Published in final edited form as:

Bioorg Med Chem Lett. 2008 July 15; 18(14): 4098–4101. doi:10.1016/j.bmcl.2008.05.091.

Synthesis and SAR of an mGluR5 allosteric partial antagonist lead: Unexpected modulation of pharmacology with slight structural modifications to a 5-(phenylethynyl)pyrimidine scaffold

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Abstract

This Letter describes the synthesis and SAR, developed through an iterative analogue library approach, of a mGluR5 allosteric partial antagonist lead based on a 5-(phenylethynyl)pyrimidine scaffold. With slight structural modifications to the distal phenyl ring, analogues demonstrated a range of pharmacological activities from mGluR5 partial antagonism to full antagonism/negative allosteric modulation to positive allosteric modulation.

Glutamate is the major excitatory transmitter in the central nervous system, exerting its effects through both ionotropic and metabotropic glutamate receptors. The metabotropic glutamate receptors (mGluRs) are members of the GPCR family C, 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: mGluR1 and mGluR5; Group II: mGluR2 and mGluR3; Group III: mGluRs 4,6,7,8) based on their sequence homology, pharmacology, and coupling to effector mechanisms.¹ In preclinical models, studies with the non-competitive antagonists MPEP (**1**) and MTEP (**2**) have demonstrated that selective antagonism of mGluR5 has therapeutic potential for chronic disorders such as pain, anxiety, depression, cocaine addiction and Fragile X syndrome.² Indeed, there is now direct clinical validation of anxiolytic activity by allosteric antagonism of mGluR5 in patients with fenobam (**3**).³

A major potential issue with MPEP (**1**), MTEP (**2**) and fenobam (**3**) is that these non-competitive, allosteric antagonists function as inverse agonists (Fig. 1).^{2,3} These compounds completely block all activity of the mGluR5 receptor, including constitutive activity. Based on the close association of mGluR5 with its signaling partner the NMDA receptor, there is growing concern that complete blockade may have several adverse effects commonly

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associated with NMDAR antagonists such as cognitive deficits and psychotomimetic effects.^{3,4} These adverse effects could severely limit the clinical utility of currently available mGluR5 antagonists for the treatment of chronic disorders.

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 MPEP (**1**) binding site together with theoretical models of allosteric function, we postulated that it might be possible to develop 'partial antagonists'.⁵ In this case, a 'partial antagonist' would fully occupy the MPEP (**1**) binding site on the mGluR5 receptor but only partially block agonist response, resulting in partial mGluR5 inhibition.⁴ To test this hypothesis, 200 analogues of MPEP (**1**) were synthesized and evaluated for displacement of [³H]methoxy-PEPy. Compounds that fully displaced the radioligand were then screened in a calcium fluorescence assay, which determined the ability of these analogues to block functional responses of mGluR5 to glutamate. The goal was to identify compounds that only partially block mGluR5 at concentrations that fully occupy the allosteric site. As reported by Rodriguez, *et. al.* in 2005, this effort identified three compounds that only partially inhibited or had no functional effects on mGluR5 response.⁴ M-5MPEP (**4**) and Br-5MPEPy (**5**) represented the first 'partial antagonists' of mGluR5 and induced a maximal blockade of ~50%, while 5MPEP (**6**) acted as a neutral allosteric site ligand (Fig. 2).⁴

Based on these data, we performed a high-throughput functional screen of our 160,000 compound screening collection to identify novel compounds with a range of activities as full and partial mGluR5 antagonists. This effort identified a number of novel full mGluR5 antagonists as well as a small number of mGluR5 partial antagonists. As shown in Figure 3, we identified several compounds, such as **7** (EC₅₀ = 297 nM) and **8** (EC₅₀ = 62 nM), that fully occupied the MPEP binding site, but induced a maximal 58% or 82.1% blockade, respectively, in the primary HTS. In this Letter, we describe the synthesis and SAR developed around partial antagonist lead **8**, and the unexpected modulation of pharmacology that resulted.

