5$ EC_{50} (μM)	Glu Fold Shift/ K_i (μM)	In Vivo Behavior Assays	Comments	Reference
1: DFB		2.6	2.0 / 8.5	i.c.v. only	1st selective PAM, 'molecular switch' prone	O'Brien 2003
4: CPPHA		0.81	7.9 / NB	None	1st non-MPEP no switches	O' Brien 2004
5: CDPPB		0.098	4.3 / 3.0	s.c. AHL, PPI, and others	1st systemic PAM no switches	Kinney 2005
6: VU-1545		0.0096	4.8 / 0.16	none		de Paulis 2006
7: VU-29		0.009	2.5 / 0.24	none	agonist in truncated $mGlu_5$	Chen 2007
8		2.43	NA / 0.023	none	highest affinity CDPPB analog	Zou 2011

[a] Abbreviations: i.c.v., intracerebroventricular; s.c., subcutaneous; AHL, amphetamine induced hyperlocomotion; PPI, pre-pulse inhibition.

extinction of memories associated with drug-addiction and drug-seeking behavior[54,55] as well as reversal of deficits in novel object recognition due to methamphetamine access.[56] More recently, the Merck team reported on the effect of CDPPB in novel object recognition and was able to examine across similar doses the effects of CDPPB on several protein markers of synaptic plasticity in prefrontal cortex and hippocampus ex vivo.[57] Interestingly, behavior outcomes and several protein markers in the prefrontal cortex were characterized by an inverted U-shaped dose−effect relationship, suggesting that, at least for CDPPB or CDPPB-like compounds, dose selection for potential compounds entering clinical trials with cognition enhancing end points could prove challenging. A subsequent study by the Merck team reported in 2010 examined a subchronic (7 day) dosing regimen of CDPPB and was shown to maintain antipsychotic efficacy as well as maintain levels of protein markers in the

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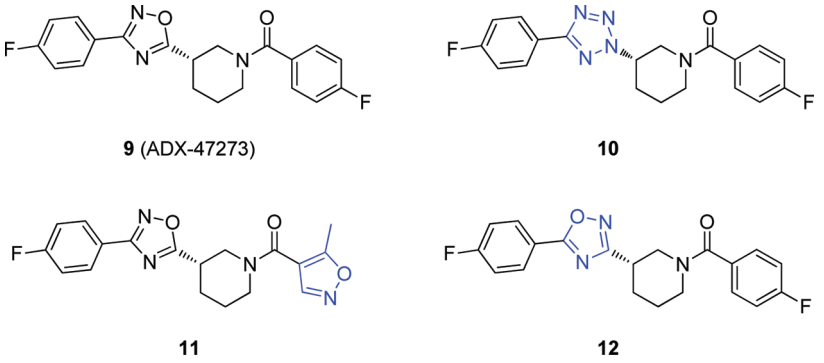


Figure 2. Piperidinylazole-based $mGlu_5$ PAMs developed by Addex: ADX-47273 (**9**), *N*-linked tetrazole (**10**), isoxazole amide (**11**), and regioisomeric oxadiazole (**12**) reported in patent applications as being efficacious in reversal of amphetamine and/or PCP models of locomotion.

striatum, including $mGlu_5$ expression levels.[58] In contrast to the antipsychotic effects, changes in $mGlu_5$ expression levels were reported to modestly decrease in the frontal cortex from 840 to 696 fmol/mg (B_{max}) and an enhancement in wakefulness observed on day one in sleep architecture was lost by the third and seventh days, indicating a potential tolerance development in cortical sleep function. Interestingly, withdrawal of CDPPB on day six resulted in the return of $mGlu_5$ receptor levels to vehicle levels on day seven. These studies are the first to examine the potential for tolerance following repeated dosing of an $mGlu_5$ PAM. It will be interesting to see if these brain-region dependent dynamic changes in protein markers persist after repeated dosing with alternate $mGlu_5$ PAM scaffolds and with varying dose. Moreover, the question of tolerance in cognition models involving cortical and hippocampal function remains to be investigated.

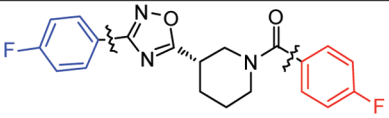
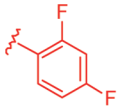
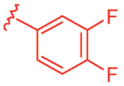
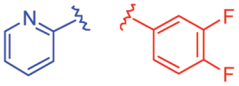
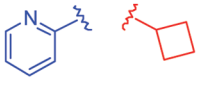
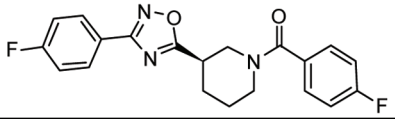
Since the discovery of CDPPB and VU-29, a small set of new pyrazol-5-yl-benzamide analogues were reported by Hauck-Newman and co-workers in 2010.[59] Inspired by a proposed overlapping NAM pharmacophore, a key compound from these efforts is represented by *N*-(1-(2-chlorophenyl)-3-phenyl-1H-pyrazol-5-yl)-2-cyano-4′-fluoro-[1,1′-biphenyl]-4-carboxamide (**8**, Table 1). Based on prior CDPPB and NAM SAR, the authors hypothesized that the benzamide ring portion of CDPPB was the dominating structural feature responsible for driving receptor affinity and the pyrazole ring for biocharacter/efficacy. A combination of the 2-chloro and 4′-fluorobiphenyl substituent was key to improving the affinity > 100-fold relative to CDPPB (K_i = 23 nM). Previous affinity/potency relationships within the CDPPB template nicely highlight a consistent cooperative allosteric interaction ($K_i/EC_{50} > 10$);[47] however, PAM **8**, which appears to be the highest affinity reported CDPPB analogue to date, loses all functional cooperatively as noted by its weak rightward shifted EC_{50} of 2.4 μM. Additional SAR from this study appears to suggest an affinity cutoff of ~300 nM where cooperativity in calcium mobilization begins to diminish. Based on cLogP values, compounds within this series are not acceptable for in vivo studies. Remarkably, in spite of its limited solubility and formulation challenges, CDPPB and CDPPB-like compounds continue to contribute new insights into ongoing questions surrounding the validity of $mGlu_5$ as a therapeutic target.

II. PIPERIDINES

In 2005, Addex disclosed a structurally distinct class of piperidinyl 1,2,4-oxadiazoles which represented the first $mGlu_5$ PAM containing a stereogenic center represented by (*S*)-(4-fluorophenyl)(3-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-piperidin-1-yl)methanone or ADX-47273 (**9**, Figure 2).[60,61] Shortly thereafter, multiple patent applications within the piperidinyl core scaffold appeared from Addex,[62−68] and in addition to **9** exemplary compounds containing alternate benzamide moieties and heterocyclic linkers were disclosed in patent figures with antipsychotic-like activity in amphetamine and PCP models of hyperlocomotion, including **10**,[67] **11**,[62] and **12**.[64] Eventually in 2008, Wyeth published a full account of ADX-47273 in vitro and in various models of antipsychotic behavior and cognition.[69] More recently, Merz disclosed similar behavioral studies using ADX-47273.[70] ADX-47273 was found to have efficacy in several models sensitive to antipsychotics after intraperitoneal (i.p.) administration at doses of 100 mg/kg and greater.[69] Interestingly, in models of cognition, including novel object recognition and five-choice serial reaction time test, ADX-47273 was shown to be efficacious at much lower doses of 1 and 10 mg/kg, respectively.[69] In a report by the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) group, ADX-47273 was shown to be efficacious at 10 mg/kg i.p. in Morris water maze, a model of hippocampus-dependent spatial learning.[49]

