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Discovery of Molecular Switches that Modulate Modes of mGlu₅ Pharmacology *In Vitro* and *In Vivo* Within a Series of Functionalized, Regioisomeric 2- and 5-(Phenylethynyl) Pyrimidines

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Abstract

This Letter describes the synthesis and SAR of a series of analogs of the mGlu₅ partial antagonist 5-(phenylethynyl)pyrimidine. New molecular switches are identified that modulate the pharmacological activity of the lead compound. Slight structural modifications around the proximal pyrimidine ring change activity of the partial antagonist lead to that of potent and selective full negative allosteric modulators and positive allosteric modulators, that demonstrate *in vivo* efficacy in rodent models for anxiolytic activity and antipsychotic, respectively.

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system and exerts its effects through both ionotropic and metabotropic glutamate receptors. The metabotropic glutamate receptors (mGluRs^γ) are members of the G Protein-coupled receptor (GPCR) family C, which are characterized by a large extracellular amino-terminal agonist-binding domain. To date, eight mGluRs have been cloned, sequenced and assigned to three groups (Group I: mGlu₁ and mGlu₅; Group II: mGlu₂ and mGlu₃; Group III: mGlu_{4,6,7,8}) based on their sequence homology, pharmacology, and coupling to effector mechanisms.¹⁻² In preclinical models, studies with the negative allosteric modulators (NAMs) **1** (MPEP) and **2** (MTEP) have demonstrated that selective antagonism of mGlu₅ has therapeutic potential for chronic disorders such as pain, anxiety, depression, addiction and Fragile X syndrome.³⁻⁷ Furthermore, there is direct clinical validation of anxiolytic activity by allosteric antagonism of mGlu₅ in patients with fenobam **3**.⁸ Alternatively receptor activity can be enhanced through positive allosteric modulators (PAMs) such as **4** (DFB), **5** (CPPHA), **6** (CDPPB) and **7** (ADX-47273), which, with the exception of **5**, share the same allosteric binding site as **1**.⁹⁻¹³ PAMs **6** and **7**, both ago-potentiators, have demonstrated *in vivo* proof of concept in

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Supporting Information Available: Experimental procedure and analytical data for compounds **8a-f** and **10a, b** are provided as well as details of the *in vitro* and *in vivo* assays. This material is available free of charge via the Internet at <http://pubs.acs.org>.

^γAbbreviations: mGluR, metabotropic glutamate receptor; NAM, negative allosteric modulator; PAM, positive allosteric modulator; GPCR, G protein-coupled receptor

preclinical schizophrenia models in which other known antipsychotics show similar positive effects.¹⁰⁻¹³ Recently, pure mGlu₅ PAMs have been developed based on **7**, by the incorporation of a basic heterocycle in the 3-position of the oxadiazole.¹⁴ Based on our experience in the development of allosteric modulators of mGluRs with a broad range of activities including negative allosteric modulators, positive allosteric modulators and neutral allosteric site ligands at the allosteric binding site occupied by **1**, together with theoretical models of allosteric function, we postulated that it might be possible to develop ‘partial antagonists’. As envisioned, a ‘partial antagonist’ would fully occupy the binding site of **1** on the mGlu₅ receptor but only partially block agonist response, resulting in partial mGlu₅ inhibition; moreover, Rodriguez et al. identified several mGlu₅ partial antagonists.¹⁵ In 2008, Sharma et al. conducted a limited optimization effort focused on the mGlu₅ partial antagonist lead **8**. Within two 24-member libraries, SAR elucidated a “molecular switch” to modulate pharmacological activity (Fig. 1).¹⁶ Lead **8**, with an unsubstituted distal phenyl ring, fully occupied the allosteric binding site of **1**, possessed an IC₅₀ of 486 nM, but only afforded partial response (29% response, 71% partial antagonism), that is, allosteric partial antagonism. Incorporation of small chemical moieties in the 3-position of the distal phenyl ring, such as a 3-methyl group, delivered **9**, a full noncompetitive mGlu₅ antagonist (IC₅₀ = 7.5 nM). When the methyl group was moved from the 3-position to the 4-position as in **10**, an efficacious (99% of glutamate max) mGlu₅ PAM resulted (EC₅₀ = 3.3 μM, 4.2-fold shift), which also represented a new mGlu₅ PAM chemotype.¹⁶ The observation of a conserved molecular switch, accessed by toggling between 3- and 4-substitution on the distal phenyl ring, within this chemical series was unprecedented. These preliminary data encouraged us to further optimize **8**, and survey the impact of incorporating substituents on the pyrimidine ring, as well as examining regioisomeric pyrimidines in an attempt to develop potent and selective mGlu₅ NAMs and PAMs suitable for *in vivo* studies to confirm the observed *in vitro* pharmacology.