We began our lead optimization campaign around partial antagonist **8** (IC₅₀ = 62 nM, 17.9% response, 82.1% partial antagonism), a 5-(phenethyl)pyrimidine scaffold. Lead **8** was resynthesized (Scheme 1) employing a microwave assisted organic synthesis (MAOS) Sonogashira protocol with commercially available 5-bromopyrimidine **9** and phenyl acetylene **10** and catalytic CuI/Pd(PPh₃)₄ to deliver 50 mg quantities of **8** in >90% yield.⁶ After screening in triplicate with full concentration response curves, the activity of **8** was right-ward shifted ~8-fold (IC₅₀ = 486 nM), but data indicated that **8** was clearly a partial antagonist (29% response, 71% partial antagonism).⁷ In radioligand binding assays with [³H]methoxy-PEPy, **8** was found to possess a K_i of 125.9 nM, indicating it binds to the MPEP site.

It was unclear from the outset if the degree of mGluR5 partial antagonism would be maintained within this chemical series, or if the degree of partial antagonism would vary over a dynamic range to include complete blockade as seen with MPEP (**1**). Our optimization effort relied on an iterative library synthesis approach in which we employed MAOS to rapidly prepare two, 24-member analogue libraries.⁸ In the event, commercially available 5-bromopyrimidine **9** was subjected to a microwave-assisted Sonogashira coupling protocol (10% mol Pd° catalyst, 20 mol% CuI, 20 equiv. diethylamine, 1:1 ratio of **9**:**11**, 70°C, 10 minutes) with a library of functionalized alkynes **11** to deliver diverse 5-ethynylpyrimidine analogues **12** (Scheme 2). By application of MAOS and the Fu catalyst (Pd(*t*BuP)₂), the palladium precipitated upon rapid cooling, and no Pd° scavenging was required prior to mass-directed preparative HPLC purification, unlike in the scale-up of **8** which employed Pd(PPh₃)₄. Moreover, excellent chemical yields (70–95%) were obtained for a diverse range of functionalized alkynes, suggesting this new protocol is quite general and broad in scope.

All 48 analogues **12** were screened in our calcium fluorescence ‘triple add’ assay that can identify agonists, antagonists and positive allosteric modulators (potentiators). Only 11 of the 48 analogues (23%) demonstrated any measurable effect on calcium transients, and these effects were unexpected (Table 1) providing only one partial antagonist (PA), but six full antagonists (A) and four positive allosteric modulators (PAM). This dynamic modulation of pharmacological response was unanticipated. Moreover, despite a rather ‘flat’ SAR, trends emerged that identified ‘chemical switches’ to modulate modes of mGluR5 pharmacology.

In general, incorporation of chemical moieties into the 3-position of the distal phenyl ring afforded full mGluR5 antagonists with IC_{50} s ranging from 7.5 nM (**12a**) to 7.8 μ M (**12f**). Small groups like 3-Me (**12a**, IC_{50} = 7.5 nM) and 3-Cl (**12b**, IC_{50} = 33.8 nM) provided the most potent noncompetitive antagonists, while larger groups such as 3- CF_3 (**12c**, IC_{50} = 454 nM) and 4-Ph (**12f**, IC_{50} = 7.8 μ M) saw a diminution in inhibitory activity. Within this library, only a 2,5-dimethyl congener (**12g**) retained partial mGluR5 antagonist activity (IC_{50} = 11.2 μ M, 42% response), but suffered a 23-fold loss in activity. Interestingly, the concept of partial antagonism is not conserved within a chemical series. More surprising was the observation that substituents in the 4-position of the distal phenyl ring of **8** produced mGluR5 positive allosteric modulators (Table 1, **12h–12k**), and the first examples of mGluR5 PAMs based on an MPEP-like scaffold. Even though these analogues possess EC_{50} s in the micromolar range, they afford significant potentiation of mGluR5. As in the case of the noncompetitive antagonists **12a–12f**, small groups in the 4-position provided the most efficacious PAMs. Unlike mGluR5 PAMs reported previously from our lab, **DFB (13)**, **CPPHA (14)** and **CDPPB (15)**, which achieved <70% of maximal glutamate response (Fig. 4), PAMs such as **12h** (EC_{50} = 3.3 μ M) afforded 99% of maximal glutamate response.⁵ In the absence of glutamate, **12h** and related congeners had no effect on receptor response, but in the presence of a sub-threshold concentration of glutamate, a concentration-dependent potentiation of mGluR5 response was observed (Fig. 5). In addition, compound **12h** displayed a robust 4.2-fold leftward shift of the glutamate agonist response curve with an increase in glutamate max (Fig. 6).