Both CDPPB and ADX-47273 represent key systemically available tool compounds which have contributed significantly to current understandings in the field. Subchronic studies using ADX-47273 to compare with the studies reported using CDPPB have not yet appeared, perhaps due in part to the poor physicochemical properties of ADX-47272. Interestingly, both of these PAMs have been reported to induce weak agonist-like responses alone above 1 μM in $mGlu_5$ cell lines, which leaves potential unanswered questions regarding the mechanism of in vivo action for these agents. In an attempt to address this agonist phenomenon, a focused lead optimization effort was reported by VCNDD utilizing a 3 × 12 library array approach.[71] A robust effect on agonist activity was observed depending on the identity of the oxadiazole ring substituent. For example, as shown in Table 2, modification of the oxadiazole substituent with heterocyclic substituents led to identification of 2-pyridyl analog **13** as being nearly equipotent to **9** and without agonist activity; the 3- and 4-pyridyl (**13**-**14**) congeners were 10−30× less active. In contrast to **13**, the 2-thiophenyl (**16**) analogue retained robust agonist activity similar to **9**. Further optimization of the benzamide portion led to a slight improvement in potency with the 3,4-difluoro derivative **18**; incorporation of the 2-pyridyl "pure-PAM" switch led to the hybrid pure-PAM **19** with an EC_{50} of 300 nM and a robust 14-fold shift of the glutamate concentration response curve. Interestingly, PAM **19** provided the first potentiator with a basic center, allowing salt formation and overall improvements

Table 2. 3-Piperidinyl Oxadiazole $mGlu_5$ PAMs[69,71,a]

Compound	Structure	$rmGlu_5$ EC_{50} (μM)	Fold Shift/ K_i (μM)	In Vivo Behavior Assays	Comments
9: ADX-47273		0.17	9.0 / 4.3	i.p. AHL, CAR, 5-CSRT	lowers NAc dopamine, ago-PAM, 'molecular switch' prone
13		0.35			pure-PAM
14		5.0			pure-PAM
15		1.4			pure-PAM
16		0.17			ago-PAM
17		0.22			ago-PAM
18		0.13			ago-PAM
19		0.30	14/ NT		pure-PAM- no agonist activity up to 30 μM
20		IC_{50} = 8.7			Weak NAM
21		1.68			*R*-ent 10x less active, full efficacy

[a]Abbreviations: AHL, amphetamine induced hyperlocomotion; PPI, pre-pulse inhibition; CAR, conditioned avoidance response; 5-CSRT, five choice serial reaction time; NAc, nucleus accumbens; NR, not reported.

in physicochemical properties (cLogP < 3.6); however, pharmacokinetic evaluation of **19** and close analogues in rats in these laboratories (unpublished results) revealed significantly diminished brain penetration relative to **9** and rapid clearance of parent compound, and thus, further in vivo studies were not possible.

The potential for stereoselective potentiation by a modulator, a first for the $mGlu_5$ PAM field, was confirmed by the 10-fold rightward shift in EC_{50} observed for the *R*-enantiomer **21** versus ADX-47273 (**9**). Interestingly, incorporation of a cyclobutyl amide as reported with the single analogue **20** led to the first non-MPEP "molecular switch" outside the DFB series. This also represented the first report of a saturated cycloalkyl derivative from within the Addex piperidinyl scaffold, and subsequently researchers at VCNDD conducted a follow up investigation into

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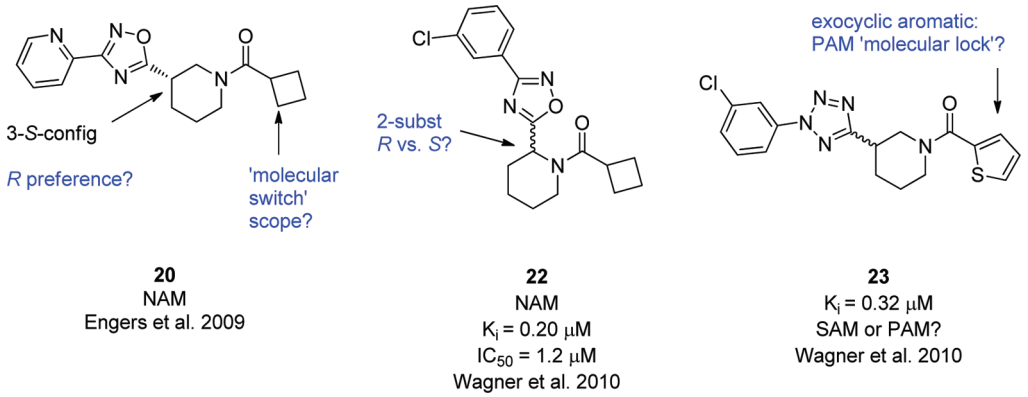


Figure 3. Pipiperidinyl cyclobutyl amides and hypotheses for "molecular switch/lock": Addex inspired cyclobutyl amide as "molecular switch" (**20**),[71] 2-piperidinyl oxadiazole HTS NAM lead reported by Gedeon Richter (**22**),[73] and functionally "inactive" thiophene amide (**23**).[73]

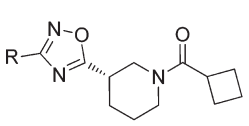
the cycloalkyl "molecular switch" to see if this structural modification might translate a pharmacological switch across other subseries utilizing alternate core structures and substituted oxadiazoles.[72] These investigations were further inspired by a report by the Gedeon Richter group[73] which disclosed a series of disubstituted oxadiazoles and tetrazoles, including **22**−**23**, as $mGlu_5$ modulators reminiscent of the Addex based PAMs (Figure 3).

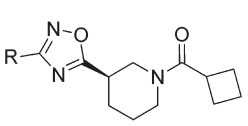
In contrast to the piperidinyl substituted Addex analogues within Table 2, the Gedeon Richter group tested various core structures, including piperidinyl as well as thiazolidinyl and pyrrolidinyl ring systems, which were linked primarily at the 2-position rather than the 3-position of the piperidine ring. The compounds described were reported functionally as either inactive or as NAMs, all with varying degrees of affinity for the MPEP site, including a handful with K_i < 100 nM; however, it is not clear from the experimental results if the calcium functional assay utilized was capable of detecting PAM activity. If activity was assessed only in antagonist mode, then based upon SAR described in Table 2 it is conceivable that the 3-substituted piperidinyl thiophene derivative **23** as well as other compounds reported as inactive may in fact have PAM activity. This premise is also built upon the hypothesis that within the 3-substituted scaffolds (i.e., 3-piperidinyl) the cyclobutyl amides are *highly* biased as "molecular switches" toward NAM activity and, therefore, can be considered in essence a "molecular lock" such that new modifications from within the template no longer have propensity to undergo further pharmacological mode switching. In addition to the apparent absence of mode switching observed within subsequent analogues of scaffold **20**, the concept of a "molecular lock" is proposed to include efficacy mode changing modifications involving more than one heavy atom.

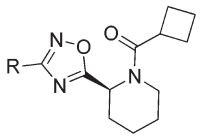
In order to further address the scope and nature of this "molecular switch", the VCNND group systematically examined 2- versus 3-substitution of the piperidine ring, as well as the impact of the chirality at the stereogenic center. Several insights were gained from this study, and key compounds are shown in Table 3. Within the 3-piperidinyl series, holding the cyclobutane ring constant, the use of lipophilic aromatic rings known to be optimal for PAM activity (i.e., **9** and **16** Table 2) in fact led to compounds which were either inactive (**24a**−**24b**) or had very weak NAM activity. The 3-fluoro phenyl congeners, 3-(*S*)-**24c** and 3-(*R*)-**24c**, both behaved as NAMs, similar to **20**, with a preference for the *S*-stereoisomer. A further improvement in potency for NAM activity was obtained for the 3-chloro derivative, particularly for the *S* preferring stereoisomer (3-(*S*)-**24d**) with an IC_{50} of 200 nM and a full blockade of the calcium response. The *R*-stereoisomer of **20**, 3-(*R*)-**24e**, proved to behave similarly with weak NAM activity. Thus, it appears within the context of the 3-piperidinyl series the cyclobutyl analogues retain an overall NAM activity profile thus far (**26**, Figure 4), demonstrating a robust "molecular switch" or "molecular lock" toward NAM activity.