For the next round of chemical lead optimization, we relied on an iterative analog library synthesis approach¹⁷ to rapidly prepare a 24-member library¹⁸ in which 2-substituted-5-bromo-pyrimidines **11** were treated with either phenylacetylene **13**, 3-methylphenyl acetylene (the NAM ‘switch’) **14** or 4-methylphenyl acetylene (the PAM ‘switch’) **15** under microwave-assisted Sonogashira conditions (Scheme 1) to provide analogs **16**. In parallel, we prepared a small 3-member library employing the regioisomeric 2-bromopyrimidine **12** and **13-15** to deliver analogs **17**.

SAR from this library was ‘flat’, with few actives (Table 1); however, unexpected modulation of the mode of mGlu₅ pharmacology was observed. All new analogs **16** containing the 4-methyl phenyl moiety were uniformly inactive, save for **16f**, a weak mGlu₅ PAM. When R₁ was an ethoxy group in combination with the NAM ‘switch’ 3-methyl phenyl, **16a** resulted, a potent mGlu₅ NAM (IC₅₀ = 21 nM). The remaining analogs **16** were either inactive, or more surprisingly, potent mGlu₅ PAMs. When an aminomethyl group was incorporated at the 2-position of the pyrimidine, in conjunction with an unsubstituted phenyl ring, **16b** resulted, which represents the most potent (EC₅₀ = 14.3 nM, 15-fold shift) rat mGlu₅ PAM reported to date (10-15-fold more potent than **6** and **7**). Addition of the NAM ‘switch’ 3-methyl phenyl moiety with the 2-aminomethyl group **16c** unexpectedly afforded a similarly potent mGlu₅ PAM (EC₅₀ = 21.1 nM, 5.9-fold shift) – suggesting the 3-methylphenyl moiety is not a conserved molecular switch for engendering NAM activity. Interestingly, the NAM **16a** differs from the PAM **16c** by substitution at the 2-position of the pyrimidine, OEt versus NHMe, respectively, with equal potency (IC₅₀ = 21 nM and EC₅₀ = 21 nM, respectively), but opposite mode of pharmacology. Other groups were tolerated in the 2-position of the pyrimidine such as SMe **16d** and *t*-Bu **16e**, and found to engender mGlu₅ PAM activity (EC₅₀ = 120 nM, 11-fold shift and EC₅₀ = 247 nM, 6-fold shift, respectively), but were inactive in the presence of the 3- or 4-Me phenyl moieties.

Overall, **16b** represents a 235-fold improvement in potency over mGlu₅ PAM **10**, was selective for mGlu₅ (>10 μ M vs. mGlu_{1,2,3,4,7,8}), and warranted further evaluation.

The PAMs reported here demonstrated no activity in the absence of glutamate, but in the presence of a sub-threshold concentration of glutamate (EC₂₀), a concentration dependent potentiation of mGlu₅ response was observed (Fig. 2). Importantly, **16b** is a pure mGlu₅ PAM, not an ago-potentiator like **6** and **7**. In addition, compound **16b** demonstrated a robust 15-fold leftward shift of the glutamate concentration response curve (EC₅₀ shifts from 493 nM to 32 nM) with an increase in glutamate max (Fig. 2).

In the regiosiomeric pyrimidine series **17**, the 4-Me congener **17c** was inactive. The unsubstituted phenyl analog **17a** was a moderately potent mGlu₅ NAM (IC₅₀ = 195 $\pm$ 65 nM). Unlike series **16**, the 3-Me NAM 'switch' performed as expected in series **17**, significantly increasing mGlu₅ NAM activity (IC₅₀ = 10.8 $\pm$ 2.7 nM) for **17b**. Moreover, **17b** was selective for mGlu₅ (>10 μ M vs. mGlu_{1,2,3,4,7,8}).

With a potent mGlu₅ PAM **16b** and a potent mGlu₅ NAM **17b**, we were poised to determine if the modes of mGlu₅ modulation observed in our *in vitro* cellular assays would be mirrored in standard *in vivo* behavioral paradigms. To evaluate the PAM **16b**, we chose to study the ability of **16b** to reverse amphetamine-induced hyperlocomotion in rats, as **6** and **7** displayed robust efficacy in this preclinical model where other known antipsychotic agents show similar positive results.¹⁰⁻¹³ In the event, **16b** was dosed i.p. at 3, 10 or 30 mg/kg thirty minutes prior to s.c. administration of 1 mg/kg amphetamine. As shown in Figure 3, a modest dose response is observed with **16b**, with significant reversal noted at the 30 mg/kg dose, and no effect (i.e, sedation) of **16b**/vehicle alone. Thus, the mGlu₅ PAM activity observed in cell-based *in vitro* assays is mirrored *in vivo* with **16b**, and comparable to the effects seen with both **6** and **7**.¹⁰⁻¹³ Moreover, the reversal of amphetamine-induced hyperlocomotion with **16b** is important, as **16b** lacks the intrinsic agonism of the ago-potentiators **6** and **7**, suggesting for the first time that positive allosteric modulation alone is sufficient for an antipsychotic profile in this preclinical model.

