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REVIEW

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Recent Progress in the Synthesis and Characterization of Group II Metabotropic Glutamate Receptor Allosteric Modulators

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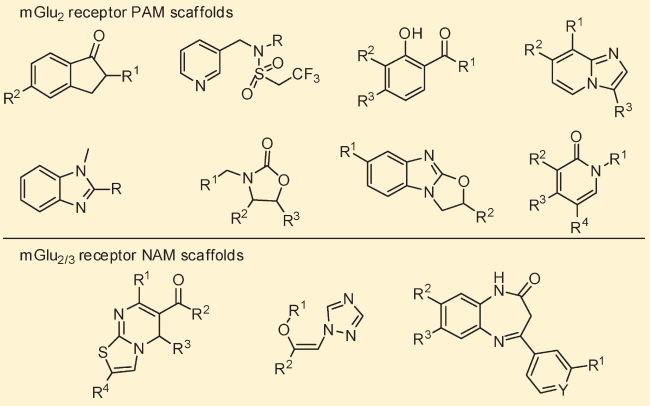
ABSTRACT: Group II metabotropic glutamate (mGlu) receptors consist of the metabotropic glutamate 2 ($mGlu_2$) and metabotropic glutamate 3 ($mGlu_3$) receptor subtypes which modulate glutamate transmission by second messenger activation to negatively regulate the activity of adenylyl cyclase. Excessive accumulation of glutamate in the perisynaptic extracellular region triggers $mGlu_2$ and $mGlu_3$ receptors to inhibit further release of glutamate. There is growing evidence that the modulation of glutamatergic neurotransmission by small molecule modulators of Group II mGlu receptors has significant potential for the treatment of several neuropsychiatric and neurodegenerative diseases. This review provides an overview of recent progress on the synthesis and pharmacological characterization of positive and negative allosteric modulators of the Group II mGlu receptors.



KEYWORDS: $mGlu_2$, $mGlu_3$, allosteric modulators, schizophrenia, memory, anxiety, drug dependence, sleep-wake architecture

Significant effort in recent years has been focused on the discovery of allosteric modulators, acting on various central nervous system receptors, as putative therapeutics for neuropsychiatric disorders. This interest is partly engendered by the rationale that such compounds may have improved therapeutic properties by subtly modulating the activity of malfunctioning receptor signaling pathways in concert with the endogenous system activity. As such, it is hypothesized that selective positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs) may have enhanced therapeutic effects, as well as improved side-effect profiles, compared with directly acting (orthosteric) receptor agonists and antagonists. Many such efforts have been pursued in the glutamate field, and in particular for the G protein-coupled family of metabotropic glutamate (mGlu) receptors. The present review focuses on positive and negative allosteric modulators of Group II metabotropic glutamate receptors that comprise metabotropic glutamate 2 ($mGlu_2$) and metabotropic glutamate 3 ($mGlu_3$) receptors. The Group II mGlu receptors modulate glutamate transmission by second messenger activation via coupling to $G_{i/o}$ proteins to negatively regulate the activity of adenylyl cyclase. Excessive accumulation of glutamate in the perisynaptic extracellular region triggers $mGlu_2$ and $mGlu_3$ receptors to inhibit further release of glutamate. Thus, there is significant potential for the development of selective Group II mGlu receptor PAMs and NAMs for the treatment of CNS diseases caused by aberrant glutamatergic signaling.

The first section of this review covers recent disclosures of $mGlu_2$ receptor PAMs in the primary literature from 2008 through 2010. In addition to the review in 2005 by Rudd and McCauley,[1] a recent review by Fraley[2] extensively covered the patent and primary literature around this class of compounds. Thus, in terms of chemistry, this review mainly focuses on publications and patents since 2008 that are not covered in the 2009 review. There have been very few reports on $mGlu_3$ receptor PAMs, and so most of the literature reviewed here is focused on $mGlu_2$ receptor PAMs and $mGlu_{2/3}$ receptor NAMs. Because these compounds are relatively new and not widely available to the scientific community, there have been very few investigations of the behavioral effects of these compounds reported in the literature. Thus, we have attempted to provide a comprehensive review of all published data on the behavioral effects of these compounds, and thus provide guidance as to the possible therapeutic indications for Group II mGlu receptor PAMs and NAMs.[3]

■ $MGLU_2$ RECEPTOR POSITIVE ALLOSTERIC MODULATORS (PAMS)

The in vitro activity of $mGlu_2$ receptor PAMs has been primarily evaluated in two manners across a number of functional

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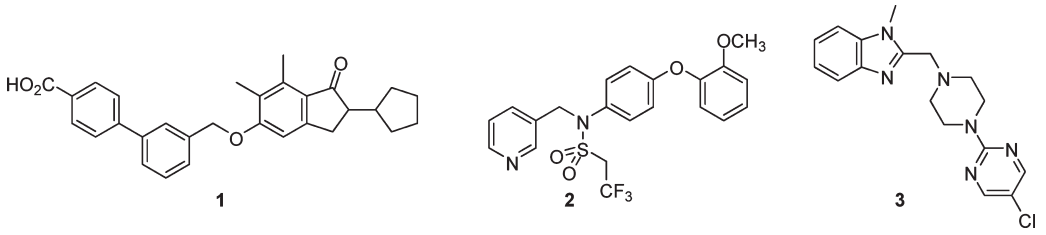


Figure 1. Structures of prototypical $mGlu_2$ receptor PAMs BINA (**1**), LY487379/4-MPPTS (**2**), and GSK1331268 (**3**).

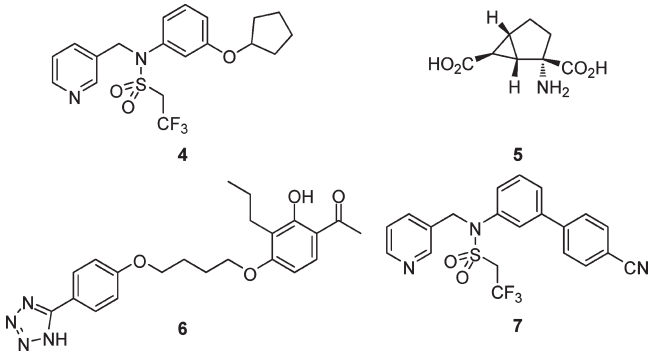


Figure 2. Structures of $mGlu_2$ receptor PAMs cyPPTS (**4**), **6**, and CBiPES (**7**), and $mGlu_{2/3}$ receptor orthosteric agonist LY354740 (**5**).