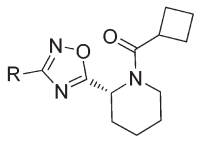
In contrast to the 3-piperidinyl series, the topologically distinct and compact 2-piperidinyl series appeared to be more susceptible to subtle molecular switches in pharmacology with single atom modifications. The first hint of promiscuous pharmacological switching was observed with **25c**, where both 3-fluorophenyl stereoisomers (2-(*S*)-**25c** and 2-(*R*)-**25c**) were found to have PAM activity. In the case of the *S*-stereoisomer, a maximum glutamate response of 71% and potency of 700 nM were found. Analogues with a 2-thiophene (**25a**) or a 3-chlorophenyl substituent (**25b**) retained NAM activity like that observed within the 3-piperidinyl series, although with diminished potency for the 3-chlorophenyl analogues (3-(*S*)-**24d** vs 2-(*S*)-**25b**). The preparation of a subsequent library of 18 analogs within either the context of a 3-CH_3 phenyl or 3-F phenyl (**27**) showed robust retention of NAM and PAM activity respectively, again reinforcing the unique mode switching behavior of the 2-piperidinyl ring system. In particular, a highly potent and efficacious PAM, **28**, was identified bearing a cyclopropyl amide. Overall **28** represents a modest 2-fold improvement over ADX-47273 (**9**) in terms of potency; however, if one considers the MW reduction for **28**, this modification translates to a >0.1 kcal/mol/non-H atom improvement in ligand efficiency (LE = 0.33 for ADX-47273 vs LE = 0.44 for **28**)[74,75] and reduction in cLogP by 2 orders of magnitude (3.79 vs 1.72, ChemBioDraw Ultra, version 12.0). In light of these promising improvements, further examination of the DMPK and pharmacological profile of **28** is warranted to better understand if a more optimal compound for in vivo studies can be identified within the larger oxadiazole-based class of PAMs. Interestingly, contraction of the core piperidine ring to a pyrrolidine allowed the 3-fluoro PAM switch to transfer giving rise to a modestly potent PAM **29** which, upon resolution of the individual enantiomers, revealed a 13-fold enantioselective potentiation, in this case for the (*R*)-**29** isomer. Shape-based modeling studies using this now sizable database of compounds within the same scaffold family with propensities for mode switching will be interesting to examine further, with the purpose to hopefully gain a fuller understanding of the molecular

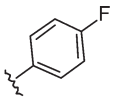
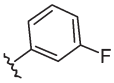
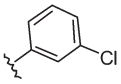
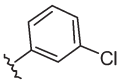
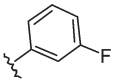
Table 3. 2- and 3-Piperidinyl Oxadiazole $mGlu_5$ Modulators[72,a]


3-(*S*)-**24**


3-(*R*)-**24**


2-(*S*)-**25**


2-(*R*)-**25**

Compound	R Group	Pharmacology	$rmGlu_5$ IC_{50} (μM)	$rmGlu_5$ EC_{50} (μM)	Glu Max (%)
3-(*S*)-**24a**		NAM	9.3	NA	67
3-(*R*)-**24a**		Inactive			
3-(*S*)-**24b**		Inactive	NA	NA	
3-(*R*)-**24b**		Inactive	NA	NA	
3-(*S*)-**24c**		NAM	2.4	NA	31
3-(*R*)-**24c**		NAM	>10	NA	60
3-(*S*)-**24d**		NAM	0.2	NA	2.4
3-(*R*)-**24d**		NAM	3.1	NA	18
20		NAM	8.7-10	NA	23-33
3-(*R*)-**24e**		NAM	9.9	NA	19
2-(*S*)-**25a**		NAM	2.6	NA	11
2-(*R*)-**25a**		NAM	3.5	NA	7
2-(*S*)-**25b**		NAM	0.9	NA	6
2-(*R*)-**25b**		NAM	10	NA	38
2-(*S*)-**25c**		PAM	NA	0.7	71
2-(*R*)-**25c**		PAM	NA	0.6	37
2-(*S*)-**25d**		NAM	10	NA	33
2-(*R*)-**25d**		Inactive	NA	NA	

[a]Abbreviations: NA, not applicable.

basis for mode switching within these chemotypes. PAMs containing saturated nitrogen heterocycle ring systems, as first discovered by Addex, continue to show great promise and display a wide range of pharmacological properties. It is anticipated that future disclosures and investigations will shed further light on their full potential relative to existing templates.

III. ACETYLENES

Despite the breadth of "molecular switches" uncovered within the DFB series and now within the ADX-47273 series, it is somewhat surprising that NAM to PAM mode switching was until recently not reported within the classical 2-methyl-6-(phenylethynyl)pyridine (MPEP **30**, Figure 5) or related acetylene based antagonist scaffolds. The first reports of variable pharmacology within the MPEP series were elegantly characterized by Rodriguez and colleagues,[76] resulting in the identification of several partial antagonists (PA) in addition to the first high affinity MPEP-site SAM 5MPEP (**31**); however, PAMs from an acetylene scaffold were not available at the time. Acetylene-based $mGlu_5$ PAMs first appeared in the patent literature[77] and at a Fall National ACS meeting in 2007[78] and were later described in the primary literature in 2008.[79] These compounds were uncovered as an indirect and serendipitous discovery by the Merz group

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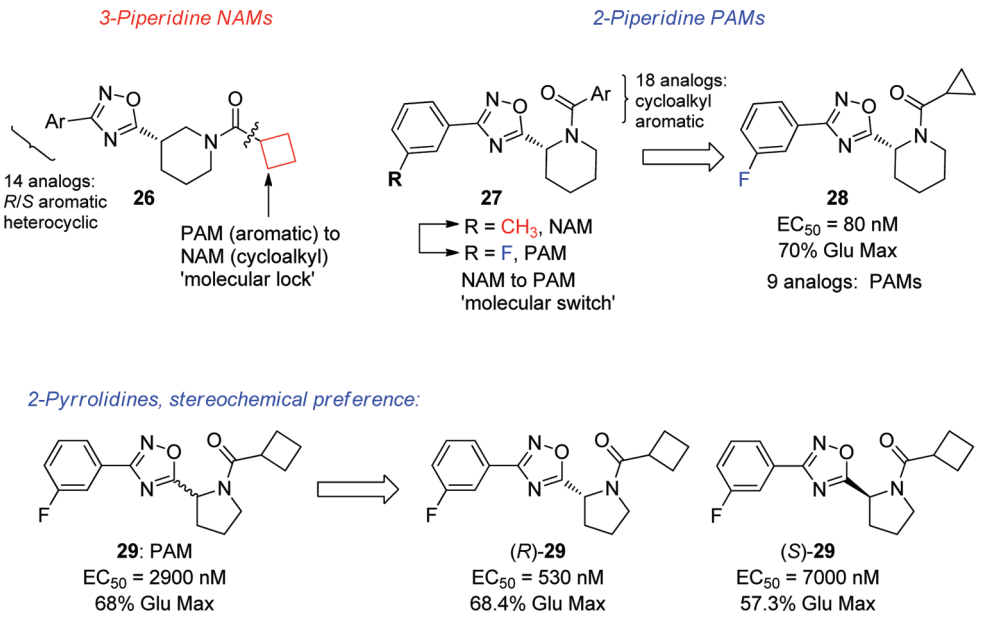

Figure 4. Structures of ADX-47273 analogues with "molecular locks" and subtle "molecular switches": 2- vs 3-substituted piperidines (**26**−**28**), transfer of "molecular switch" to 2-substituted pyrrolidine ring system and stereochemical preference (**29**).

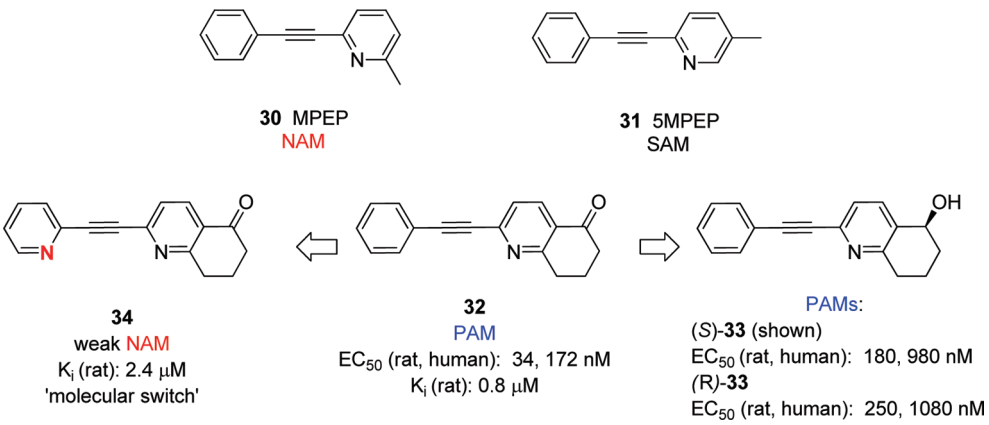

Figure 5. Structures of MPEP and 5MPEP (**30**, **31**), first reported MPEP-based $mGlu_5$ PAMs (**32**, **33**) and example of "molecular switch" within dihydroquinolinone scaffold (**34**) reported by Merz.

