ACS Medicinal Chemistry Letters

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Metabotropic Glutamate Receptor 5 Negative Allosteric Modulators as Novel Tools for in Vivo Investigation

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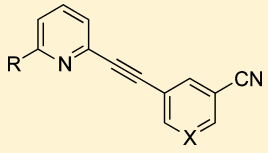
Supporting Information

ABSTRACT: Negative allosteric modulators (NAMs) of metabotropic glutamate receptor subtype 5 (mGluR5) have shown promising results in preclinical models for anxiety and drug abuse. Here, we describe a series of aryl-substituted alkynyl analogues of the prototypic mGluR5 NAM 2-methyl-6-(phenylethynyl)pyridine (MPEP, **1**). Displacement of [^{3}H]**1** binding in rat brain membranes showed that several of these novel compounds displayed high affinity binding (K_i < 10 nM) for mGluR5, with up to a 24-fold increase in affinity over **1**. Replacements of the 2-position Me on the pyridyl ring of **1** along with various 3′-CN, 5′-substitutions were generally well tolerated. All of the active analogues in this series had cLog *P* values in the 2−5 range and displayed inverse agonist characteristics in an ELISA-based assay of $G_q\alpha$-mediated IP3 production. Compounds **7i** and **7j** produced in vivo effects in mouse models of anxiety-like behaviors more potently than **1** or 3-3-((2-methyl-4-thiazolyl)ethynyl)pyridine (MTEP, **2**), supporting their utility as in vivo tools.

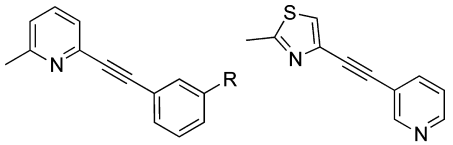


KEYWORDS: *glutamate, negative allosteric modulator, inverse agonist, anxiety, light−dark box*

The excitatory neurotransmitter glutamate regulates neuronal firing via ionotropic and eight metabotropic glutamate receptor (mGluR) subtypes. The metabotropic glutamate receptor subtype 5 (mGluR5) is a G protein-coupled receptor (GPCR) highly expressed in mesocorticolimbic regions of the brain, primarily localized on postsynaptic glutamatergic synapses of the cortex, amygdala, hippocampus, and basal ganglia (including nucleus accumbens, striatum, and olfactory tubercle). Upon receptor activation, mGluR5 modulates neuronal firing through $G_q\alpha$-mediated signaling pathways, including the activation of phospholipase C, enhanced production of D-myo-inositol 1,4,5 trisphosphate (IP3), and increased cytosolic calcium.[1] Attenuation of mGluR5 signaling has shown promising results in preclinical models for conditions as diverse as Parkinson's disease, anxiety, fragile X syndrome, gastresophageal reflux disease, and drug abuse.[2−4] Mice lacking functional mGluR5 show reduced anxiety-like behavioral responses[5] and do not self-administer cocaine.[6]

The orthosteric glutamate binding site on the mGluR5 protein is located within a large bilobed N-terminal domain, a region highly homologous across the mGluRs. Targeting an allosteric binding site located within the transmembrane region provides an opportunity for greater mGluR subtype selectivity, and highly selective agents have been discovered that can either negatively or positively modulate glutamate's actions at mGluR5. The prototypic mGluR5 negative allosteric modulators (NAMs; Figure 1), 2-methyl-6-(phenylethynyl)pyridine (MPEP, **1**, K_i = 16 nM) and 3-((2-methyl-4-thiazolyl)ethynyl)pyridine (MTEP, **2**, K_i = 42 nM), have served as critical research tools, but notable limitations on receptor selectivity and metabolic stability have limited clinical development.[7]

We have previously evaluated structure−activity relationships (SARs) of mGluR5 NAMs with a wide variety of structural motifs, including amides, diaryl amides,[8,9] heterobicyclics,[10] and



