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Synthesis and SAR of a 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 activities of them GluR5 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 affects commonly 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.

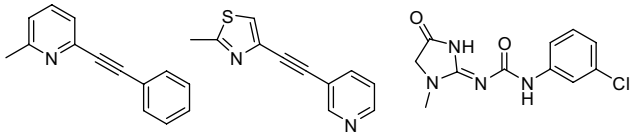

Figure 1. Representative non-competitive mGluR5 antagonists that bind at an allosteric site on the mGluR5 receptor engender mGluR subtype selectivity.

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 [^{3}H]methoxy-PEPy. Compounds that fully displaced the radioligand were then screened in a calcium fluorescence

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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** (EC_{50} = 297 nM) and **8** (EC_{50} = 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** (IC_{50} = 62 nM, 17.9% response, 82.1% partial antagonism), a 5-(phenylethynyl)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/$Pd(PPh_3)_4$ 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 (IC_{50} = 486 nM), but data indicated that **8** was clearly a partial antagonist (29% response, 71% partial antagonism).[7] In radioligand binding assays with [^{3}H]methoxy-PEPy, **8** was found to possess a K_i of 125.9 nM, indicating it binds to the MPEP site.

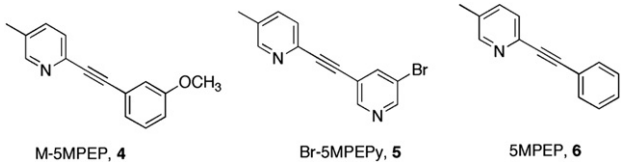


Figure 2. MPEP analogues with partial antagonists activity **4** and **5**, as well as a neutral allosteric site ligand **6**. mGluR5 partial antagonists **4** and **5** completely occupy the MPEP binding site, but induce a maximal blockade of approximately 50% without further blockade at higher concentrations.

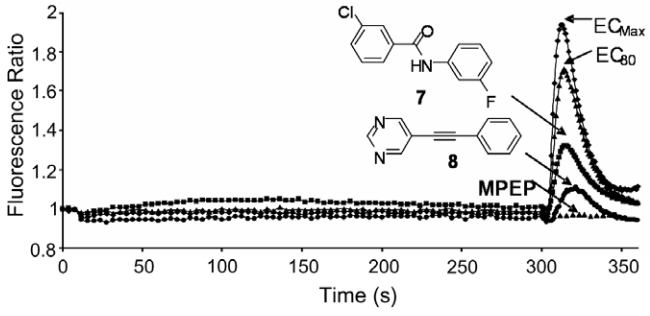
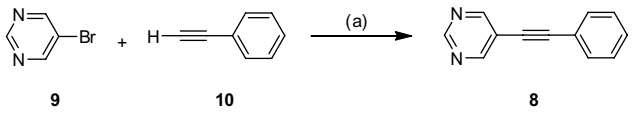


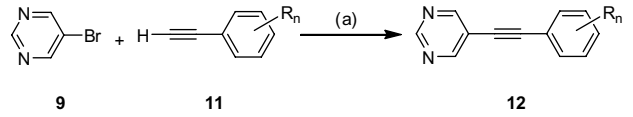
Figure 3. MPEP (10 μM) blocks calcium fluorescence response to glutamate, whereas saturating concentrations of representative mGluR5 partial antagonists from the HTS screen only partially inhibit calcium fluorescence response to glutamate.



Scheme 1. Reagents and conditions: (a) **9**/**10** (1:1), 10 mol% $Pd(PPh_3)_4$, 20% CuI, 20 equiv diethylamine, DMF, mw, 70 °C, 10 min, >90% yield.

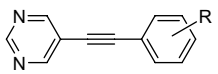
It was unclear from the outset if the degree of mGluR5 partial antagonism would be maintained within this chemical series, or if the degree of partial antagonism would vary over a dynamic range to include complete blockade as seen with MPEP (**1**). Our optimization effort relied on an iterative library synthesis approach in which we employed MAOS to rapidly prepare two, 24-membered analogue libraries.[8] In the event, commercially available 5-bromopyrimidine **9** was subjected to a microwave-assisted Sonogashira coupling protocol (10% mol Pd^0 catalyst, 20 mol% CuI, 20 equiv diethylamine, 1:1 ratio of **9**/**11**, 70 °C, 10 min) with a library of functionalized alkynes **11** to deliver diverse 5-ethynylpyrimidine analogues **12** (Scheme 2). By application of MAOS and the Fu catalyst ($Pd(t\text{BuP})_2$), the palladium precipitated upon rapid cooling, and no Pd^0 scavenging was required prior to mass-directed preparative HPLC purification, unlike in the scale-up of **8** which employed $Pd(PPh_3)_4$. 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



Scheme 2. Reagents and conditions: (a) **9**/**11** (1:1), 10 mol% $Pd(t\text{-BuP})_2$, 20% CuI, 20 equiv diethylamine, DMF, mw, 70 °C, 10 min, 70–95% yield.