during an $mGlu_1$ antagonist virtual screening campaign and subsequent hit-to-lead optimization. This investigation led to 2-(phenylethynyl)-7,8-dihydroquinolin-5(6H)-one (**32**) and the reduced alcohols (*S*)-**33** and (*R*)-**33** as PAMs with excellent to moderate potency against both a rat (34−250 nM) and human (172−1080 nM) cell line with moderate fold shifts of 2−3-fold. Interestingly, complete reduction of the carbonyl group to form the parent tetrahydroquinoline or ring-opening to form the methyl nicotinate ester resulted in modulators with antagonist activity (not shown). In addition, replacement of the pendant phenyl group with a more polar 2-(pyridin-2-ylethynyl) substituent (**34**) resulted in a switch to antagonist activity with only a modest loss in affinity. The authors concluded that the presence of the appropriate hydrogen bond acceptor in a fixed geometry was optimal for conferring the PAM activity and a pharmacophore was proposed based upon the disclosed SAR. No in vivo characterization of **32**−**34** was reported.

Concurrent with the work by Merz, the VCNDD team reported on efforts to further develop functionally partial antagonists[76] targeted to fully occupy the MPEP site.[80] The Vanderbilt team mined results from an HTS campaign of a 160 000 compound library which examined both potentiator and antagonist activity. From this effort, pyrimidine **35** was identified as a partial antagonist lead (Figure 6).[80] Modification of **35** led to a single atom "molecular switch" to give weak PAM **36**, via introduction of a 4-methyl substituent. Additional 4-substituted analogues, including the ethyl homologue, also conferred weak PAM activity. PAM **36** had no effect on receptor response in the absence of glutamate, and despite its potency in the micromolar range **36** displayed a robust fold-shift of 4.2. A subsequent report by the VCNDD group disclosed further subtleties in pharmacology and was able to arrive at a much more potent acetylenic pyrimidine PAM **37** (MPPA), bearing a 2-aminomethyl substituent.[81] The aminomethyl PAM-switch was able to override previously identified NAM molecular switches, and this respect can be considered a "molecular lock". Remarkably despite the >200-fold improvement in potency, this compound still behaved as a pure-PAM, similar to **36**, with no effect on receptor response when

dx.doi.org/10.1021/cn2000519 |*ACS Chem. Neurosci.* 2011, 2, 450–470

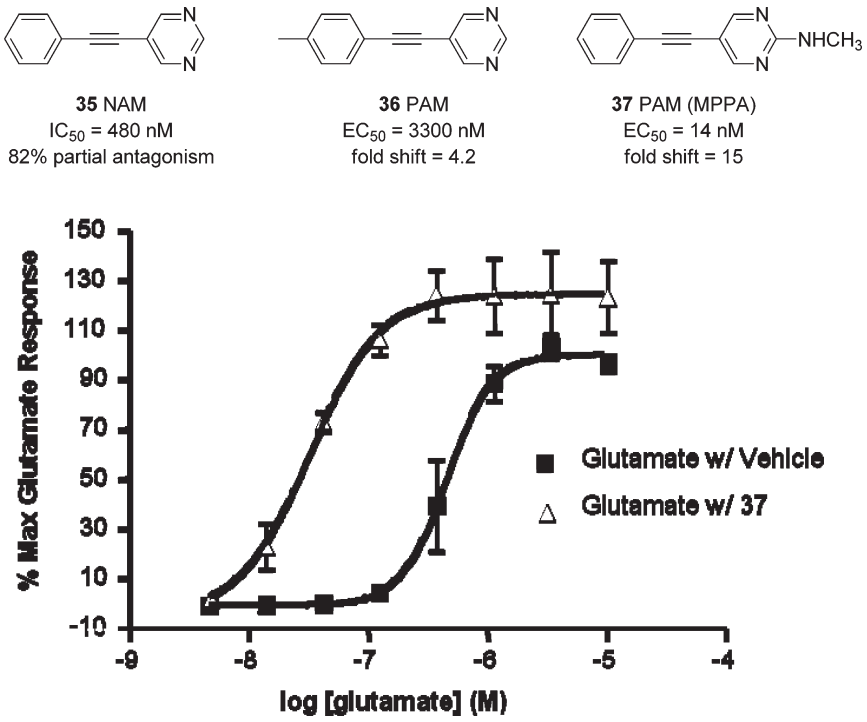

Figure 6. Acetylenic pyrimidine $mGlu_5$ modulators: (top panel) partial antagonist discovered from Vanderbilt HTS (**35**), 4-methyl substituted PAM (**36**), and optimized 2-aminomethyl PAM (**37**). (bottom panel) PAM **37** potentiates the response of the glutamate concentration response curve 15-fold and increases agonist sensitivity.

tested alone. In addition, an improved and robust leftward fold-shift of 15-fold was observed for **37** (MPPA, Figure 6), which appears to be largest fold-shift for an $mGlu_5$ PAM with MW below 300! With the potency improvements and the unique pure-PAM profile for **37** (MPPA) relative to the previous comparator PAMs **5** and **9** utilized in vivo, compound **37** (MPPA) was evaluated for its properties in reversal of amphetamine induced hyperlocomotion. PAM **37** (MPPA) when dosed i.p. at 3, 10, or 30 mg/kg 30 min prior to amphetamine administration provided a modest dose response effect, with significant reversal in hyperlocomotion noted at 30 mg/kg. Although not highly efficacious, these data suggest that ago-potentiation is not required for efficacy in this antipsychotic model and pure-PAMs alone may be sufficient.

A recent report by AstraZeneca in 2011 utilized **37** as a starting point to examine several aminopyrimidine replacements with the goal to ultimately incorporate suitable nonacetylene linkers.[82] Solubility was a key feature they wished to understand within the series while maintaining an appropriate cLogP. Replacement of the pyrimidine with various groups including azaindoline (**38**−**39**), benzimidazole (**40**), azabenzimidazole (**41**), or *N*-methyl 8-azaoxazine (**42**) was incorporated with the phenyl acetylene moiety retained throughout. A select set of the more interesting results from these studies is shown in Table 4. The direct azaindoline **38** proved to be nearly equipotent to **37**, methylation resulted in a 7-fold loss in activity (**39**), and cLogP exceeded 4.0. The benzimidazole **40** and in particular the *N*-methyl azabenzimidazole **41** was also suitable as an amiopyrimidine replacement with good potency and solubility (∼40× improved vs **37**) and excellent cLogP of 3.1. The bicycle could also be expanded to an azaoxazine ring system **42** which was nearly equipotent and with low solubility. Interestingly, modification of this ring system to give the regioisomeric 5-azaoxazine **43** was also effective but rather steep SAR was noted for the des-methyl analog **45** which was inactive, while the 8-isomer retained activity, suggesting a flexible bidentate interaction with the receptor for the aminopyrimidine isostere that is clearly not permitted within the 5-azaoxazine core. Acetylation of **45** however to reintroduce a hydrogen bond acceptor afforded **46**, which returned PAM activity to below 100 nM with a cLogP below 3.0. Importantly, **46** improved solubility to levels which previously had not been observed for the other active acetylene containing PAMs (>200 μM) described, supporting further investigation (vide infra). No additional pharmacological or in vivo characterization for **38**−**46** was reported, and it is not clear if these potentiators lack intrinsic activity of their own as noted for **37** (MPPA). In addition, no pharmacological mode switching was reported for these analogues described using the reported cell lines.