readouts. First, the effects of fixed concentrations of $mGlu_2$ receptor PAMs have been evaluated on the concentration-responses of orthosteric agonists in a fold shift assay, whereby PAMs left-shift the concentration-response of an orthosteric agonist. Second, the concentration-response for PAM potentiation of an EC_{10}-EC_{20} concentration of an orthosteric agonist has been utilized to provide the potency for PAM potentiation. Numerous functional readouts have been employed to initially characterize $mGlu_2$ receptor PAMs in vitro including [^{35}S]GTPγS binding[4−12] and coupling of $mGlu_2$ receptors via either promiscuous (Ga15 or Ga16) or chimeric (Gqi5) G proteins to either calcium mobilization[5,10−13] or to inositol phosphate accumulation.[3,11] More recently, coupling of $mGlu_2$ receptors to modulation of G protein-regulated inwardly rectifying potassium (GIRK) channel thallium flux has also been utilized to characterize the $mGlu_2$ receptor PAM BINA (Figure 1).[14] A few PAMs have been further characterized for their mechanism of $mGlu_2$ receptor potentiation. For example, LY487379 (Figure 1) has been demonstrated to increase the B_{max} of saturation [^{35}S]GTPγS binding and to slightly decrease the K_d for [^{3}H]-DCG-IV binding, implying that LY487379 both increases the coupling to G proteins and slightly increases orthosteric agonist affinity, providing two mechanisms by which $mGlu_2$ receptor PAMs can increase orthosteric agonist efficacy.[11] Mutational analyses have generally defined the binding pocket for $mGlu_2$ receptor PAMs. Initial studies demonstrated that three amino acids in the 7TM domain (Ser885, Gly689, and Asp735), which reside in TMIV and TMV, are critical for the activity of LY487379.[11] Further studies demonstrated that multiple, structurally diverse, $mGlu_2$ receptor PAMs require these residues for functional activity and that mutation of the corresponding residues in $mGlu_3$ receptors to those of $mGlu_2$ receptors allows $mGlu_2$ receptor PAMs to display activity at $mGlu_3$ receptors.[3]

A number of $mGlu_2$ receptor PAMs, including BINA (**1**), LY487379/4-MPPTS (**2**), and GSK1331268 (**3**) (Figure 1) have been evaluated in electrophysiological studies, where they have been demonstrated to have effects in potentiating Group II mGlu receptor agonist responses in many brain regions including the medial preforant path-dentate gyrus (MPP-DG) synapse,[5,11,15] within the globus pallidus (GP) (LY487379),[16] and within the medial prefrontal cortex (mPFC) (BINA).[17] While these studies have focused on potentiation of Group II agonist-mediated electrophysiological effects, two important studies have demonstrated activity of $mGlu_2$ receptor PAMs in the absence of exogenously added Group II agonists. First, the PAM cyPPTS (**4**) (Figure 2) demonstrated a clear dependence on the frequency of presynaptic stimulation of corticostriatal excitatory postsynaptic potentials (EPSPs), whereas the orthosteric agonist LY354740 (**5**) inhibited EPSPs even under low frequency stimulation.[18] This study demonstrated that $mGlu_2$ receptor PAMs act in a synaptic activity-dependent manner and may therefore be better tolerated therapeutically than direct-acting agonists. Second, the $mGlu_2$ receptor PAM BINA has been demonstrated to attenuate serotonin (5-HT) induced excitatory postsynaptic currents (EPSCs) in the mPFC,[17] a region where Group II mGlu receptor effects are thought to be relevant for therapeutic benefit in schizophrenia. This study provided the first example of an $mGlu_2$ receptor PAM not requiring either synaptic stimulation or exogenously applied Group II orthosteric agonists to demonstrate efficacy. In addition to these electrophysiological studies, a limited number of microdialysis studies have evaluated the ability of $mGlu_2$ receptor PAMs to alter neurotransmitter release, with effects that may be potentially relevant for the antipsychotic action of $mGlu_{2/3}$ receptor agonists. For example, pretreatment with an $mGlu_2$ receptor PAM (**6**) (Figure 2) inhibited norepinephrine release in the ventral hippocampus induced by the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine.[10] Recently, the receptor PAM CBiPES (**7**) was also demonstrated to inhibit ketamine-evoked histamine release in the mPFC.[12] Further, in studies expanding on this initial finding with CBiPES, the $mGlu_2$ receptor PAM THIIC (**24**) was found to attenuate the dark phase increase in histamine efflux in the mPFC and to dose-dependently inhibit levels of the histamine metabolite tele-methylhistamine (t-MeHA) found in rat cerebrospinal fluid (CSF) after both acute and five day dosing.[19]

As can be seen, the majority of studies with $mGlu_2$ receptor PAMs have focused on determining the potential of these compounds as a novel approach for the treatment of schizophrenia (see below). However, $mGlu_2$ receptor PAMs reported to date have only been characterized to a limited degree in vitro, and the majority of characterization of these compounds has focused on non-native coupling of $mGlu_2$ receptors to functional responses. In future studies, further characterization of $mGlu_2$ receptor PAMs utilizing native responses, including inhibition of cAMP accumulation,[20] activation of ERK1/2 phosphorylation,[21] activation of phosphatidylinositol-3 (PI3) kinase activity,[21] and

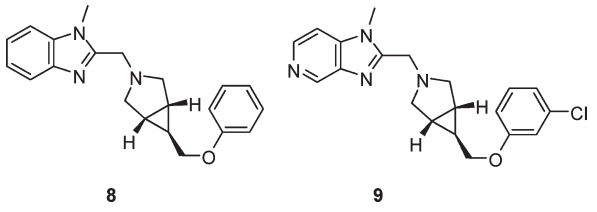


Figure 3. Structures of $mGlu_2$ receptor PAMs from Pfizer.

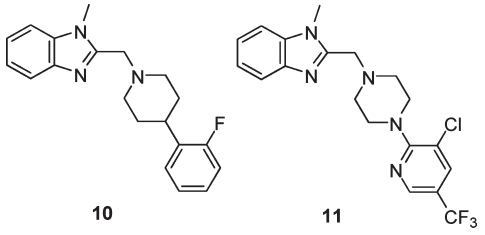


Figure 4. Structures of $mGlu_2$ receptor PAMs from GSK.

coupling to GIRK channels,[14] will prove useful for fully evaluating $mGlu_2$ receptor PAMs as a novel therapeutic approach.

There have been several new reports describing structure–activity relationship (SAR) studies around various $mGlu_2$ receptor PAMs. For example, workers from Pfizer recently published on a series of benzimidazole derivatives as $mGlu_2$ receptor PAMs (Figure 3).[22] Compound **8** was identified through a high-throughput screening (HTS) campaign. Although HTS hit **8** was very potent (EC_{50} = 24 nM), it displayed poor pharmacokinetic (PK) properties, with high in vitro clearance in rat and human liver microsomes (>65.7 and 14.2 mL/min/kg, respectively) and poor oral bioavailability in the rat (<2% after a 5 mg/kg dose p.o.). Holding aza-bicyclo [3.1.0] hexanyl core constant, the benzimidazole and phenyl ring were examined extensively, eventually leading to compound **9**, which displayed similar potency (EC_{50} = 64 nM) but much improved rat PK (%F = 79 after 5 mg/kg dose p.o.). However, no data from in vivo efficacy models were presented for this series, nor was any mention made of selectivity against other mGlu receptors.

Researchers at GSK have disclosed a series of orally bioavailable benzimidazole-based, $mGlu_2$ receptor PAMs (Figure 4)[15] that are structurally similar to the series of compounds previously disclosed by Pfizer.[22] For this series of compounds, a screening hit (**10**) was discovered that had some activity as an $mGlu_2$ receptor PAM (EC_{50} = 126 nM) but also was active at dopamine D2 receptors (EC_{50} = 40 nM). Some SAR studies were performed around the methyl group and aromatic portion of the benzimidazole moiety, although no major improvements were seen. SAR studies around the phenylpiperidine were more successful, eventually culminating in the discovery of a pyrimidinylpiperazine analogue (**11**; GSK1331258) that had both improved potency as an $mGlu_2$ receptor PAM (EC_{50} = 79 nM) and minimal cross reactivity with dopaminergic receptors (pK_i of 5.3, 5.3, and 6.2 for D2, D3, and D4, respectively). However, it was not profiled for selectivity over $mGlu_3$ receptors or other mGlu receptor subtypes. GSK1331258 (**11**) also exhibited low clearance (Cl = 18 mL/min/kg), a half-life of 4.1 h, and a brain/blood ratio of 3.2 after a 1 mg/kg dose p.o. in rats.