Table 1
Structures and allosteric activities of analogues **12**



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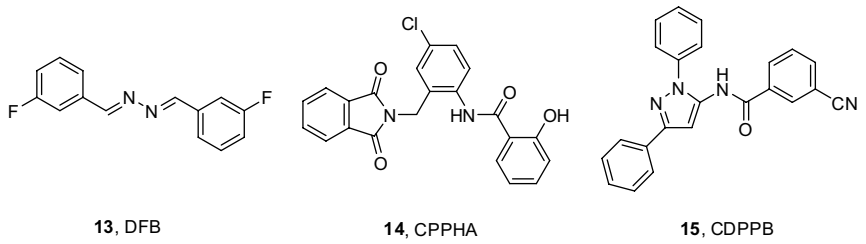
Compound	R	Allosteric activity	IC_{50}/EC_{50}[a] (μM)	Antag%[a]	Fold shift[a]
8	H	PA	0.48	71	N/A
12a	3-Me	A	0.007	100	N/A
12b	3-Cl	A	0.034	100	N/A
12c	3-CF_3	A	0.45	100	N/A
12d	3-Me, 4-F	A	0.35	100	N/A
12e	3,5-diF	A	6.4	100	N/A
12f	4-Ph	A	7.8	100	N/A
12g	2,5-diMe	PA	11.2	58	N/A
12h	4-Me	PAM	3.3	N/A	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

[a] IC_{50}s, EC_{50}s, Antag%, and fold shift are the average of at least three determinations. N/A, not applicable; PA, partial antagonist; A, antagonist; PAM, positive allosteric modulator.



13, DFB **14**, CPPHA **15**, CDPPB

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

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 μM (**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 non-competitive antagonists, while larger groups such as 3-CF_3 (**12c**, IC_{50} = 454 nM) and 4-Ph (**12f**, IC_{50} = 7.8 μM) 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 μM, 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 non-competitive antagonists **12a**–**12f**, small groups in the 4-position provided the most efficacious PAMs. Unlike mGluR5 PAMs reported previously from our laboratory, **DFB** (**13**), **CPPHA** (**14**), and **CDPPB** (**15**), which achieved <70% of maximal glutamate response (Fig. 4), PAMs such as **12h** (EC_{50} = 3.3 μM) 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 left-ward 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), that is, 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 μM, 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, that is, '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.

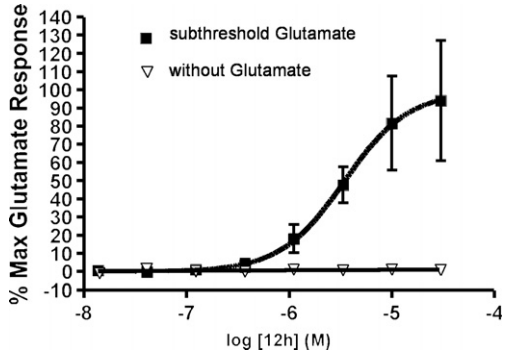


Figure 5. Compound **12h** potentiates mGluR5 activation by glutamate. In the absence of glutamate, **12h** does not activate mGluR5. In the presence of a sub-threshold quantity of glutamate, **12h** potentiates mGluR5 in a concentration-dependent manner.

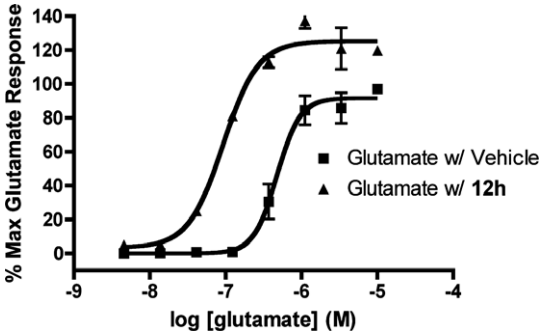


Figure 6. Compound **12h** potentiation of response to glutamate is manifested as increased mGluR5 agonist sensitivity. The glutamate EC_{50} value is shifted from 389 nM to 93 nM, or a 4.2-fold shift.

Acknowledgments

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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) were 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 min 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. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.15 (s, 1H), 8.86 (s, 2H), 7.59-7.54 (m, 2H), 7.42-7.36 (m, 3H); ^{13}C NMR ($CDCl_3$, 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; LC–MS (214 nm) >99%, single peak, 2.98 min, *m*/*e* 181.8 (M+1); HR-MS 181.0760 (calc. 181.0766), $C_{12}H_9N_2$.
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% CO_2. Media were 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 min (37 °C, 5% CO_2) to allow for dye loading. Dye was removed, 20 μL assay buffer was added, and the cell plate was allowed to incubate for 10 min. After incubation in assay buffer, cell plates were loaded into Flexstation II (Molecular Devices Corp.). Test compound in assay buffer was added 19 s into the assay and subsequently, a submaximal (150–165 nM) or nearly maximal (3 μM) amount of glutamate was added 109 s 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 concentration of glutamate. Compounds were tested in concentrations ranging from 46 pM to 100 μM. 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 μM) or vehicle. A concentration response curve was generated using glutamate concentrations ranging from 4.6 nM to 10 μM. Controls included compound vehicle (0.3% DMSO) plus assay buffer, EC_{max} (100 μM 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 analogue [^{3}H]methoxy-PEPy 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 5× stock and 100 μL of test compound was added to each well of a 96-well assay plate. Aliquots of membranes (300 μL) diluted in assay buffer (40 μg/well) were added to each well. [^{3}H]methoxy-PEPy (100 μL) (2 nM final concentration in assay buffer) was added, and the reaction mixture 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). Scintillation fluid (40 μL) was added to each well, and the membrane-bound radioactivity was determined by scintillation counting (TopCount). Non-specific binding was estimated using 5 μM MPEP. Assays were performed in triplicate on three different days. Concentration response curves were generated using GraphPad Prism 4.0.
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