The analogues of HTS partial antagonist lead **8** in this small library elucidated a ‘molecular switch’ to modulate pharmacological activity. Lead **8**, with an unsubstituted distal phenyl ring, fully occupied the MPEP binding site, possessed an IC_{50} of 486 nM, but only afforded partial response (29% response, 71% partial antagonism), i.e., an allosteric partial antagonist.

Incorporation of small chemical moieties in the 3-position of the distal phenyl ring, such as a 3-methyl group delivers **12a**, a full non-competitive mGluR5 antagonist (IC_{50} = 7.5 nM). When the methyl group is moved from the 3-position to the 4-position as in **12h**, an efficacious (99% of glutamate max) mGluR5 positive allosteric modulator (EC_{50} = 3.3 μ M, 4.2-fold shift) results. The observation of a conserved ‘molecular switch’, accessed by toggling between 3- and 4- substitution on the distal phenyl ring, within this chemical series is unprecedented and once again, highlights the complexities involved in the optimization and development of allosteric ligands.

In summary, a lead optimization campaign based on mGluR5 allosteric partial antagonist lead, **8**, resulted in relatively ‘flat’ SAR (only 23% of analogues possessed any mGluR5 activity), and subtle structural variants, i.e., ‘molecular switches’, gave rise to a range of mGluR5 pharmacological activities from partial antagonism to full non-competitive antagonism to positive allosteric modulation. Moreover, this study demonstrates that allosteric partial antagonism is neither conserved within a series nor an inherent property of a given chemical scaffold. Further studies in this arena are in progress and will be reported in due course.

Acknowledgement

The authors thank the National Institute of Mental Health the Vanderbilt Department of Pharmacology and the Vanderbilt Institute of Chemical Biology for support of this research.