Workers from Pfizer have also described a series of oxazolidinones as $mGlu_2$ receptor-selective PAMs (Figure 5).[23] Starting

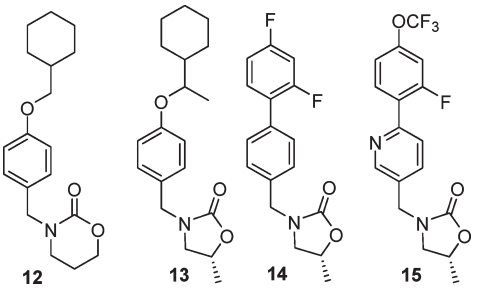


Figure 5. Structures of $mGlu_2$ receptor PAMs from Pfizer (second series).

from a screening hit (**12**), which had reasonable potency (EC_{50} = 117 nM), a parallel synthesis effort focusing on the cyclic carbamate and pendant methylcyclohexyl group was undertaken to rapidly assess this chemotype. Compound **13** was identified from these efforts, which displayed excellent potency (EC_{50} = 5 nM) but had high in vitro human liver microsomal clearance (14.7 mL/min/kg) and a high cLogP (5.12). Further optimization around the methylcyclohexyl group, which led to a biaryl motif, and the central phenyl ring by incorporating a nitrogen atom led to the discovery of **14** and **15**, which addressed many of the issues of compound **13**. Although in vitro potency was somewhat lower (EC_{50} = 70 and 380 for **14** and **15**, respectively), the in vitro human liver microsomal clearance (7.3 and <5.3 mL/min/kg) and cLogP (4.42 and 4.19) were improved. However, compound **14** displayed binding activity at human 5-HT_{2A} receptors (K_i = 323 nM), whereas **15** showed no off-target effects at these receptors, although no data from a functional assay were reported. Interestingly, testing both **14** and **15** on blockade of methamphetamine-induced hyperactivity in rat, despite similar $mGlu_2$ receptor potency and acceptable brain levels (2230 and 10 090 ng/g for **14** and **15**, respectively), only **14** showed any biological activity (MED = 10 mg/kg for **14**, s.c. vs >32 mg/kg, s.c. for **15**) which raised the question that 5-HT_{2A} cross-reactivity may be responsible for at least some of the observed in vivo effects. In addition, the activity of compounds, such as **14**, against $mGlu_3$ or other mGlu receptor subtypes was not reported.

Researchers at Merck have recently described a series of phenyl-oxazolidinone compounds as $mGlu_2$ receptor PAMs (Figure 6)[24] which are similar in structure to the oxazolidinone series of compounds described by Pfizer.[23] Compound **16** was discovered through an HTS effort, showing moderate potency (EC_{50} = 450 nM in a FLIPR assay with 74% maximum efficacy) but relatively poor clearance in dog (Clp = 36.5 mL/min/kg). SAR studies demonstrated that *meta*-substitution on the aryl ring was preferred, with a nitrile group giving the best potency. Several replacements for the pendant hexyl group on the oxazolidinone ring were examined, and a methylene-phenoxy group was found to be preferred. In addition, activity was found to reside in the *R*-isomer of **16** as well as the other compounds in this series. Extensive SAR around the phenoxy group led to the discovery of **17**, with a *para*-*t*-butyl group. Compound **17** displayed improved potency (EC_{50} = 82 nM) and somewhat improved PK in dog (Clp = 17.7 mL/min/kg). Selectivity versus $mGlu_3$ or other mGlu receptor subtypes was not reported. In addition, compound **16** showed good brain penetration in rat with a high CSF/plasma ratio (after a 100 mg/kg dose i.p. in PEG400 at 2 h, [brain] = 10.5 μM, [plasma] = 3.5 μM,

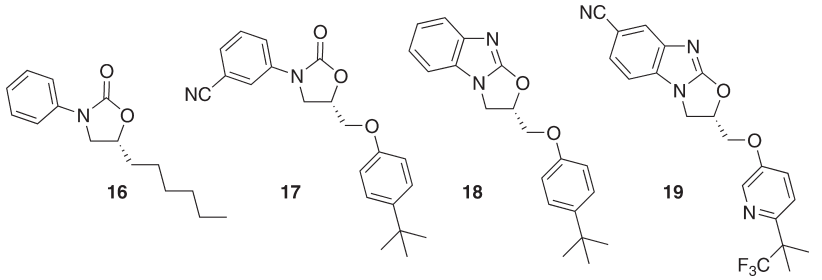


Figure 6. Structures of $mGlu_2$ receptor PAMs from Merck.

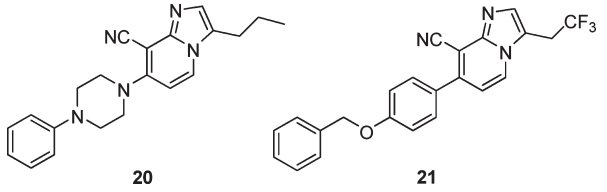


Figure 7. Structures of $mGlu_2$ receptor PAMs from Johnson & Johnson.

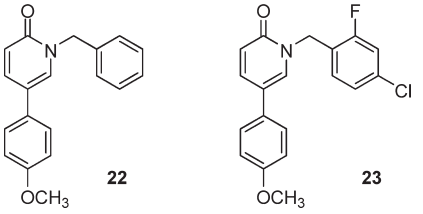


Figure 8. 1,5-Disubstituted pyridone $mGlu_2$ receptor PAMs from Johnson & Johnson and Addex.

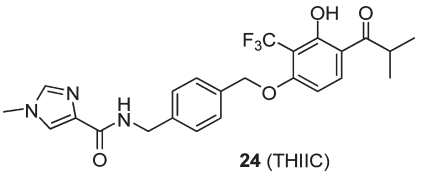


Figure 9. Acetophenone $mGlu_2$ receptor PAM from Eli Lilly.

[CSF] = 118 nM). At a dose of 100 mg/kg i.p., **17** was subsequently shown to attenuate ketamine-induced hyperactivity in rats, albeit only for the first 30 min after administration, presumably due to rapidly declining compound levels in vivo.

Continued optimization of this series by researchers at Merck via introduction of a central ring constraint on the oxazolidinone core to form oxazolobenzimidazole compound **18** (Figure 6) has been reported recently.[25] Compound **18** was found to be extremely potent (FLIPR mGluR2 EC_{50} = 12 nM), albeit with poor aqueous solubility and high clearance in rat (103 mL/min/kg) and dog (45.9 mL/min/kg). Further optimization focusing on incorporating polar groups, for example, adding a cyano group and a change from phenyl to pyridyl and blocking metabolism on the *t*-butyl group by replacement of one of the methyls with a trifluoromethyl, led to compound **19**. Compound **19** was somewhat less potent (FLIPR mGluR2 EC_{50} = 33 nM) but displayed excellent solubility and PK parameters in rat and dog (Cl = 9.05 mL/min/kg, %F = 60 and Cl = 8.85 mL/min/kg, %F = 76.5, respectively). In addition, compound **19** had no activity against other mGlu receptors (mGlu receptors 1, 3, 4, 5, and 6). Compound **19** was shown to be active in the blockade of phencyclidine-induced hyperlocomotion in rats after oral doses of 30 and 100 mg/kg (which gave CSF levels of 550 and 1500 nM, respectively). This represents the only reported example to date of efficacy in these tests after oral dosing.