The second series of acetylenic PAMs from the Vanderbilt group was disclosed in a patent application in late 2008[83] and then in more detail in 2010−2011.[84,85] These PAMs contained a basic acetylene core with either a benzamide or nicotinamide or related isoquinolinone and naphthridinone bicyclic ring systems. Several PAMs **47**−**49** were disclosed as being active in reversal of amphetamine induced hyperlocomotion (Figure 7).[83−85] Independently, in 2009, the Lundbeck group disclosed a similar series of 16 acetylenic PAMs,[86] and then a second report followed in 2011.[87]

Several select key compounds from the monocyclic PAMs[84,86] are summarized in Table 5 from human or rat $mGlu_5$ expressing cell lines. PAMs containing para-substituted amides generally proved to have robust activity with a range of substituents tolerated. Overall, SAR between the Lundbeck and VCNDD efforts were quite similar with small cycloalkyl secondary amides, **52** (VU0360172) and **53**, having potency below 50 nM. Interestingly,

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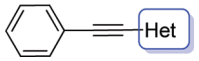
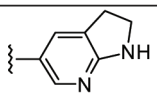
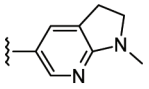
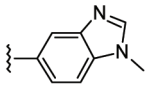
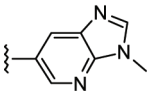
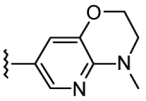
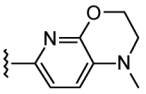
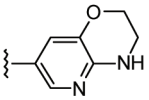
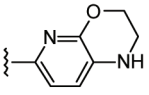
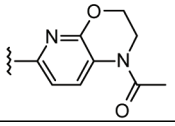
Table 4. Bicyclic Acetylenes Containing Aminopyrimidine HBD/HBA Isosteres[82,a]



Compound	Heterocycle	hmGlu$_5$ EC$_{50}$ (nM)	Solubility pH 7.4	cLogP
38		15.8	NT	3.9
39		108.4	NT	4.3
40		37.2	10	4.2
41		17.4	42	3.1
42		30.2	4	4.5
43		56.7	100	4.5
44		20.3	NT	4.1
45		>25,000	NT	4.1
46		61.7	210	2.7

[a]Abbreviations: NT, not tested.

incorporation of the nicotinamide ring nitrogen displayed differential SAR depending upon the identity of the amide substituent. For example, a 9-fold improvement in potency was observed between benzamide **54** to nicotinamide **55** when using the 3-thiophenyl methyl secondary amide. Additional examples of this type of SAR have been noted in these laboratories using a rat mGlu$_5$ expressing HEK cell line (unpublished). In contrast, secondary amides in the Vanderbilt cell line showed the opposite trend between the benzamide and nicotinamide cores with a 10−40-fold loss in potency observed (i.e., **58** vs **59** and **60** vs **61**). Fortunately, fluorination of the pendant phenyl ring at the 3-position modestly improved the potency 2−3 fold for the morpholino amide **61** and 4-hydroxypiperidinyl amide **59**, with activity below 200 nM. In addition this modification appeared to improve metabolic stability. Incorporation of more basic heterocyclics, such as piperazine **62**, was deleterious, resulting in micromolar activity. The Lundeck group fully characterized **53** and confirmed in a [^{3}H]-quisqualic acid binding assay that indeed the functional activity of **53** was due to an allosteric interaction. Not surprisingly, **53** as well as **52** and other analogues from Vanderbilt were found to have a significant MPEP-site interaction: K_i = 1.8 and 0.195 μM, respectively. Notably for these amide based acetylenes and similar to the early CDPPB analogues, the affinity was considerably lower than the potency (12−60 fold shifted), indicative of high cooperativity within the orthosteric and allosteric binding events. PAM **53** was also shown to have sufficient solubility and moderate brain penetration in rats and mice, b/p 0.3−0.5, after oral administration. The Vanderbilt team discovered their acetylenic amide PAMs after an HTS screen utilizing an in-house triple-add functional calcium mobilization

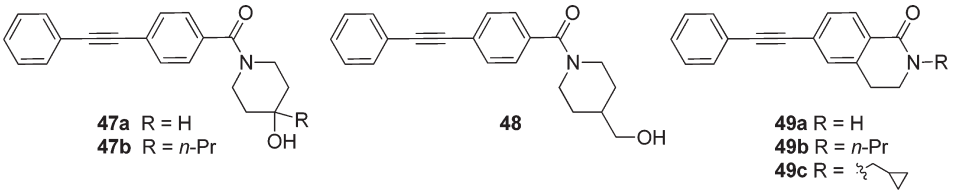
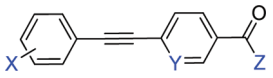
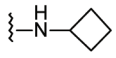
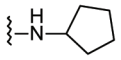
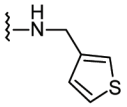
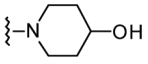
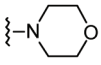
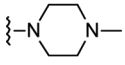


Figure 7. Phenyl and naphthridinone acetylenic PAMs disclosed by VCNND in 2008 as active in vivo using an amphetamine based antipsychotic model.[83]

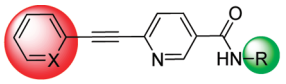
Table 5. Acetylene Amide $mGlu_5$ PAMs[a]



Compound	X	Y	Z	$hmGlu_5$ EC_{50} (μM)	$rmGlu_5$ EC_{50} (μM)	K_i (μM)	Comments	Reference
50	H	N	NH_2	6.6	--	--		Ritzen 2009
51	H	N	$HNCH_2CH_2CH(CH_3)_2$	0.51	--	--		"
52 (VU0360172)	3-F	N		--	0.016	0.195	selective, orally active in AHL	Rodriguez 2010
53	H	N		0.030	--	1.8	fold shift = 5.5, rat b/p = 0.3	Ritzen 2009
54	H	CH		1.2	--	--		"
55	H	N	"	0.14	--	--		"
56	H	N		0.28	--	--		"
57 (VU0092273)	H	CH		--	0.010	0.97	HTS lead, $mGlu_3$ IC_{50} = 6.3 μM	Rodriguez 2010
58 (VU0366026)	3-F	CH	"	--	0.0052	1.12		"
59 (VU0361747)	3-F	N	"	--	0.214	--		"
60 (VU0366031)	3-F	CH		--	0.0038	0.040		"
61 (VU0360175)	3-F	N	"	--	0.049	--		"
62	H	N		1.6	--	--		Ritzen 2009

[a]Abbreviations: AHL, amphetamine induced hyperlocomotion; b/p, brain/plasma.

assay. From this screen, 1400 confirmed PAMs were identified, including **57** (VU0092273), which was one of 63 PAMs with potency below 500 nM. Initial pharmacological characterization of **57** identified modest $mGlu_3$ antagonist activity (IC_{50} = 6.3 μM) with notable agonist activity found in the examined cell line in the absence of added glutamate. PAM **52**, containing a *N*-cyclobutyl moiety, was identified as an optimal compound with the best overall properties and was isolated as a stable HCl salt. DMPK assessment of **52** also indicated favorable properties for in vivo studies: microsomal stability > 80% remaining; p.o. dose in Sprague−Dawley rats at 10 mg/kg as a 1 mg/mL 20% β-CD formulation gave sustained exposure with a plasma C_{max} = 21 μM and moderate b/p = 0.13. Administration of **52** significantly reversed amphetamine-induced hyperlocomotion at doses of 30,



R / X group		Efficacy	EC_{50}/IC_{50} (nM)	Ki (μM)
63 R = cyclopentyl	X = N	NAM	227	7.6
64 R = CH_3	X = CH	SAM	--	15
65 R = *i*-Pr	X = CH	PAM	62	7.1

Figure 8. First report of PAM to NAM/SAM mode switching within monocyclic MPEP scaffold.[87]

56.6, and 100 mg/kg after i.p. administration and at doses of 56.6 and 100 mg/kg orally using the aqueous vehicle 20% β-CD. Importantly, the vehicle−56.6 mg/kg group of animals was not significantly different from the vehicle−vehicle group, indicating that efficacy was not due to sedation. In contrast to the aminomethylprimidine **37** (MPPA), several of the amide based PAMs reported by VCNDD were found to have agonistic activity at higher concentrations alone and, thus, are considered ago-PAMs in this particular rat $mGlu_5$ cell line. In addition to CDPPB, ADX-47273, and **37** (MPPA), nicotinamide **52** (VU0360172) represented the fourth systemically available $mGlu_5$ PAM shown to be efficacious in a preclinical assay predictive of antipsychotic efficacy and the first to be suitable for oral dosing using an aqueous vehicle.