Previously antagonists for mGlu₅ NAMs such as **1** and **2** have demonstrated anxiolytic activity in numerous preclinical models. Therefore compound **17b** was tested in a modified Geller-Seifter conflict model wherein an increase in punished responding is consistent with an anxiolytic-like profile.²⁰ As seen in Figure 4, **17b** produced a significant dose-dependent increase in punished responding with the 30 mg/kg dose approaching a 300% increase in response rate [$F(4,17) = 22.69, p < 0.0001$] (upper panel) with no significant effect on unpunished responding (lower panel). Post hoc analysis indicated that both the 10 and 30 mg/kg doses in the punished component of the schedule differed significantly from vehicle ($p < 0.05$, Newman-Keul). Therefore, the NAM activity observed in cell-based *in vitro* assays was again paralleled in a standard anxiolytic *in vivo* assay where classical mGlu₅ NAMs display similar positive results.^{3-6,20}

In summary, slight structural changes to an mGlu₅ allosteric partial antagonist lead resulted in a shift in activity from partial antagonist to potent full antagonist to potent positive allosteric modulator. Two new molecular switches were elucidated through these changes. A regiosiomeric pyrimidine congener **17b** resulted in full NAM activity *in vitro* and *in vivo*. The incorporation of an amino methyl group into the 2-position of the pyrimidine core resulted in PAM activity and this new molecular 'switch' was able to override previously identified NAM molecular 'switches'. In this series, **16b** represents the most potent mGlu₅ PAM reported to date, and the first example of *in vivo* efficacy of a pure mGlu₅ PAM in reversing amphetamine-induced hyperlocomotion. The resulting mGlu₅ NAM **17b** and PAM **16b** showed *in vivo* efficacy in rodent models of anxiety and schizophrenia, respectively,

which mirrored the observed *in vitro* mode of pharmacology. With such subtle structural modifications capable of fully reversing modes of pharmacology, lead optimization campaigns focused on ligands that bind to the allosteric site occupied by **1** are especially challenging. Further work in this area is in progress and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Identification of ‘molecular switches’ that converts an mGlu₅ partial antagonist **8**, to either a full non-competitive antagonist (NAM) **9**, or a weak, but fully efficacious mGlu₅ positive allosteric modulator (PAM) **10**.

Figure 2.

Compound **16b** potentiates mGlu₅ activation by glutamate. In the absence of glutamate, **16b** does not activate mGlu₅. In the presence of a subthreshold quantity of glutamate, **16b** potentiates mGlu₅ in a concentration-dependent manner. Compound **16b**'s potentiation of response to glutamate is manifested as increased mGlu₅ agonist sensitivity. The glutamate EC₅₀ value is shifted from 493 nM to 32 nM, or a 15-fold shift with 10 μ M **16b**.

Figure 3. Reversal of amphetamine-induced hyperlocomotion with mGlu₅ PAM **16b** in dose-dependent manner with the non-toxic vehicle, 10% Tween 80.

Figure 4.

Dose-response curves for the effects of **17b** on punished (upper panel) and unpunished (lower panel) responding. The data are the mean number of punished and unpunished responses that animals made when tested on 1, 3, 10, 30 mg/kg **17b** and vehicle. Each value represents the mean $\pm$ SEM for eighteen animals. For punished responding animals tested on 10 and 30 mg/kg **17b** made significantly greater number of responses than animals tested on vehicle ($p < 0.05$). Unpunished responding did not change significantly at any of the doses tested.

Scheme 1. Synthesis of Analogs of **16** and **17**^a.

^aReagents and conditions: (a) 10 mol% Pd(PPh₃)₄, 20 mol% CuI, 20.0 equiv. diethylamine, DMF, mw, 70 °C, 10 min, 16-95%; all compounds purified by mass-directed HPLC to >98% purity.¹⁹

Chart I. mGlu₅ Allosteric Ligands

Table 1

Structures, Activity and Mode of Pharmacology of Analogs **16** and **17**.

	R_1	R_2	Allosteric Activity	IC_{50}/EC_{50} (nM) ^a	% Antagonism ^a	Fold Shift ^a
8	H	H	PA	486±28	71	N/A
9	H	3-Me	NAM	7.5±1.2	100	N/A
10	H	4-Me	PAM	3,300±290	N/A	3.3
16a	OEt	3-Me	NAM	21.1±2.8	100	N/A
16b	NHMe	H	PAM	14.3±2.3	N/A	15
16c	NHMe	3-Me	PAM	21.1±1.8	N/A	5.9
16d	SMe	H	PAM	120±25	N/A	11
16e	<i>t</i> -Bu	H	PAM	247±24	N/A	6.0
16f	NHMe	4-Me	PAM	704±86	N/A	5.7
17a	N/A	H	NAM	195±65	100	N/A
17b	N/A	3-Me	NAM	10.8±2.1	100	N/A
17c	N/A	4-Me	N/A	>10,000	N/A	N/A

^a IC_{50} s, EC_{50} s, % Antagonism and Fold Shift are the average of at least three independent determinations. N/A = not applicable PA = Partial Antagonist NAM = Negative Allosteric Modulator PAM = positive Allosteric Modulator. Fold-shift at 10 μ M fixed concentration of cmpd