A new class of imidazopyridines has also been described by workers at Johnson & Johnson (Figure 7).[26] This series of compounds was developed by a scaffold hopping approach starting from a number of pyridone compounds that were previously reported as $mGlu_2$ receptor potentiators.[27] Using an overlay hypothesis looking for similarity in 3D shape and electrostatics, an imidazopyridine core was used as a replacement for a pyridone, giving a moderately active compound (**20**, EC_{50} = 977 nM). Further optimization and incorporation of fragments from other classes of previously reported $mGlu_2$ receptor ligands led to **21**, with an EC_{50} = 158 nM. No in vivo or selectivity data against other mGlu receptor subtypes were presented for this class of $mGlu_2$ receptor PAMs.

Researchers at Johnson & Johnson and Addex have also described a series of 1,5-disubstituted pyridones as $mGlu_2$ receptor PAMs (Figure 8).[28] Compound **22** was identified through an HTS campaign as a modestly potent $mGlu_2$ receptor PAM (EC_{50} = 6.29 μM). Optimization around the benzyl group led to analogue **23**, which was one of the most potent compounds found in this series (EC_{50} = 0.53 μM). It was also selective against $mGlu_3$ receptors but did show some $mGlu_7$ receptor antagonism (EC_{50} = 4.94 μM). Compound **23** was found to have very poor stability in human liver microsomes (0% remaining after 15 min incubation) but was nevertheless dosed in a PCP-induced locomotor assay in mice. It was shown to be active, albeit at a dose of 200 mg/kg i.p. Compound **23** exhibited a reasonable brain to plasma ratio of 1.4.

Researchers at Eli Lilly recently reported the in vivo activity of a $mGlu_2$ receptor PAM that is structurally related to acetophenone compounds from Merck (see, e.g., compound **6** in Figure 2).[19] *N*-(4-((2-(Trifluoromethyl)-3-hydroxy-4-(isobutyryl)phenoxy)methyl)benzyl)-1-methyl-1H-imidazole-4-carboxamide (THIIC, **24** in Figure 9) was found to be a very potent $mGlu_2$ receptor PAM (EC_{50} = 23 nM). It was also selective against the other mGlu receptor subtypes as well as a panel of CNS receptors. THIIC showed in vivo activity in numerous rodent models, including anxiolytic activity in a stress induced hyperthermia model in rat at 3, 10, and 30 mg/kg p.o., as well as antidepressant effects in a forced-swim model in mice at 30 mg/kg i.p. Of note is that the effects displayed by THIIC in the mouse model were absent in $mGlu_2$ receptor null mice.

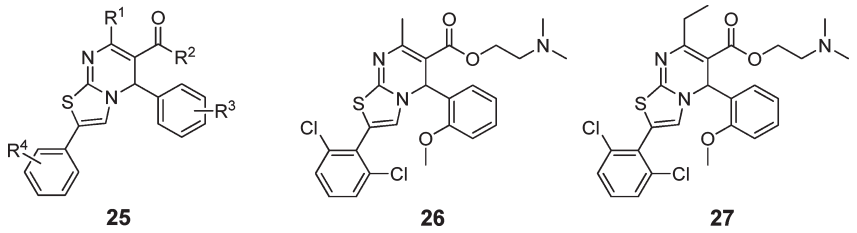


Figure 10. Wichmann and co-workers discovered a series of thiazolopyrimidine $mGlu_{2/3}$ receptor NAMs with activity in a GTPγS binding assay.

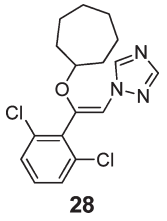


Figure 11. Kolczewski described a series of enolether-based $mGlu_{2/3}$ receptor NAMs, including **28**, with activity in a GTPγS binding assay.

MGLU$_{2/3}$ RECEPTOR NEGATIVE ALLOSTERIC MODULATORS (NAMS)

Workers at Hoffmann-La Roche disclosed the first series of $mGlu_{2/3}$ receptor NAMs to be identified, based around a 5H-thiazolo[3,2-a]pyrimidine scaffold **25**[29] (Figure 10). These compounds were identified and characterized utilizing a [^{35}S]-GTPγS binding assay on rat $mGlu_2$ receptor transfected CHO cell membranes by determining the inhibition of the $mGlu_{2/3}$ receptor agonist (1*S*,3*R*)-ACPD-induced GTPγS binding response. From this report, compounds **26** and **27** demonstrated the most potent inhibition of GTPγS binding, with IC_{50} values of 1.5 and 1.0 μM, respectively. Compound **26** was further characterized and found to have an IC_{50} value of 10 μM for reversal of (1*S*,3*R*)-ACPD-induced inhibition of forskolin-stimulated cAMP production in CHO cells transfected with rat $mGlu_2$ receptors. Compound **27** was also demonstrated to be inactive toward $mGlu_{1a}$ and $mGlu_{4a}$ receptors. These compounds represent the first example of non-amino acid antagonists of $mGlu_{2/3}$ receptors. Following this report, Hoffmann-La Roche presented $mGlu_{2/3}$ receptor NAMs centered on a heterocyclic enolether scaffold (Figure 11).[30] These compounds were characterized in the GTPγS binding assay described above, with compound **28** providing the most potent $mGlu_{2/3}$ receptor NAM with an IC_{50} of 110 nM. Interestingly, compound **28** was found to inhibit [^{3}H]-DCG-IV (radio-labeled orthosteric agonist)[31] binding on rat $mGlu_2$ receptor CHO cell membranes with an IC_{50} value of 500 nM.

Following the disclosure of these series, researchers at Hoffmann-La Roche next characterized a series of 1,3-dihydro-benzo-[b][1,4]diazepine-2-ones as $mGlu_{2/3}$ receptor NAMs (**29**, Figure 12).[32] The initial lead from this series was discovered from HTS of a random library of small molecules utilizing a [^{3}H]-LY354740 (a radio-labeled $mGlu_{2/3}$ receptor orthosteric agonist[33]) binding assay in rat $mGlu_2$ receptor CHO cell membranes. These compounds, exemplified by **30**, were demonstrated to act noncompetitively, decreasing the maximal efficacy but not altering the potency of (1*S*,3*R*)-ACPD in a GTPγS binding assay. Because these compounds inhibited [^{3}H]-LY354740 binding,

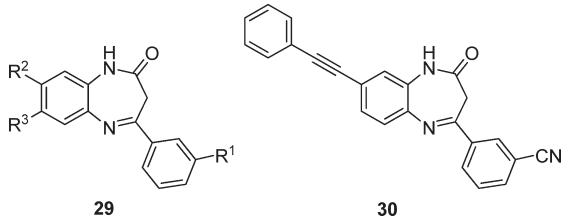


Figure 12. Benzodiazepinone $mGlu_{2/3}$ receptor NAMs. R^1 = CN, amides, halogen, CF_3, OMe; R^2 = Me, Ar—C≡C—, R−C≡C—; and R^3 = H, NR(R), OR.