References and Notes

1. a) Schoepp DD, Jane DE, Monn JA. *Neuropharmacology* 1999;38:1431–1476. [PubMed: 10530808] b) Conn PJ, Pin J-P. *Annu. Rev. Pharmacol. Toxicol* 1997;37:205–237. [PubMed: 9131252]
2. a) Gasparini F, Lingenhohl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I. *Neuropharmacology* 1999;38:1493–1503. [PubMed: 10530811] b) Roppe JR, Wang B, Huang D, Tehrani L, Kamenecka T, Schweiger EJ, Anderson JJ, Brodtkin J, Jiang X, Cramer M, Chung J, Reyes-Manalo G, Munoz B, Cosford NDP. *Bioorg. Med. Chem. Lett* 2004;14:3993–3996. [PubMed: 15225713] c) Lea PM IV, Faden AI. *CNS Drug Reviews* 2006;12(2):149–166. [PubMed: 16958988]
3. Porter RHP, Jaeschke G, Spooren W, Ballard TM, Buttelmann B, Kolczewski S, Peters J-U, Prinseen E, Wichmann J, Vieira E, Muhlemann A, Gatti S, Mutel V, Malherbe P. *J. Pharm. Exp. Ther* 2005;315:711–721.
4. Rodriguez AL, Nong Yi, Sekaran NK, Alagille D, Tamagnan GD, Conn PJ. *Mol. Pharm* 2005;68:793–1802.
5. a) Williams DL Jr, Lindsley CW. *Curr. Topics in Med. Chem* 2005;5:825–846. b) O'Brien JA, Lemaire W, Chen T-B, Chang RSL, Jacobson MA, Ha SN, Lindsley CW, Sur C, Pettibone DJ, Conn J, Williams DL. *Mol. Pharmacol* 2003;64(3):731–740. [PubMed: 12920211] c) O'Brien JA, Lemaire W, Wittmann M, Jacobson MA, Ha SN, Wisnoski DD, Lindsley CW, Schaffhauser HJ, Sur C, Duggan ME, Pettibone DJ, Conn J, Williams DL. *J. Pharmacol. Exp. Therapeut* 2004;309(2):568–577. d) Zhao Z, Wisnoski DD, O'Brien JA, Lemaire W, Williams DL Jr, Jacobson MA, Wittman M, Ha S, Schaffhauser H, Sur C, Pettibone DJ, Duggan ME, Conn PJ, Hartman GD, Lindsley CW. *Bioorg. Med. Chem. Lett* 2007;17:1386–1391. [PubMed: 17210250] e) Lindsley CW, Wisnoski DD, Leister WH, O'Brien JA, Lemaire W, Williams DL Jr, Burno M, Sur C, Kinney GG, Pettibone DJ, Tiller PR, Smith S, Duggan ME, Hartman GD, Conn PJ, Huff JR. *J. Med. Chem* 2004;47:5825–5828. [PubMed: 15537338] f) Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, Wisnoski DD, Lindsley CW, Tiller PR, Smith S, Jacobson MA, Sur C, Duggan ME, Pettibone DJ, Williams DW Jr. *J. Pharmacol. Exp. Therapeut* 2005;313(1):199–206. g) de Paulis T, Hemstapat K, Chen Y, Zhang Y, Saleh S, Alagille D, Baldwin RM, Tamagnan GD, Conn PJ. *J. Med. Chem* 2006;49(11):3332–3344. [PubMed: 16722652]
6. Representative experimental for the synthesis of **8** (5-(phenylethynyl)pyrimidine): To a solution of 5-bromopyrimidine (50 mg, 0.32 mmol) in DMF (4 mL) was added phenylacetylene (32 mg, 0.32 mmol), Pd(PBu₃)₂ (16 mg, 0.03 mmol), CuI (12 mg, 0.06 mmol) and diethylamine (1 mL). The reaction vessel was sealed and heated at 70°C for 10 minutes in a microwave reactor. The reaction was cooled to room temperature, diluted with EtOAc:hexanes (2:1) and washed with water. The crude product was purified on the Agilent 1200 preparative LC/MS. Concentration of purified fractions afforded 5-(phenylethynyl)pyrimidine as an orange solid. ¹H NMR (CDCl₃, 400MHz) δ 9.15 (s, 1H), 8.86 (s, 2H), 7.59–7.54 (m, 2H), 7.42–7.36 (m, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 158.6, 156.7, 131.8, 131.8, 131.7, 129.3, 128.6, 128.5, 121.8, 119.9, 96.3, 82.3; LCMS (214 nm) >99%, single peak, 2.98 min, m/e 181.8 (M+1); HR-MS 181.0760 (calc. 181.0766), C₁₂H₉N₂.
7. **Functional Assay.** HEK293A cells expressing rat mGluR5 receptor were plated (BD Falcon Poly-D-Lysine Cellware) at 50,000 cells/well in assay media (DMEM, 20 mM HEPES, 10% dialyzed FBS, and 1 mM sodium pyruvate). The plates were incubated overnight at 37°C in 5% CO₂. Media was removed and assay buffer (Hanks Balanced Salt Solution, 20 mM HEPES, 2.5 mM Probenecid, pH 7.4) containing 4.0 μM Fluo4-AM dye (Invitrogen) was added. Cells were incubated for 45 minutes (37°C, 5% CO₂) to allow for dye loading. Dye was removed, 20 μL assay buffer was added and the cell plate was allowed to incubate for 10 minutes. After incubation in assay buffer, cell plates were loaded into Flexstation II (Molecular Devices Corp). Test compound in assay buffer was added 19 seconds into the assay and subsequently, a submaximal (150–165 nM) or nearly maximal (3 μM) amount of glutamate was added 109 seconds into the assay for potentiators and antagonists respectively. Controls included compound vehicle (0.3% DMSO) plus assay buffer, EC_{max} (100 μM glutamate), and submaximal or nearly maximal concentrations of glutamate. Compounds were tested