1, R=H, MPEP **2, MTEP**
3, R=CN

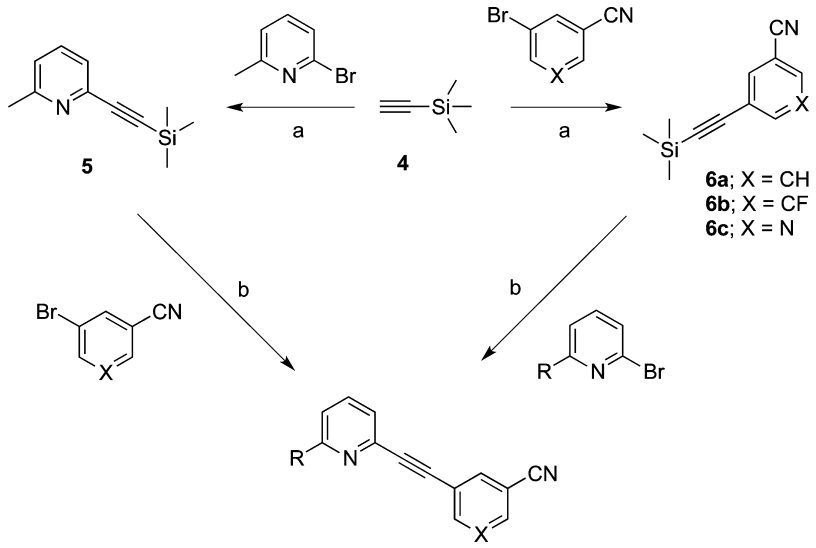
Figure 1. Chemical structures of selective mGluR5 NAMs.

quinolines.[11] In this report, we have extended previous work on aryl-substituted alkynyl analogues to optimize binding affinity at mGluR5 and expand the pharmacological toolbox for in vivo studies. A 3′-CN substitution has been previously reported to enhance binding affinity at mGluR5 (e.g., compound **3**).[11,12] More recently, high affinity at mGluR5 resulting from the 3′-CN, 5′-F substitution on the template of either **1** or **2** has been described.[13−15] In the present series of compounds, we retained the template of compound **3** and explored substitutions in the 2- and or 5′-positions as the combination of modifications in these two positions has yet to be explored.

The aryltrimethylsilylacetylenes **5** and **6a−c** were prepared through the coupling of trimethylsilylacetylene **4** and the respective aryl bromides according to a literature procedure.[12] The diaryl alkyne analogues **7a−q** were synthesized through the coupling of the aryl bromides with aryltrimethylsilylacetylenes **5** or **6a−c** under Sonagashira coupling conditions with good yields (Scheme 1).

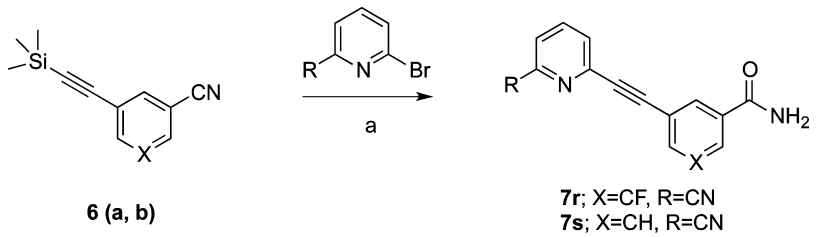
Received: March 29, 2012
Accepted: May 22, 2012
Published: May 22, 2012

Scheme 1. Synthesis of Compounds 7a−q[a]



[a]Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, RT, overnight, 92−99%. (b) $Pd(PPh_3)_4$, CuI, Et_3N, DMF, tetrabutylammonium fluoride (TBAF, 1.0 M in THF), 65−70 °C, 28−98%.

Scheme 2. Synthesis of Compounds 7r and 7s[a]



[a]Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, Et_3N, DMF, TBAF, 85 °C, 20−57%.

Generally, the coupling reaction was not sensitive to higher temperatures. However, if the substituents on the phenyl ring (b ring) were 3′-CN, 5′-F, allowing the reaction temperature to exceed 70 °C resulted in the hydrolysis of the 3′-CN to give the amide as a side product. If the temperature remained >70 °C and the reaction time was long enough, the amide was the only product isolated as shown in Scheme 2. The same result was obtained in the 3′-pyridyl (b ring) series, with the temperature higher than 65 °C.