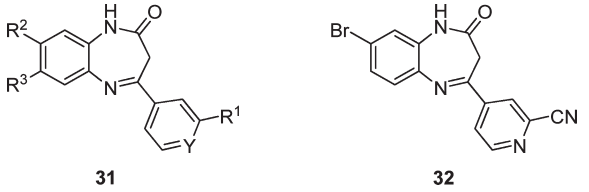


Figure 13. Benzodiazepinone-derived $mGlu_{2/3}$ receptor NAMs. R^1 = CN, I, 3-pyridyl; R^2 and R^3 = H, CH_3, halogen, OMe, NHMe; and Y = CH or N.

IC_{50} values for binding inhibition were reported as an indirect measure of affinity, with **30** having an IC_{50} value of 34 nM for this inhibition of binding. Multiple analogues were synthesized varying the R^1-R^3 positions, and these were tested in the in vitro binding assay, resulting in potent NAMs (Figure 12). The R^1 position showed a preference for CN substitution, although halides, methoxy, and carboxamides were also tolerated. Arylalkyne substitution at R^2 was found to provide analogues with superior affinity for $mGlu_2$ receptors. While hydrogen substitution at the R^3 position provided the most active compounds, the authors attempted to increase the solubility of this series by introducing polar and/or ionizing groups which resulted in a concomitant drop in activity. Compound **30** (Figure 12) was further characterized for subtype selectivity in a calcium mobilization assay with no effects at either $mGlu_{1a}$ or $mGlu_{5a}$ rat receptors. In addition, this compound did not show binding to *N*-methyl-D-aspartate (NMDA) or γ-aminobutyric acid A ($GABA_A$) receptors. Compound **30** was further characterized electrophysiologically in CHO cells expressing Kir3.2c and Kir3.1 GIRK channels and found to noncompetitively inhibit glutamate-induced K^+ currents.

Following this report by Woltering and colleagues, researchers at Vanderbilt University further evaluated the SAR around the dihydro-benzo[b][1,4]diazepine-2-ones (**31**, Figure 13).[34] SAR studies revealed a preference for H, halogen, or methyl substitution at R^2 and R^3, with methoxy and methylamine substitution

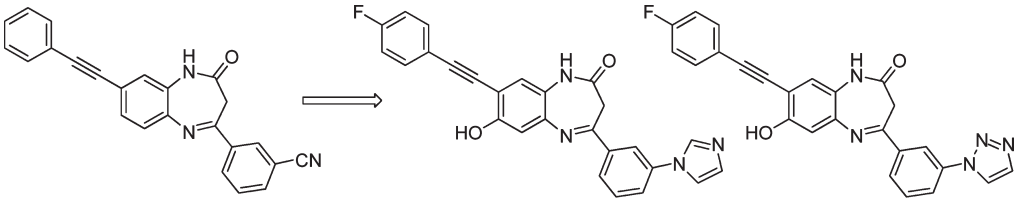


Figure 14. Benzodiazepinone NAMs with improved physical properties.

resulting in a loss of activity. The data also suggested a preference for cyano-pyridine substitution at R^1 and Y, with MNI-137 (**32**) being the most potent analogue (Figure 13). In addition, several derivatives (**31**) were tested in the GTPγS binding assay and a calcium mobilization assay, displaying potent nanomolar IC_{50} values against $hmGlu_2$ receptors of 12.6−170 nM; IC_{50} ($rmGlu_3$) = 8.3−158 nM. MNI-137 (**32**) blocked glutamate-induced calcium mobilization at both human (IC_{50} = 12.6 nM) and rat (IC_{50} = 8.3 nM) $mGlu_2$ receptors utilizing the chimeric G protein Gqi5 to couple $mGlu_2$ receptors to calcium mobilization, blocked glutamate-induced GTPγS binding at human $mGlu_2$ receptors (IC_{50} = 72.7 nM), and blocked glutamate-induced GTPγS binding at rat $mGlu_3$ receptors (IC_{50} = 20.3 nM). The compounds identified at Vanderbilt University did not displace [^{3}H]-LY341495 (a radiolabeled $mGlu_{2/3}$ receptor orthosteric antagonist) binding.[35] As previous findings had demonstrated that $mGlu_{2/3}$ receptor agonists inhibit excitatory neurotransmission at the MPP-DG synapse, the effects of MNI-137 at this synapse were evaluated. The $mGlu_{2/3}$ receptor agonist DCG-IV was found to inhibit field excitatory post synaptic potentials (fEPSPs) at the MPP-DG synapse, consistent with known $mGlu_{2/3}$ receptor agonist effects, and MNI-137 (**32**) reversed the effects of DCG-IV. Initial mutagenesis studies were performed to define the binding site of the $mGlu_2$ receptor NAMs. The $mGlu_2$ receptor mutations S668L/G689 V/N735D, which are thought to comprise the $mGlu_2$ receptor PAM binding site,[3,11] did not affect the ability of MNI-137 (**32**) to inhibit glutamate responses, implying that the $mGlu_{2/3}$ receptor NAMs may occupy a different allosteric site from the $mGlu_2$ receptor PAMs.

Following these studies, another disclosure by the Hoffmann-La Roche group[36] further exploring the SAR around the 1,3-dihydro-benzo[*b*][1,4]diazepine-2-one series noted that although these compounds partially inhibit [^{3}H]-LY354740 binding, they do not displace the radio-labeled orthosteric antagonist [^{3}H]-LY341495 in a binding assay, consistent with the findings of the Vanderbilt group. In this report, the Hoffmann-La Roche group followed up on their earlier SAR studies around the benzodiazepinone scaffold (**30**, Figure 14) with an expansion of substitution at the various key positions. In this iteration, it was reported that 5-membered heterocycles were suitable replacements for the cyano group at R^1 (Figure 12) and added much needed solubilizing properties (Figure 14). A requirement of the arylalkyne at R^2 did not change. However, the authors report tolerance of a phenolic OH at R^3, further decreasing the logP of the previous generation of these NAMs. Within this study, the most potent compounds were **33** (IC_{50} = 26 nM for [^{3}H]-LY354740 binding and 11 nM for [1*S*,3*R*]-ACPD-cAMP) and **34** (Figure 14) (IC_{50} = 20 nM for [^{3}H]-LY354740 binding and 17 nM for cAMP). Both of these compounds were also found to inhibit GIRK currents stimulated by glutamate at both rat $mGlu_2$ and $mGlu_3$ receptors, and **33** was further shown to reverse the LY354740-induced inhibition of fEPSPs at the MPP-DG synapse with an IC_{50} value of 230 nM. Although **33** was inactive in selectivity studies versus $mGlu_{1a}$, $mGlu_{5a}$, and $mGlu_8$ receptors, **34** did inhibit [^{3}H]-L-AP4 binding to $mGlu_8$ receptors with an IC_{50} value of 5 μM. In addition, compound **33** (Figure 14) was evaluated for activity at NMDA and $GABA_A$ receptors and was found to have no binding affinity for either class of receptors.