in concentrations ranging from 46 pM to 100 μ M. Fold shifts were determined using the same functional assay by varying the amount of glutamate in the presence of either a fixed concentration of compound (30 μ M) or vehicle. A concentration response curve was generated using glutamate concentrations ranging from 4.6 nM to 10 μ M. Controls included compound vehicle (0.3% DMSO) plus assay buffer, EC_{max} (100 μ M glutamate), and glutamate concentration response curve. Assays were performed in triplicate on three different days. Concentration response curves were generated using GraphPad Prism 4.0. **Radioligand Binding Assay.** The allosteric antagonist MPEP analog [3 H] methoxyPEPy was used to evaluate the interaction of the test compounds with the allosteric MPEP site on mGluR5. Membranes were prepared from HEK293A cells containing rat mGluR5. Compounds were diluted in assay buffer (50 mM TRIS, 0.9% NaCl, pH 7.4) to a 5x stock and 100 μ L of test compound was added to each well of a 96-well assay plate. 300 μ L aliquots of membranes diluted in assay buffer (40 μ g/well) were added to each well. 100 μ L of [3 H] methoxyPEPy (2 nM final concentration in assay buffer) was added, and the reaction was incubated at room temperature for 60 min with shaking. After the incubation period, the membrane-bound ligand was separated from free ligand by filtration through glass fiber 96 well filter plates (Unifilter-96, GF/B). The contents of each well were transferred simultaneously to the filter plate and washed four times with assay buffer (Brandel cell harvester). 40 μ L of scintillation fluid was added to each well, and the membrane-bound radioactivity was determined by scintillation counting (TopCount). Nonspecific binding was estimated using 5 μ M MPEP. Assays were performed in triplicate on three different days. Concentration response curves were generated using GraphPad Prism 4.0.

8. Kennedy JP, Williams L, Bridges TM, Daniels RN, Weaver D, Lindsley CW. J. Comb. Chem. 2008ACS ASAP

Figure 1.

Representative non-competitive mGluR5 antagonists that bind at an allosteric site on the mGluR5 receptor engender mGluR subtype selectivity.

Figure 2. MPEP analogues with partial antagonist activity **4** and **5**, as well as a neutral allosteric site ligand **6**. mGluR5 partial antagonist **4** and **5** completely occupy the MPEP binding site, but induce a maximal blockade of approximately 50% without further blockade at higher concentrations.

Figure 3. MPEP (10 μ M) blocks calcium fluorescence response to glutamate whereas saturating concentrations of representative mGluR5 partial antagonists from the HTS screen only partially inhibit calcium fluorescence response to glutamate.

Figure 4. Structures of mGluR5 PAMs previously reported from our laboratory: **DFB (13)**, **CPPHA (14)** and **CDPPB (15)**.

Figure 5.

Compound **12h** potentiates mGluR5 activation by glutamate. In the absence of glutamate, **12h** does not activate mGluR5. In the presence of a sub-threshold quantity of glutamate, **12h** potentiates mGluR5 in a concentration-dependent manner.

Figure 6.

Compound **12h** potentiation of response to glutamate is manifested as increased mGluR5 agonist sensitivity. The glutamate EC₅₀ value is shifted from 389 nM to 93 nM, or a 4.2-fold shift.

Scheme 1.

Reagents and conditions: (a) **9:10** (1:1), 10 mol% Pd(PPh₃)₄, 20% CuI, 20 equiv. diethylamine, DMF, mw, 70°C, 10 min, >90% yield.

Scheme 2.

Reagents and conditions: (a) **9**:**11** (1:1), 10 mol% $\text{Pd}(t\text{-BuP})_2$, 20% CuI , 20 equiv. diethylamine, DMF, mw, 70°C , 10 min, 70–95% yield.

Table 1

Structures and allosteric activities of analogues **12**. **12**

Cmpd	R	Allosteric Activity	IC ₅₀ /EC ₅₀ (μM) ^a	Antag % ^a	Fold-Shift ^a
8	H	PA	0.48	71	N/A
12a	3-Me	A	0.007	100	N/A
12b	3-Cl	A	0.034	100	N/A
12c	3-CF ₃	A	0.45	100	N/A
12d	3-Me,4-F	A	0.35	100	N/A
12e	3,5-diF	A	6.4	100	N/A
12f	4-Ph	A	7.8	100	N/A
12g	2,5-diMe	PA	11.2	58	N/A
12h	4-Me	PAM	3.3	N/A	4.2
12i	4-Et	PAM	18.8	N/A	3.1
12j	4-Cl	PAM	3.9	N/A	2.3
12k	4-CHO	PAM	7.9	N/A	2.0

^aIC₅₀s, EC₅₀s, Antag % and Fold-Shift are the average of at least 3 determinations. N/A not applicable. PA partial antagonist, A antagonist, PAM positive allosteric modulator.