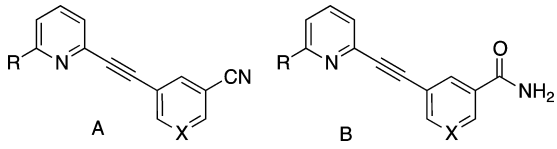
To evaluate the binding affinities of this series of compounds for mGluR5, we developed an assay utilizing [^{3}H]**1** as a competitive radioligand for binding site competition in membranes prepared from rat brains. To assess the functional activity of these novel compounds in vitro, we used a competitive immunoassay that measured the production of IP3, a second messenger of $G_q\alpha$-mediated signaling. The results of these in vitro tests for prototypical ligands, **1** and **2**, and the novel alkynyl analogues are listed in Table 1.

Binding studies showed that several of these compounds had affinities in the low nanomolar range for mGluR5: **3**, **7a**, **7f**, **7h**, **7i**, **7j**, and **7k**; compound **7i** had the highest mGluR5 binding affinity (K_i = 0.67 nM), with an approximately 24-fold improvement over compound **1**. Replacement of the 6-Me group with some substituents (e.g., CN, Et, CH_2OH) was better tolerated than others, but none led to compounds with higher affinity than the parent pharmacophore. The 5′-F substituents could be replaced with an OMe group (**7h**; K_i = 1.3 nM) with good results, but modifying the ring to pyridyl—with the nitrogen in this position—significantly reduced or completely abolished activity. Furthermore, when the 5′-CN was reduced to $CONH_2$, inactive compounds resulted. All of the active analogues (K_i < 150 nM) had cLog *P* values in the 2−5 range.

To evaluate the efficacy of these novel compounds, we utilized an enzyme-linked immunosorbent assay (ELISA)-based immunocompetitive assay in HEK 293A cells stably transfected with rat mGluR5[19] (Karen O'Malley, Washington University School of Medicine, St. Louis, MO). Agonist stimulation of $G_q\alpha$

dx.doi.org/10.1021/ml3000726 | ACS Med. Chem. Lett. 2012, 3, 544−549

Table 1. In Vitro Data for Alkynyl mGluR5 NAMs



	structure				nM	
compd ID	template	R	X	cLog P^a	K_i^b	IC_{50}^c
1, MPEP				3.8	16.3 ± 0.5	31 ± 8
2, MTEP				2.1	42 ± 1	110 ± 50
3[d]	A	CH_3	CH	2.9	1.04 ± 0.03	13 ± 4
7a	A	CN	CH	3.7	8.2 ± 0.5	20 ± 10
7b	A	$HOCH_2$	CH	4.8	37 ± 4	30 ± 20
7c	A	CH_3O	CH	2.9	200 ± 90	300 ± 200
7d	A	$CH_3C(O)$	CH	2.5	114 ± 3	300 ± 100
7e	A	F	CH	3.5	150 ± 20	210 ± 40
7f	A	Et	CH	3.5	3.4 ± 0.3	80 ± 50
7g	A	*n*-Bu	CH	3.0	45 ± 3	700 ± 400
7h	A	CH_3	$C(OCH_3)$	3.4	1.29 ± 0.05	3.4 ± 0.8
7i[e]	A	CH_3	CF	3.2	0.67 ± 0.09	2.4 ± 0.3
7j	A	CN	CF	2.7	2.7 ± 0.2	9 ± 4
7k	A	$HOCH_2$	CF	2.3	7.5 ± 0.3	50 ± 20
7l	A	CH_3O	CF	3.7	40 ± 10	400 ± 100
7m	A	$CH_3C(O)$	CF	3.1	64 ± 2	240 ± 90
7n	A	F	CF	3.1	41 ± 3	80 ± 50
7o	A	CN	N	1.2	52 ± 6	500 ± 200
7p	A	$HOCH_2$	N	0.8	>10000	
7q	A	CH_3O	N	2.2	55 ± 6	200 ± 100
7r	B	CN	CF	1.9	>10000	
7s	B	CN	CH	1.6	>10000	

[a]cLog *P* values were determined using ChemBioDraw. [b]K_i values determined by competitive inhibition of [^{3}H]MPEP binding. [c]IC_{50} values determined by IP-One ELISA. [d]Compound reported previously.[12,16] [e]Compound reported previously.[14,17,18]