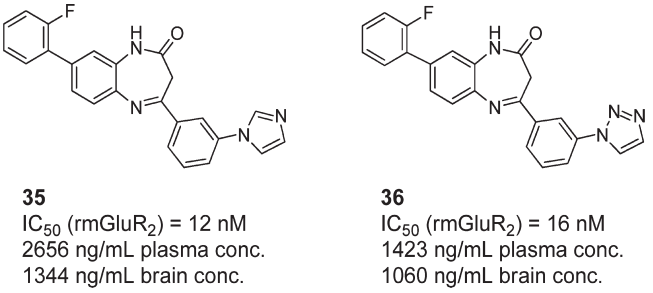
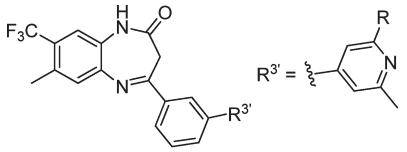


Figure 15. Orally available $mGlu_{2/3}$ receptor NAMs. Shown are plasma and brain concentrations after 10 mg/kg p.o. dose.

A third report on this series from Woltering and co-workers focused on further alterations of the benzodiazepinone scaffold that would improve the physicochemical properties of this series and enable in vivo evaluation. Specifically, the group focused on finding a more suitable, preferably smaller, replacement for the phenylalkyne moiety in the R^2 position (Figure 15).[37] It was found that this group could be effectively replaced with the smaller, less-lipophilic 2-fluorophenyl group. Thus, compounds **35** and **36** were more extensively characterized and were found to inhibit glutamate-induced GIRK currents at $mGlu_2$ receptors (**35**, IC_{50} = 11 nM; **36**, IC_{50} = 22 nM) and $mGlu_3$ receptors (**35**, IC_{50} = 33 nM; **36**, IC_{50} = 42 nM) and to reverse the LY354740-induced inhibition of fEPSPs at the MPP-DG synapse. Initial PK studies were performed with **35** and **36**. Compound **35** demonstrated CYP3A4 inhibition with an IC_{50} value of 1 nM; however, **36** only showed weak CYP3A4 activity (IC_{50} = 4.3 μM). Dosing **35** and **36** at 10 mg/kg p.o. in rats demonstrated reasonable brain exposure for both compounds (**35**, 2656 ng/mL, brain/plasma 0.5; **36**, 1423 ng/mL, brain/plasma 0.9). The most recent report from Woltering et al. at Hoffman-La Roche described the further optimization of the previously disclosed benzodiazepinone series of $mGlu_{2/3}$ receptor NAMs.[38] In efforts to develop antagonists with more druglike properties and in vivo efficacy, the group explored replacements for the heterocyclic moiety in the R^3 position (Figure 16). The optimal $mGlu_{2/3}$ receptor NAMs were



37; R = H, IC_{50} (rmGluR$_2$) = 4 nM
38; R = CH_3, IC_{50} (rmGluR$_2$) = 2 nM

Figure 16. In vivo active mGlu$_{2/3}$ receptor NAMs. The present study looked at variations of the R3′ position to enhance druglike properties. Compounds **37** and **38** (RO4491533) also rescue memory impairment caused by mGluR2/3 agonists in rat behavioral models.

found to contain the combination of trifluoromethyl in the 8-position and methyl in the 7-position of the benzodiazepinone core. With respect to substitution at the R3′ position, 2-methyl and 2,6-dimethyl-substituted *para*-pyridine produced derivatives that were potent and active in vivo. The exemplified compounds and their respective activities are shown in Figure 16.

BEHAVIORAL FINDINGS WITH ALLOSTERIC MODULATORS OF GROUP II METABOTROPIC GLUTAMATE RECEPTORS

Published findings on the behavioral effects of PAMs of Group II mGlu receptors are rather limited and mostly extend previous findings with Group II mGlu receptor orthosteric agonists. In terms of Group II mGlu receptor NAMs, the behavioral data are even more limited. Herein we first summarize the few reported behavioral effects of Group II mGlu receptor NAMs on tests of locomotion and memory in rats. Next, we summarize the behavioral effects of Group II mGlu receptor PAMs that primarily involve the evaluation of compounds in rats or mice in (i) putative animal models of schizophrenia; (ii) measures of anxiety-like behavior and animal models of anxiety; (iii) animal models with relevance to dependence on drugs of abuse; and (iv) two reports on sleep−wake architecture.

NAMS, Locomotion and Memory. An initial brief report showed that the mGlu$_{2/3}$ receptor NAMs (compounds **35** and **36** in Figure 15) reversed the hypoactivity induced by the Group II mGlu receptor agonist LY354740 in C57BL/6J mice.[37] A more extensive report by the same group from Hoffmann-La Roche extended this finding to the mGlu$_{2/3}$ receptor NAM compound RO4491533 (**38**) that also dose-dependently reversed locomotor hypoactivity induced by the mGlu$_{2/3}$ receptor agonist LY354740 in rats, without inducing hyperactivity when administered on its own.[38] The latter finding is significant in that it suggests that the NAM specifically reversed the effect of the mGlu$_{2/3}$ receptor agonist, rather than having nonspecific additive effects (see below as to how the hypoactivity induced by mGlu$_{2/3}$ receptor PAMs complicates the interpretation of the effects of PAMs on NMDA-induced hyperactivity).

The most interesting effect of mGlu$_{2/3}$ receptor NAMs relates to their property of reversing deficits in delay-dependent memory. A task used to assess memory involves testing rats in a chamber that contains two levers and a light above each of the levels. During a trial, the light above a lever is illuminated for a brief period of time and after some delay, that ranges from zero to several seconds, the subject is given the opportunity to respond on either of the two levels. It is required that the rat responds on the lever associated with the light illuminated at the beginning of the trial, while responding on the other lever is not reinforced. Thus, this task is called delay match-to-sample and assesses memory. As the delay between the stimulus presentation and the opportunity to make a response increases, the performance of the subjects deteriorates, particularly after administration of compounds, such as the muscarinic receptor antagonist scopolamine, that impair memory. Compound RO4491533 (**38**) reversed the impairment in episodic memory induced by either the mGlu$_{2/3}$ receptor agonist LY354740 or scopolamine in this task, without having any effects when administered on its own. In addition, compound RO4491533 enhanced the subthreshold effects of the acetylcholinesterase inhibitor donepezil on reversal of scopolamine-induced disruptions in performance in the delay match-to-position task in rats.[38] In conclusion, a mGlu$_{2/3}$ receptor NAM had memory-enhancing effects when performance was deteriorated by another drug manipulation. Such effects suggest that mGlu$_{2/3}$ receptor NAMs may be useful therapeutics for the treatment of memory deficits seen in several neuropsychiatric disorders, such as Alzheimer's, dementia, and schizophrenia. Of interest here are the potential for deleterious effects of mGlu$_{2/3}$ receptor agonists in this task, that were the subject of some debate.[39] However, in a study evaluating the effects of pretreatment with the mGlu$_{2/3}$ receptor agonist LY354740, this compound was found to produce a significant dose-related improvement in working memory during infusion of the NMDA glutamate receptor antagonist ketamine in healthy human subjects.[78] It would be of great interest to examine whether mGlu$_{2/3}$ receptor PAMs replicate the effects of mGlu$_{2/3}$ receptor orthosteric agonists in vivo.