A second communication by Lundbeck in 2011 revealed efficacy mode modulation initially within the monocyclic nicotinamides wherein the *N*-methyl homologue **64** was shown to elicit neutral activity (Figure 8) and the des-methyl congener **50** (Table 5) weak PAM activity.[87] Branched iso-propyl derivative **65** gave rise to a potent PAM with weak MPEP site interaction (Figure 8). A more pronounced and reproducible "molecular switch" was identified on the pendant phenyl ring wherein replacement with a 2-pyridyl ring (Figure 8, **63**), a similar modification and switch as first reported by Merz within tetralone **34**,[79] provided a modulator with NAM activity.

In addition to the patent disclosure by VCNDD in 2008,[83] extension of monocyclic acetylene amide PAMs into various bicyclic lactam constraints was the subject of recent reports. (**49a**−**c**, **66**−**75** Table 6)[82,85,87] A series of 6-(phenylethynyl)-3,4-dihydroisoquinolin-1(2*H*)-ones, 5-phenylethynyl-isoindoline-1,3-diones, and 5-phenylethynyl-isoindolinones were shown to have activity ranging from PAM to ago-PAM, and in the case of **71** and **72** displayed exquisite PAM activity below 10 nM (Table 6). In contrast to bicyclics with a phenyl core, several "molecular switches" were identified by both the Lundbeck and VCNDD laboratories within the dihydronaphthyridinone ring system which change the mode of pharmacology from PAM to NAM (Table 6).[85,87] The dihydronaphthyridinone ring system places a rigid hydrogen bond acceptor nitrogen proximal to the bicyclic tether site which appears to be the molecular determinant responsible for the increased "molecular switch" propensity. In general, unsubstituted lactams within the dihydronaphthyridinone series were among the more potent compounds identified, and lower alkyl groups tended to act as "molecular switches" with weak NAM activity and higher alkyl retained PAM activity. An impressive example of opposing and equipotent pharmacology within modulators using the dihydronaphthyridinone chemotype that have similar affinity for the MPEP site below 1 μM can be found between NAM **70** and PAM **71**, both of which elicit functional potency below 10 nM. The AstraZeneca group conducted their bicyclic study using Merz compound **32** as a lead. A number of rigid hydrogen bond acceptor bicyclics were examined which also included lactams **49a** and **72**. Some of the more interesting chromanone ring systems exemplified by **73** and **74** revealed the importance of HBA disposition for not only activity but also solubility at neutral pH as both azachromanones **32** and **76** in particular have enhanced potency below 20 nM and solubility above 20 μM relative to **73** and **74**. Based on the similar potency observed for **32** and **74**, the authors speculate that the two rigid HBA groups are participating in bifurcated hydrogen bond within the allosteric binding site. Further characterization of **74** was not reported as the AstraZeneca group moved forward to identify nonacetylene linker replacements which were compatible with the newly identified azachromanone heterocycle (vide infra).

Collectively, taking into consideration the recent reports described herein, it appears that simple introduction of the amide functionality on a MPEP scaffold is not necessarily sufficient for PAM activity and that additional molecular switches can override efficacy depending upon the context of both the amide or other hydrogen bond acceptor functionality and the pendant phenyl group; however, the presence of a hydrogen bond acceptor in some form appears to be required distal from the acetylene core for which numerous variations on this theme are now apparent.

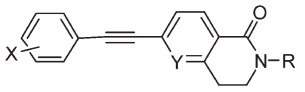
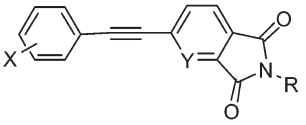
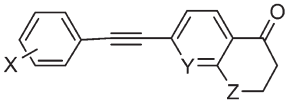
IV. *N*-ARYL PIPERAZINES

Outside the first generation potentiator series CPPHA and CDPPB and PAMs developed by Addex, identification of PAMs lacking an acetylene based core structure have been slow to emerge and overall are still relatively few. The first promising report of a nonacetylene containing $mGlu_5$ PAM appeared in the patent literature from AstraZeneca in 2007−2008 (**76**, Figure 9).[88,89] No specific information regarding in vitro or in vivo activity was disclosed from these applications. However, structurally they contained either a piperidine or piperazine core structure substituted with an *N*-aryl substituent and *N*-acyl substituent at the periphery. In particular, the *N*-acyl moiety was primarily exemplified as either a benzyloxy acetamide or anilido acetamide. In December 2008, GSK disclosed a patent application[90] claiming similar structures with an alternate linker containing a propionamide spacer (**77**) and then published an application targeting a single compound, **78**,[91] which is quite similar to the genus described under **76**.

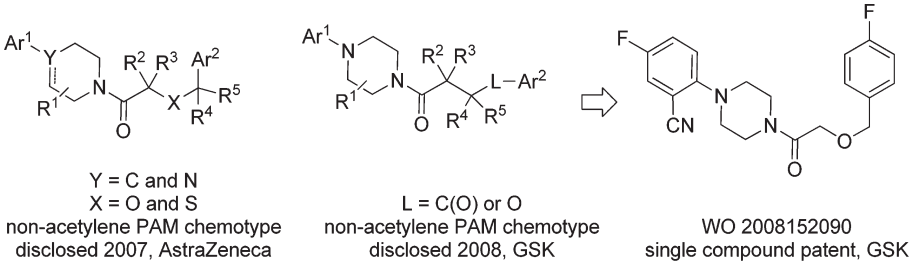
Concurrent with reports emerging in the patent literature covering piperazine chemotypes described in Figure 9, the VCNDD group was following a NAM lead, **80** (Figure 10), from their HTS screen based upon a 2-pyridylpiperazine motif.[92] Subsequent optimization of the *N*-aryl substituent identified a 2-thiazolyl replacement **79** as an improvement in potency for NAM activity; however, within the same chemical library, a surprising switch in pharmacology for derivative **81** with weak PAM activity was discovered (EC_{50} of 5.4 μM and an 86% response of the glutamate maximum) employing a 2-(benzyloxy)acetate amide moiety, similar to that reported in the patent literature, as a "molecular switch".[93]

Based upon its structural novelty versus known $mGlu_5$ PAMs and physicochemical properties (MW < 300, cLogP = 2.4), an optimization campaign ensued exploring the N-aryl ring leading to a full profile of compound **82** (VU0364289, Figure 11). Shortly thereafter, AstraZeneca released details of their preferred tool compound piperazine CPPZ (**83**), also containing a benzyloxy acetamide substituent (Figure 11).[94,95] A summary of the pharmacological and DMPK profiles for **82** and **83** described to

Table 6. Bicyclic Acetylene PAMs Containing an Amide or Rigid HBA Moiety[a]

Cmpd	Substructure	X	Y	R/Z	EC_{50}/ IC_{50} (nM)	Efficacy	Comments	Reference
49a		H	CH	H	50 44	Ago-PAM		Williams, 2011; Varnes, 2011
49b		H	CH	*n*-Pr	160	Ago-PAM	dosed i.p. in AHL	"
66		3-F	N	H	290	PAM		"
67		3-F	N	CH_3	170	PA	34% glu max	"
68		3-F	N		130	PAM	weak 53% glu max	"
69		H	N	CH_3	30	NAM	42% glu max, K_i = 0.76 μM	Sams, 2011
70		3-Cl	N	*i*-Pr	5.7	NAM	K_i = 0.1 μM	"
71		H	N		5.9	PAM	K_i = 0.89 μM	"
72		H	CH	H	5.9 11.6	Ago-PAM		Williams, 2011; Varnes 2011
73		H	CH	CH_2	588	PAM	sol < 1 μM	Varnes, 2011
32		H	N	CH_2	14	PAM	sol = 22 μM	"
74		H	CH	O	29.9	PAM	sol = 1 μM	"
75		H	N	O	11.5	PAM	sol = 23 μM	"

[a]Abbreviations: PA, partial antagonist; AHL, amphetamine induced hyperlocomotion.