GPCRs, such as mGluR5, induces production of the second messenger IP3; the ELISA indirectly measures IP3 production by measuring the accumulation of D-*myo*-inositol 1 phosphate (IP1), a degradation product of IP3. Activation of mGluR5 by the group I mGluR agonist quisqualic acid (QA) dose dependently increased IP1 levels, as shown in Figure 2. All tested compounds from this series showed inverse agonism, dose dependently reversing IP1 production stimulated by 1 μM QA to levels below vehicle treatment baseline. As shown in Figure 2, compounds **1**, **2**, **7i**, and **7j** potently decreased IP1 levels from baseline in the absence of QA. IC_{50} values, calculated from dose–response curves of the compounds in the absence of QA stimulation, were statistically significantly correlated with K_i values determined via radioligand binding (Pearson *r* coefficient = 0.4790, two-tailed *P* value = 0.0326). Compounds **3**, **7h**, **7i**, and **7j** were all more potent in this assay than the parent compounds. In further experiments, dose–response curves of each compound were generated in the presence of 1 μM QA, a dose that produces full agonism. In these experiments, the concentration of each compound that induced a 50% reversal of the agonist response could be calculated. The 50% reversal value was highly correlated to the IC_{50} value of inverse agonism (Pearson *r* coefficient = 0.6503, two-tailed *P* value = 0.0019) and very similar to K_i values determined from radioligand binding (Pearson *r* coefficient = 0.4410, two-tailed *P* value = 0.0408; best-fit linear regression: 50% reversal (nM) = 0.96 × K_i + 59.8).

Compounds **7i** (K_i = 0.67 nM) and **7j** (K_i = 2.8 nM) were evaluated in mouse models of anxiety-like behaviors and compared to compounds **1** and **2**, which exhibit anxiolytic-like activity in a variety of rodent models.[20−24] We utilized two behavioral tests, a novel open-field test and the light–dark box

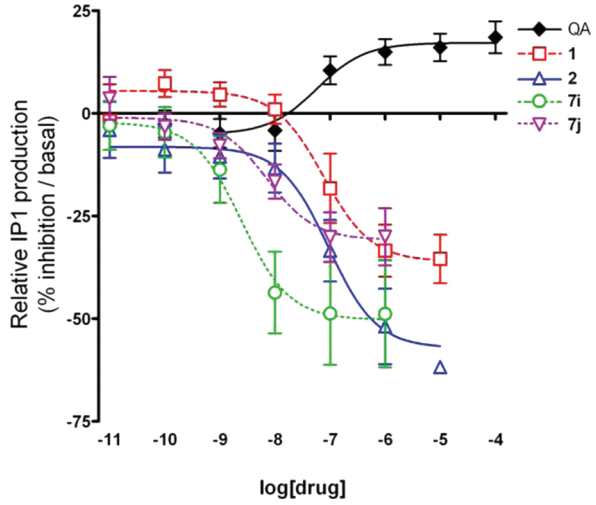


Figure 2. In vitro IP1 accumulation in mGluR5-expressing HEK293 cells is increased by quisqualic acid (QA) and decreased by mGluR5 NAMs. The group I mGluR agonist QA (black ◆) dose dependently enhanced IP3 production, resulting in increased IP1 accumulation. All tested NAMs, including the prototypical mGluR5 NAMs, **1** (red □) and **2** (blue △), and the novel NAMs, **7i** (green ○) and **7j** (purple ▽), showed dose-dependent inverse agonism, reducing IP1 accumulation in the absence of agonist. Values presented are means ± SEMs from at least three experiments.