PAMs and Schizophrenia-Related Tests. Several studies have assessed the effects of mGlu$_2$ receptor PAMS on behaviors induced by the administration of NMDA glutamate receptor antagonists, such as phencyclidine and ketamine. This approach is based on the hypothesis that dysfunction of NMDA receptors contributes to schizophrenia pathophysiology.[40,41] Blockade of NMDA receptors by noncompetitive NMDA receptor antagonists produces a schizophrenia-like state in healthy humans[42] that mimics several aspects of schizophrenia, including negative and positive symptoms, as well as cognitive deficits.[43−50] It is important to note, however, that not all changes in behavior induced by NMDA receptor antagonists in rodents constitute a valid animal model of schizophrenia.[51]

The two NMDA-induced behaviors that have been most studied with mGlu$_2$ receptor PAMs are increases in locomotor activity and disruptions in prepulse inhibition (PPI) of the startle response. Similar to Group II mGlu receptor agonists,[52,53] the mGlu$_2$ receptor PAM **6**,[10] compound **39**,[7] compound **22**[28] and the indole derivative **40**[6] (Figure 17), CBiPES (**7**),[18] LY487379 (**2**),[54] compound **23**,[28] or BINA[5,55] (Figure 1) reversed ketamine- or phencyclidine-induced hyperactivity in either rats or mice. However, a very important limitation of all of the aforementioned studies is that the rodents were habituated to the locomotor activity chambers before NMDA receptor antagonist administration, resulting in very low baseline activity levels that preclude the detection of potential locomotor suppressant effects of the test compounds. This issue is a problem for data interpretation because the apparent reversal of NMDA receptor antagonist effects by metabotropic glutamate modulators (i.e., agonists or PAMs) may simply reflect an additive effect of the NMDA receptor antagonist and the PAM, in which the NMDA antagonist increases activity while the PAM decreases activity, and not a pharmacological interaction within a neurobiological system relevant to schizophrenia psychopathology. Indeed, the

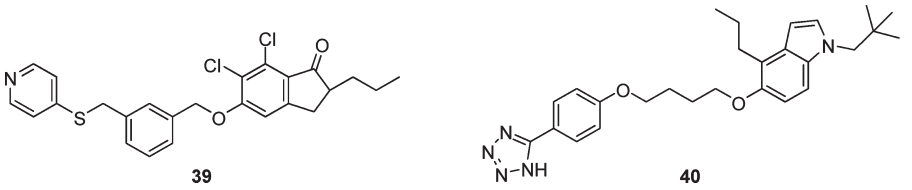


Figure 17. Structures of $mGlu_2$ receptor PAMs with activity reported in behavioral assays.

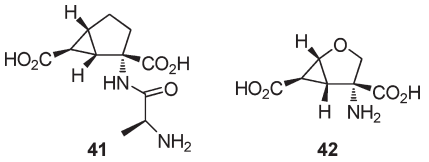


Figure 18. Structures of $mGlu_{2/3}$ receptor agonist prodrug LY544344 (**41**) and $mGlu_{2/3}$ receptor agonist LY379268 (**42**).

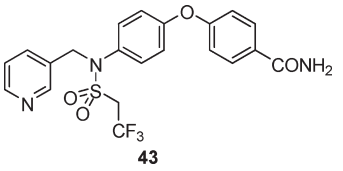


Figure 19. Structure of $mGlu_2$ receptor PAM APPES (**43**).

$mGlu_2$ receptor PAM BINA (Figure 1) decreased activity when administered alone.[5] Furthermore, arguing that NMDA receptor antagonist-induced increases in locomotor activity reflect a particular construct relevant to schizophrenia symptomatology is difficult.[51] Thus, although the above studies with $mGlu_2$ receptor PAMs indicate that these compounds have biological activity and in the vast majority of cases good brain penetration, the locomotor activity findings are not necessarily relevant to schizophrenia.

More relevant to schizophrenia may be the effects of $mGlu_2$ receptor PAMs on NMDA receptor antagonist-induced disruptions of PPI of the startle response. PPI has been shown to be disrupted in schizophrenia patients and has been hypothesized to be an endophenotype and biomarker of schizophrenia.[56] The $mGlu_2$ receptor PAM LY487379 (**2**) (Figure 1)[54] reversed amphetamine- but not phencyclidine-induced disruptions of PPI, whereas the $mGlu_2$ receptor PAM BINA (Figure 1) reversed phencyclidine-induced disruptions of PPI in mice (amphetamine-induced disruptions were not assessed with BINA[5]). This pattern of results is somewhat puzzling because Group II mGlu receptor agonists have no effects on phencyclidine-induced disruptions of PPI,[57−59] and because the effects of the $mGlu_2$ receptor PAMs LY487379 and BINA (Figure 1) were not consistent with each other.[5,54] Thus, further investigations with additional compounds and in additional species are warranted before any firm conclusions can be made about the effects of $mGlu_2$ receptor PAMs on PPI deficits induced by NMDA receptor blockade.

PAMs and Anxiety-Related Measures. Similar to Group II mGlu receptor agonists (for review, see ref 60), $mGlu_2$ receptor PAMs exhibit anxiolytic properties in a variety of procedures reflecting anxiety-like behavior. Specifically, the positive modulator 4-MPPTS (**2**) reversed the increased startle response seen in the fear-potentiated startle procedure.[13,18] This effect was reversed by a Group II mGlu receptor antagonist without an effect of the antagonist alone.[13] In this procedure, a shock is paired repeatedly with a light cue. In a subsequent session, the presentation of the cue light alone potentiates the startle response.[61,62] Importantly, the fear-potentiated startle response is sensitive to the effects of anxiolytics, such as benzodiazepines, in both humans and experimental animals.[78] Most relevant to the present review, fear-potentiated startle in humans was also attenuated by putative anxiolytic test compounds (i.e., the $mGlu_{2/3}$ receptor agonist LY354740 (**5**) (Figure 2) and its pro-drug LY544344 (**41**) (Figure 18), and reduced generalized anxiety disorder symptoms in human proof-of-principle trials.[63] Thus, preclinical and clinical data demonstrate the predictive validity of this rat model for novel treatment targets for anxiety outside of the benzodiazepine class of $GABA_A$-modulatory compounds.

Similar effects to those noted with 4-MPPTS (**2**)[13] were also seen with the $mGlu_2$ receptor PAM 4-APPES (**43**) (Figure 19) in the fear-potentiated startle procedure.[18] At the highest doses tested, these two compounds completely reversed the potentiation of the startle response induced by the presentation of stimuli previously associated with footshock, similar to diazepam, in rats. Furthermore, the related optimized molecule CBiPES (**7**) (Figure 2) reversed the transient increase in body temperature seen in mice after exposure to a stressor,[64] such as exposure to a cage that contained soiled rat shavings, in mice.[18] However, interpreting this effect of CBiPES (**7**) (Figure 2) as a clear anxiolytic effect is difficult because this compound, at a dose that reversed stress-induced hyperthermia, decreased body temperature on its own. Thus, the "reversal" of the effects of stress-induced hyperthermia may have been simply an additive effect (i.e., the compound decreased body temperature while stress increased body temperature), rather than an interaction through stress pathways.

More conclusive anxiolytic-like effects were seen with the $mGlu_2$ receptor PAM BINA[5] in the elevated plus maze[65] and in a stress-induced hyperthermia procedure different from the one described above in mice.[66] The elevated plus maze consists of an elevated plus-shaped narrow platform that has two open arms and two closed arms. This behavioral test is based on the approach-avoidance conflict inherent in rodents caused by their tendency to balance exploration of novel environments against their anxiety-like responses elicited by exposure to an open elevated narrow platform. Anxiolytic compounds increase the amount of time that rodents spend in the open arms of the plus maze and the number of entries into the open arm (e.g., ref 67). BINA, similar to the benzodiazepine chlordiazepoxide, increased the amount of time that mice spent in the open arms of the plus maze and the number of times they entered the open arms, without altering total locomotor activity in the maze.[5] The lack of effect on ambulations indicates that the compound did not increase general locomotor activity and that the increased time

spent on the open arms likely reflects an anxiolytic-like effect. Thus, BINA likely exerted an anxiolytic-like response in the plus maze test.