76 **77** **78**

Figure 9. *N*-Aryl piperazines disclosed in patent literature as $mGlu_5$ PAMs.[88−91]

date are summarized in Figure 11. As part of their report, the AstraZeneca group disclosed a revealing investigation of the benzyloxy acetamide SAR, demonstrating the importance of the two hydrogen bond acceptor units, as steep SAR was noted in the

dx.doi.org/10.1021/cn2000519 |*ACS Chem. Neurosci.* 2011, 2, 450–470

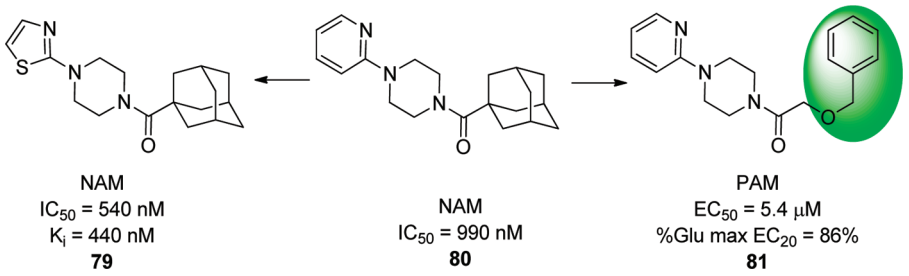
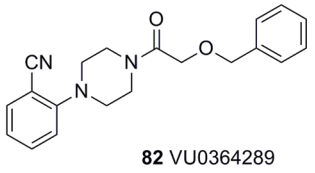


Figure 10. Vanderbilt piperazine based PAM **81** from NAM **80**.[93]

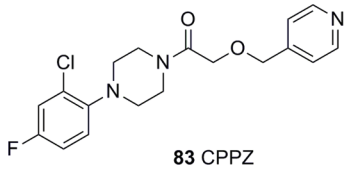


82 VU0364289

In vitro profile:
rat mGlu5 EC_{50} = 1.6 μM
pure-PAM
fold-shift: 12
$mGlu_{1\text{-}4,\ 6\text{-}8}$: inactive at 10 μM
Ki > 30 μM, incomplete [3H]MePEPy

DMPK profile:
Protein Binding (human, rat): 94, 90%
Rat brain binding %Fu = 7.1%
Permeability = 22 x 10^{-6} cm/sec
In vitro metabolic stability: poor
Solubility (pH 7.4) = 30 μg/mL (90 μM)
Vehicle: 20% β-CD 1-3 mg / mL, solution
3-5 mg / mL, micro-susp
Rat PK i.p. (10 mg/kg):
C_{max} = 3.8 μM, AUC_{brain}/AUC_{sys} = 1.0
T_{max} = 0.25 h

In vivo behavior:
reverses AHL



83 CPPZ

In vitro profile:
human mGlu5 EC_{50} = 0.55 μM
pure-PAM
fold-shift: NR
$mGlu_{1\text{-}4,\ 6\text{-}8}$: inactive at 25 μM
Ki = 2.37 μM, full [3H]MPEP

DMPK profile:
Protein Binding (human, rat): 53, 74%
Rat brain binding: NR
Permeability = 25 x 10^{-6} cm/sec
In vitro metabolic stability: poor
Solubility: NR
Vehicle: 20% β-CD 1.8 mg / mL, solution
20% SBE-CD (s.c.)
Rat PK i.v./po (3 mg/kg):
%F = 41, $T_{1/2}$ = 7.3 h, Vd_{ss} = 4.8 L/kg
Cl_p = 55 mL/min/kg

In vivo behavior:
reverses MK801-induced hyperlocomotion
reduces conditioned avoidance response

Figure 11. Optimized *N*-aryl piperazines **82** and **83** recently utilized in vivo.[93−95]

context of amide replacements, such as thioamide, and several alternate ether oxygen replacements (data not shown), all of which proved deleterious for activity. In addition, it is interesting to note that despite the similar functional potencies for **82** and **83**, there is an apparent disconnect in their MPEP site affinities, K_i > 30 μM and 2.37 μM, respectively, although it should be noted that both the radioligand and receptor source utilized are slightly different for these MPEP affinity studies. CPPZ (**83**) was also shown to enhance binding of orthosteric agonist quisqualic acid, potentiate calcium flux in rat cortical neurons using dihydroxyphenylglycine (DHPG), and modulate LTP in rat hippocampal slices.[95] Collectively based upon the SAR described to date, including the fact that no overriding additional NAM "molecular switches" were noted from neither the AstraZeneca or VCNDD studies, it appears that within the piperazine chemotype the benzyloxyacetamide motif behaves as a PAM preferring "molecular lock".

PAMs **82** and **83** are major advances for the field, particularly when one considers the ease with which these compounds can be formulated for in vivo studies relative to previous tool compounds described. Although neither compound is stable metabolically (Figure 11), they are readily soluble in aqueous vehicles, rapidly absorbed, and highly distributed to tissues with excellent fraction unbound (>5%) in relevant biological matrices. These properties are in major contrast to previous tool compounds CDPPB and ADX-47273, which are poorly soluble and highly bound to plasma protein (sol < 0.01 mg/mL, >98% bound). Reversal of amphetamine induced hyperlocomotion was readily achieved using VU0364289 (**82**) at a single dose of 56.6 mg/kg and orderly effects have been observed across multiple doses (unpublished results).[93] Recent i.p. PK studies using VU0364289 (**82**) indicate rapid and passive CNS penetration (Figure 11). In vivo in mice CPPZ (**83**) was shown to reverse MK-801 induced hyperlocomotion after subcutaneous (s.c.) dosing of 10 mg/kg and reduce rat conditioned avoidance responding with a minimum effect dose of 10 mg/kg (i.p.), the latter of which has previously only been reported for ADX-47273.[69]

V. ETHERS

Extensive efforts to replace the triple bond moiety starting from MPEP-based structures for $mGlu_5$ NAM programs have been highly successful,[96,97] and naturally efforts within the $mGlu_5$

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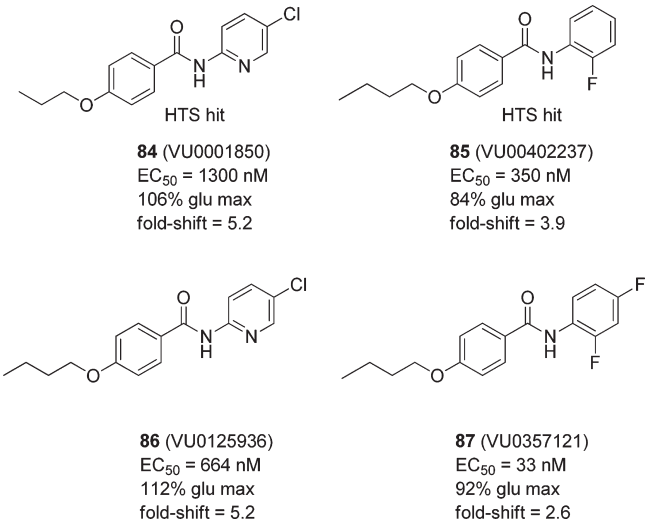


Figure 12. Non-MPEP site VCNDD HTS benzamide hits containing an ether linker **84** and **85** and evolved PAMs **86** and **87** from parallel synthesis.[99]

PAM field have begun to target acetylene replacements in order to avoid potential metabolic and subsequent toxicological liabilities associated with drug molecules containing a triple bond.[98] In addition to the piperazine scaffold as a nonacetylene chemotype, a series of ether containing benzamides was recently identified from the Vanderbilt HTS screen as illustrated by the lead structures **84** and **85** (Figure 12).[99]

These benzamides share the same amide backbone structure as that found in CPPHA with a unique 1,4-substitution pattern displaying a lipophilic ether tail. Alternatively, the amide bond can also be envisioned as an acetylene isostere which has been successfully demonstrated previously using a diarylamide backbone.[100] In fact, several of these NAM acetylene isosteres were shown to retain affinity for the MPEP site, particularly for analogues containing a 3-CN group, and X-ray and modeling studies strongly support overlapping pharmacophore features.[100] However, at concentrations up to 100 μM, 3 orders of magnitude above that required for PAM activity, the amide based PAMs described, including **84** and **85** and the more optimized compounds **86** and **87**, did not inhibit binding of [3H]methoxyPEPy, suggesting that these amides are enhancing glutamate activity via an allosteric site outside the MPEP-site. In addition, PAM **87** (VU0357121) was unable to surmount the effect of the SAM 5MPEP (**88**, Figure 13) on potentiation, showing a progressive rightward shift in the concentration response curve and diminution of the maximal response with increasing 5MPEP concentrations, suggesting a noncompetitive interaction between these two allosteric ligands and $mGlu_5$. The same report disclosed a streamlined approach to uncover SAMs within the butyl ether series of amides, whereby a screen was performed with **87** (VU0357121), submaximal glutamate, and test compounds within the same chemical series but functionally inactive on their own. Using a single concentration of 10 μM of test compound, SAM **89** (Figure 13) was identified as the most efficacious non-MPEP site neutral allosteric ligand in terms of its ability to inhibit potentiation of the glutamate-induced calcium flux by **87**. Mutagenesis studies conducted using **87** did not correlate with the radioligand binding studies, in that mutants known to be functionally sensitive to the MPEP site PAM **7** (VU29, Table 1)