dx.doi.org/10.1021/ml3000726 | *ACS Med. Chem. Lett.* 2012, 3, 544−549

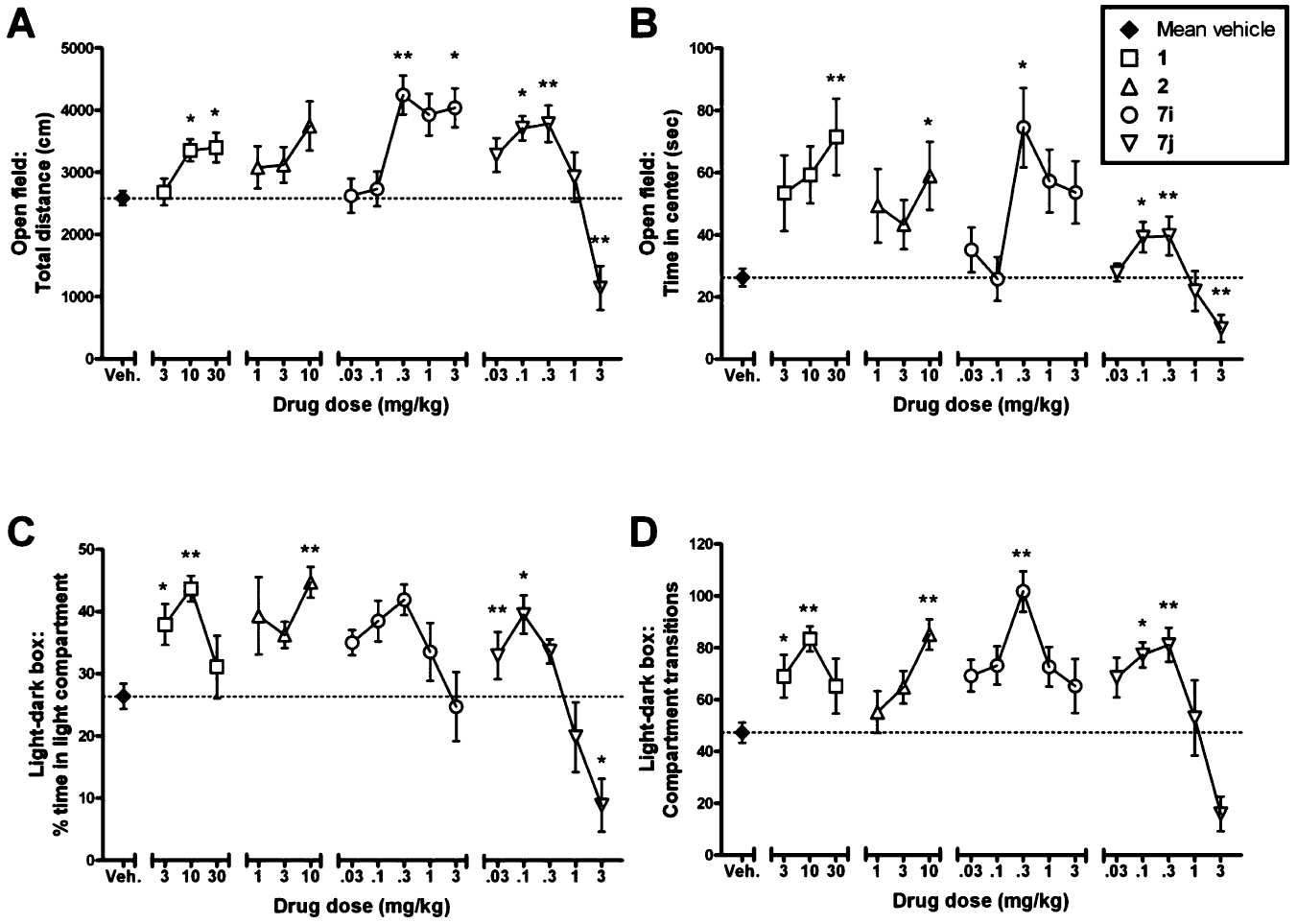


Figure 3. Anxiolytic-like behavioral effects of mGluR5 NAMs in mice, including the prototypical mGluR5 NAMs, **1** (□) and **2** (Δ), and the novel NAMs, **7i** (○) and **7j** (▽). For each test, a combined vehicle-only mean (±SEM) is presented as a baseline (♦), but all statistics were evaluated against the within-group vehicle cohort for each compound. In the novel open field test, mGluR5 NAMs generally increased locomotor activity (A) and also increased the amount of time spent in the center of the open field (B), an indication of anxiolysis. In the light−dark box test, mGluR5 NAMs generally increased proportion of time spent in the light compartment (C) and also increased the number of compartment transitions (D), both indications of anxiolysis. All drugs were suspended in 2% ethanol, 10% Tween 80, and water, except for MTEP, which was dissolved in saline. Drugs were delivered via ip injection 15 min prior to testing. Values presented are means ± SEMs; n = 10−12 for each tested dose. *P < 0.05, **P < 0.01 as compared to vehicle, one-way ANOVA, and Bonferonni's posthoc test.