The stress-induced hyperthermia procedure involves two successive measures of rectal body temperature in mice or rats, a test shown to be sensitive to the effects of anxiolytic compounds.[66] BINA in mice, similar to the benzodiazepine chlordiazepoxide[5] and the $mGlu_2$ receptor PAM THIIC in rats,[19] reversed this type of stress-induced hyperthermia. This effect of BINA was not observed when BINA was coadministered with a Group II mGlu receptor antagonist.[5] In addition, THIIC exhibited anxiolytic-like effects in an additional test of anxiety, the marble burying test in mice.[19] The marble burying test involves the measurement of the number of marbles buried in sawdust by mice placed in a cage that contains the marbles. The construct of anxiety measured by this test is not clear, although this test has been shown to be responsive to effects of compounds clinically used for anxiety, such as selective serotonin reuptake inhibitors (e.g., 68). The Fell and colleagues paper[19] also describes positive effects of THIIC in three animal models of depression-like behavior, the forced-swim test, the differential reinforcement of low rates 72 s (DRL-72 s) test, and the dominant-submissive test. However, these findings are difficult to interpret in the context of previous literature suggesting that $mGlu_2$ receptor antagonists have antidepressant properties in the forced swim test and the tail suspension test,[69−71] the fact that performance may be affected by nonspecific effects of THIIC in the DRL-72 s schedule (as decreases in responding are interpreted as antidepressant effects in this test), and the high variability seen in the dominant submissive test in the Fell and colleagues study. Additional research in this field is warranted before firm conclusions are made about the antidepressant properties of $mGlu_2$ receptor PAMs or NAMs.

PAMs and Behaviors Related to Drug Dependence and Sleep-Wake Architecture. The effects of the $mGlu_2$ receptor PAM BINA were assessed in rat models relevant to cocaine dependence. BINA decreased cocaine self-administration in rats, indicating that this compound decreased the reinforcing effects of cocaine.[14] Furthermore, in a procedure that is a putative model of relapse to drug taking in humans, BINA decreased cue-induced reinstatement of cocaine seeking.[14] In this procedure, stimuli, such as a cue light previously associated with cocaine delivery, acquire motivational properties. Subsequently, the presentation of these cues reinstates cocaine-seeking behavior in subjects whose drug-seeking behavior was extinguished. Importantly, these effects of BINA were observed at doses that had no effect on responding for a food reinforcer using identical procedures and conditions.[14] This pattern of results indicates a better behavioral profile of BINA compared with the Group II mGlu receptor agonist LY379268 (**42**) (Figure 18) which affected cocaine-related behaviors at doses that also affected behaviors motivated by a food reinforcer. Although not directly assessed yet, there is no indication that PAMs or NAMs would have abuse liability based on their overall behavioral profile discussed here.

Finally, BINA and THIIC also affected sleep-wake architecture in rats. BINA suppressed rapid eye movement (REM) sleep, lengthened its onset, and slightly increased passive waking.[72] BINA had synergistic effects with the Group II mGlu receptor agonist LY354740 on these sleep−wake measures.[72] Similarly, THIIC decreased REM sleep and increased non-REM sleep.[19] Considering that REM sleep abnormalities, such as early onset REM sleep and high-density REM sleep, characterize depression,[73] these findings have important implications for $mGlu_2$ receptor PAM potential normalization of sleep abnormalities in depressed patients.

CONCLUSIONS

There has been a significant increase in publication activity in the primary scientific literature focused on the medicinal chemistry and pharmacology of $mGlu_2$ receptor PAMs in the past few years.[79] Multiple structural classes of $mGlu_2$ receptor PAMs have been identified, and in many cases extensive structure−activity relationship studies have been disclosed. In addition to additional studies around some of the earlier structural classes, such as the biphenylindanone,[80] *N*-(pyridin-3-ylmethyl)ethanesulfonamide (e.g., APPES, 4-MPPTS, cyPPTS, CBiPES), and 2-hydroxyphenylethanone (e.g., THIIC) scaffolds, new classes including the benzimidazole, oxazolidinone, dihydrobenzo[4,5]imidazo-oxazolidinone, imidazo[1,2-a]pyridine, and 1-methylpyridin-2(1H)-one derivatives have appeared. Importantly, several of these disclosures include compounds with systemic activity in vivo, paving the way for therapeutic proof-of-concept studies.

Disclosures on the medicinal chemistry around Group II mGlu receptor NAMs in the primary scientific literature have been restricted primarily to benzodiazepinone derivatives. This work has been spearheaded by groups at Hoffmann-La Roche and Vanderbilt University who have shown that this structural class of $mGlu_{2/3}$ receptor NAMs exhibits promising pharmacological properties in vitro and in vivo. Behavioral data with $mGlu_{2/3}$ receptor NAMs are limited but quite interesting, since the data suggest that such compounds may have cognitive enhancing properties, and in particular memory enhancing properties. Thus, $mGlu_{2/3}$ receptor NAMs could be useful for the treatment of memory deficits seen in Alzheimer's, aging, schizophrenia, and other neuropsychiatric conditions.

Although Group II mGlu receptor agonists or PAMs have received much attention in recent years for the treatment of schizophrenia, the preclinical data are not as conclusive as the data for other indications, such as anxiety. This statement is not meant to imply that agonistic or positive modulatory actions on these receptors will not have efficacy in treating aspects of schizophrenia. Indeed, a clinical study demonstrated the efficacy of the Group II mGlu receptor agonist LY2140023, the prodrug of LY404039, in schizophrenia,[74] although this clinical effect has not been replicated in a follow up study. Furthermore, strong neurobiological evidence implicates these receptors in the pathophysiology of schizophrenia (for review, see ref 75). Instead the comments here are about the use of inadequate and severely limited procedures, with little construct validity for schizophrenia symptoms,[51,76] in the assessment of these compounds. More intensive preclinical work is warranted in the area of schizophrenia models, and to determine whether some schizophrenia symptoms may be treated with PAMs and other symptoms may be treated with NAMs, and how the two opposite pharmacological approaches may coexist.

While repeated dosing of the Group II mGlu receptor agonist LY379268 (**42**) (Figure 18) has been shown to lead to tolerance for behavioral effects[54,77] and downregulation of mGlu2/3 receptor function in the prefrontal cortex as assessed by [^{35}S]-GTPγS binding assays,[77] it is possible that the modulatory actions of Group II mGlu receptor PAMs may result in less or no tolerance to their effects, perhaps allowing for the development of efficacious treatments for psychiatric disorders even after

chronic administration. The most promising preclinical findings with $mGlu_2$ receptor PAMs suggest that these compounds may have efficacy for the treatment of anxiety disorders, sleep abnormalities in depressed individuals, and other psychiatric populations characterized by increased REM sleep density, and drug dependence on psychomotor stimulant drugs, such as cocaine.

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■ AUTHOR INFORMATION

Author Contributions

D.J.S., A.B.P., R.D., A.M., and N.D.P.C. all researched the scientific literature, wrote sections of the manuscript and prepared diagrams and figures. N.D.P.C. compiled, reviewed and edited the collated manuscript.

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