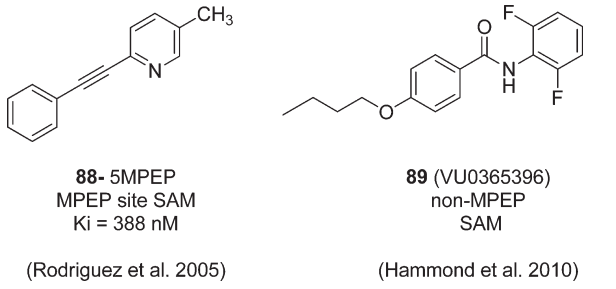
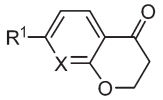
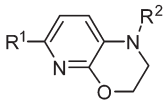
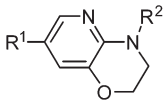


Figure 13. Neutral or silent allosteric modulators (SAMs) for $mGlu_5$.[76,99]

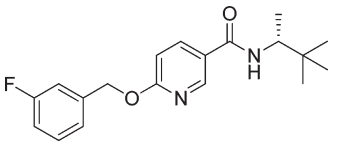
were in fact inhibitory to responses mediated by **87**, and conversely mutants known to be sensitive to CPPHA (**4**, Figure 1) were unaffected by **87**. In light of the MPEP binding studies, which suggest a lack of interaction, this result is somewhat surprising. A number of possible scenarios were proposed from these data, including the possibility that ethers within the series may be interacting at a third site; however, more studies are needed to definitively understand the nature of the allosteric binding site for this class of $mGlu_5$ PAMs.

Starting from the optimized heterocycle replacements **42**, **46**, and **75** (Tables 4 and 6), identified as improved PAMs relative to the acetylene PAMs **37** (Figure 6) and **72** (Table 6), the AstraZeneca team disclosed in the same study a series of alternate linkers containing variations between the pendant phenyl and right-hand side heterocycle, wherein in the triple bond was replaced with either $-OCH_2-$, $-CH_2O-$, ester, or stilbene based linkages. A number of the promising and key SAR highlights focusing on the ether-based linkers are summarized in Table 7. Chromanone ring system **74** readily allowed replacement of the triple bond with both benzyloxy and phenoxy types of linker systems (**90**−**93**). Chromanone benzyloxy derivative **90** was 5-fold less active than acetylene **74** and the phenoxy analogue **92** was slightly less preferred. Installation of the more water-soluble azachromanone ring systems exemplified in **91** and **93** appeared to retain most of the activity with EC_{50}'s below 500 nM. Based upon the lessons learned within the two isomeric oxazine acetylene series (Table 4), a similar investigation was performed within this ring system (**94**−**102**) and shown to offer promising PAM activity. In contrast to the chromanone ring system, the authors state that derivates containing a benzyloxy linker were weakly active or inactive, and thus, only phenoxy based oxazines were shown to have promise with activity below micromolar levels. The 2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]oxazine ring system exemplified by **96**−**100** was stated as being superior to regioisomeric oxazine **94**, and this appears to be driven by enhancements in solubility mostly, although only one example was provided. The previous "steep SAR" and restoration of activity noted within the 2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]oxazine acetylene series (i.e., **45** and **46**, Table 4) is unknown for the parent ring system **94** using the new ether linkage. Thus, both the *N*-methyl and progenitor NH compound would be interesting compounds to establish if "steep SAR" will parallel in this series or not. From **96**, however, several alternate oxazine capping groups, including a carbamate (**99**), acetyl (**100**), and a sulfonamide (**101**), were shown to generate analogues with modest $mGlu_5$ PAM activity. The simple *N*-methyl congener (**97**) was shown to have the best overall potency and aqueous solubility with an EC_{50} of 50 nM. Details regarding mGlu selectivity, glutamate fold-shift, and profiles in DMPK and in vivo behavior assays were not disclosed.

Table 7. Ether PAMs Reported by AstraZeneca[82,a]

Cmpd	Substructure	R^1	X	R^2	EC_{50} (nM)	Solubility (μM)
74		PhCC	CH	--	29.9	1
90		$PhCH_2O$	CH	--	167.0	7
91		$PhCH_2O$	N	--	156.3	64
92		$PhOCH_2$	CH	--	349.4	12
93		$PhOCH_2$	N	--	453.6	100
94		$PhCH_2O$	--	$C(O)CH_3$	349.4	12
95		PhCC	--	H	20.3	NT
96		$PhOCH_2$	--	H	371.5	NT
97		$PhOCH_2$	--	CH_3	50.1	18
98		$PhOCH_2$	--	CH_2CH_3	60.3	169
99		$PhOCH_2$	--	$C(O)OCH_3$	475	>520
100		$PhOCH_2$	--	$C(O)CH_3$	713.4	40
101		$PhOCH_2$	--	SO_2CH_3	609.5	10
102		$CyOCH_2$	--	CH_3	831.8	>440

[a]Abbreviations: NT, not tested.



103

$rmGlu_5$ EC_{50} = 2.2 nM

Figure 14. Ether containing $mGlu_5$ PAM (**103**) disclosed by VCNDD as active in AHL.[101]

In addition to the single report from AstraZeneca a patent application from Vanderbilt and Janssen,[101] a joint industry sponsored research collaboration, was recently disclosed which described a series of monocyclic nicotinamide ethers containing ether linkers similar to that described by AstraZeneca. The application describes both benzyloxy and alkoxy ethers as $mGlu_5$ PAMs with a number of chiral examples displaying enantioselective potentiation, in some cases >250 fold for one stereoisomer. In addition, a single benzyloxy ether, **103** (Figure 14) was disclosed as having significant activity in reversal of amphetamine induced hyperlocomotion after oral dosing at 100 mg/kg and was reported to have an EC_{50} of 2.2 nM in a HEK cell line expressing the rat $mGlu_5$ receptor.

The newly identified nonacetylene low MW PAMs such as **97** and **103** appear to offer tremendous improvements in both solubility and ligand efficiency (LE ~0.5), and the field will undoubtedly look forward to future disclosures describing the full scope of their pharmacological properties including, for example, propensity for mode switching and activity in behavior models of cognition.

VI. CONCLUSIONS AND OUTLOOK

Evidence for clinical efficacy in patients with schizophrenia using glutamate signaling modulation based therapies using either the GlyT1 inhibitor RG1678[24] or the group II mGlu2/3 agonist LY404039[102] brings forth exciting potential treatment options with the promise to impact a broader range of symptoms without the adverse effects of currently available treatments. $mGlu_5$ PAMs also continue to offer promise as a novel treatment option for pateints with schizophrenia; however, direct clinical evidence of the $mGlu_5$ mechanism as being an effective approach for the treatment of this disorder remains to be seen. The recent insights from subchronic studies in sleep and protein markers related to NMDA receptor function using first generation PAMs such as CDPPB, a tool compound first reported in 2005, raise interesting questions which can now be addressed by other groups using multiple chemotypes beyond CDPPB. The recently advanced PAMs described, including acetylenes **37** (MPPA) and **52** (VU0360172), piperazines **82** and **83** (CPPZ), and potentially the recent nonacetylene ether **97**, provide three new chemotypes

in addition to ADX-47273 and CDPPB, each with a unique pharmacological profile worthy of further investigation. Relative to the short list of first generation $mGlu_5$ PAMs long available to the field, a rennaisance of new chemotypes appears to be emerging and it is likely others are on the horizon, with several of the current compounds described herein offering improvements in both physicochemical and pharmacokinetic properties that make these molecules and their scaffolds potentially more attractive for further advancement into preclinical toxicity studies and ultimately proof-of-concept studies in humans.

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