test, to evaluate the locomotor effects and the potential anxiolytic-like effects of **7i** and **7j**; compounds **1** and **2** served as positive controls in these tests. Anxiolysis in the novel open-field test is indicated by increased time spent in the center region of the open field compared to vehicle: the center region is well established to be the most anxiogenic region of the open field.[25] Anxiolysis in the light−dark box test is indicated by an increased time spent in the light portion of the test chamber and increased transitions between the light and the dark compartments.[26]

Overall, all tested compounds produced behavioral effects consistent with anxiolysis. Compounds **1**, **2**, **7i**, and **7j** each dose dependently increased locomotor activity (Figure 3A) and the amount of time spent in the center of the novel open-field (Figure 3B). In the light−dark box test, **1**, **2**, **7i**, and **7j** each dose dependently increased the proportion of time spent in the light compartment (Figure 3C) and the number of compartment transitions (Figure 3D). Importantly, **7i** and **7j** were considerably more potent in producing anxiolytic-like effects than **1** or **2**. Statistically significant effects consistent with anxiolysis necessitated doses of 3−30 mg/kg for **1** and 10 mg/kg for **2**; in comparison, compound **7i** was active at 0.3 mg/kg, and **7j** was active at 0.1−0.3 mg/kg.

At the highest tested doses for **1**, **7i**, and **7j**, there appeared to be a loss of anxiolytic effects. For **7j**, this is clearly associated with a depression in overall activity at these same doses, but for **1** and **7i**, there is no corresponding loss of locomotor activity. The highest tested doses, however, did result in qualitative changes in behavior that suggested sedative effects. Previously, Anderson et al. determined that **1** at 10 mg/kg produced full mGluR5 receptor occupancy in the mouse brain;[27] doses above this may produce nonspecific effects. In the absence of pharmacokinetic and metabolic analyses and considering that **7i** and **7j** have approximately 24- and 6-fold greater binding affinity than **1** at mGluR5, respectively, the behavioral response to doses of **7i** and **7j** at or above 1 mg/kg may represent nonspecific effects of these compounds.

In summary, although numerous pharmacophores have been mined to optimize mGluR5 binding affinity and in vivo activity, the aryl-substituted alkynyl template of the classic mGluR5 NAMs, compounds **1** and **2**, has yielded some of the most promising leads for therapeutic development.[15] Herein, we extend SAR in this chemical class and identify two high affinity and potent NAMs (**7i** and **7j**) that demonstrate comparable

dx.doi.org/10.1021/ml3000726 | ACS Med. Chem. Lett. 2012, 3, 544−549

anxiolytic activity to the parent compounds but at significantly lower doses. We have recently obtained screening data in 63 additional receptors and ion channels (NIDA-ATDP−DPMCDA-Caliper Life Sciences contract) for **7i**. At a concentration of 100 nM, there was no significant binding (<13% inhibition) at any of these other targets, highlighting the selectivity of this compound (Supporting Information, Table S1). Further evaluation of **7i** in rat models of cocaine self-administration, incubation of cocaine craving, and reinstatement of cocaine-seeking behavior are presently underway.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the synthesis and purification of the compounds and the in vitro and in vivo pharmacological characterizations of the compounds in this manuscript. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This research was funded by the NIDA Intramural Research Program. T.M.K. was supported by an NIH Postdoctoral Intramural Research Training Award (IRTA) Fellowship, and R.P.R. was supported by an NIH Postbaccalaureate IRTA Fellowship.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

mGluR5-transfected HEK 293A cells were generously provided by Dr. Karen O'Malley of Washington University in St. Louis. We acknowledge the NIDA-DPMCDA ATDP contract N01DA-8-8877 for providing off-target screening data. Thanks to Theresa Kopajtic for help in developing the radioligand binding assay and members of Sergi Ferre's laboratory for help with the ELISA assay. Thanks to Jonathan Katz and Gianluigi Tanda for useful discussions regarding data and figure presentation.

■ ABBREVIATIONS

mGluR, metabotropic glutamate receptor; GPCR, G protein-coupled receptor; NAM, negative allosteric modulator; MPEP, 2-methyl-6-(phenylethynyl)pyridine; MTEP, 3-((2-methyl-4-thiazolyl)ethynyl)pyridine; SAR, structure−activity relationships; IP3, D-*myo*-inositol 1,4,5 trisphosphate; IP1, D-*myo*-inositol 1 phosphate; TBAF, tetrabutylammonium formate

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