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Development of 1*H*-Pyrazolo[3,4-*b*]pyridines as Metabotropic Glutamate Receptor 5 Positive Allosteric Modulators

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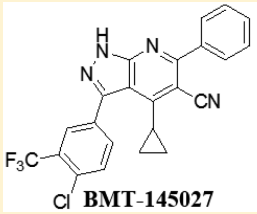
Supporting Information

ABSTRACT: The metabotropic glutamate receptor 5 (mGluR5) is an attractive target for the treatment of schizophrenia due to its role in regulating glutamatergic signaling in association with the *N*-methyl-D-aspartate receptor (NMDAR). We describe the synthesis of 1*H*-pyrazolo[3,4-*b*]pyridines and their utility as mGluR5 positive allosteric modulators (PAMs) without inherent agonist activity. A facile and convergent synthetic route provided access to a structurally diverse set of analogues that contain neither the aryl-acetylene-aryl nor aryl-methyleneoxy-aryl elements, the predominant structural motifs described in the literature. Binding studies suggest that members of our new chemotype do not engage the receptor at the MPEP and CPPHA mGluR5 allosteric sites. SAR studies culminated in the first non-MPEP site PAM, 1*H*-pyrazolo[3,4-*b*]pyridine **31** (BMT-145027), to improve cognition in a preclinical rodent model of learning and memory.



BMT-145027

non-MPEP site mGluR5 PAM
mGluR5 PAM EC_{50} = 47 ± 4 nM
fold shift = 3.4 ± 1.4
efficacious in mouse NOR

KEYWORDS: *mGluR5, positive allosteric modulator, schizophrenia, novel object recognition, glutamate*

Schizophrenia is a debilitating psychiatric disorder and afflicts approximately 1% of the general population. Schizophrenia-related health care costs are measured in the tens of billions of dollars in the US market alone. More importantly, the chronic nature of schizophrenia leads to a 2−3-fold increased rate of all-cause mortality, which translates into a 10 year decrease in life expectancy for those suffering from this disease.[1] Symptoms of schizophrenia are characterized as positive, negative, or cognitive in nature.[2] Currently available antipsychotics can successfully treat positive symptoms; however, negative and cognitive symptoms remain areas of significant unmet medical need. Treatment is further complicated by poor patient compliance due to extrapyramidal and metabolic side effects associated with existing medications.

Metabotropic glutamate receptor 5 (mGluR5) is a member of the mGluR family of G-protein-coupled receptors that contains a large extracellular glutamate-binding domain.[3,4] When mGluR5 binds to endogenous glutamate, enhanced *N*-methyl-D-aspartate receptor (NMDAR) function occurs with neuronal signal transduction.[5] Antagonism of NMDAR with ketamine or phencyclidine recapitulates all three symptomatic domains associated with schizophrenia and suggests NMDAR hypofunction may contribute to the disease.[6] Direct activation of NMDAR generates excitotoxic increases in levels of neuronal calcium and subsequent neuronal death. Allosteric mGluR5 activation could provide a potentially advantageous approach to modulation of mGluR5 because NMDAR function is only enhanced in the presence of an mGluR5 positive allosteric modulator (PAM) and an orthosteric agonist.[7,8] Such activation should maintain both spatial and temporal components of glutamate signaling.

Most reported mGluR5 allosteric modulators contain an aryl-acetylene-aryl element, exemplified by **1** and MPEP, the negative allosteric modulator (NAM) for which the first and most-studied mGluR5 allosteric binding site is named,[9,10] or the aryl-methyleneoxy-aryl motif found in the positive allosteric modulator VU0409551 (Figure 1).[12] Our initial development candidate, oxazolidinone BMS-955829, contained the former motif and provided a low fold shift (2.3) without agonism.[14] This compound progressed into a one month pre-IND toxicology study, during which it demonstrated drug-induced liver injury at all doses studied (3−30 mg/kg QD). While biotransformation studies failed to demonstrate involvement of the acetylene moiety, this functionality has been repeatedly linked with CYP-mediated bioactivation *in vivo*.[15] In an effort to discover a new, structurally divergent chemotype, we performed a functional high-throughput screen (HTS) of more than 1.1 million compounds in the presence of an EC_{10} of glutamate. This effort identified 1*H*-pyrazolo[3,4-*b*]pyridine **2** (Figure 1), which represented a novel mGluR5 chemotype with promising submicromolar PAM activity. Importantly, this compound was a pure PAM, devoid of inherent mGluR5 agonist activity. We

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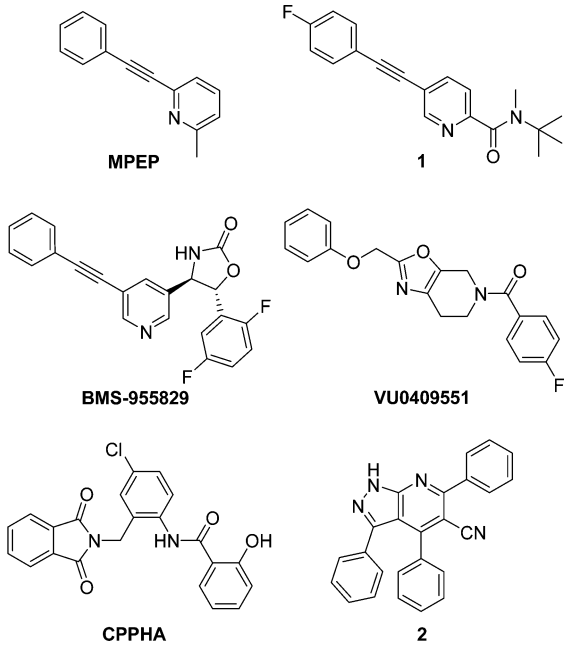
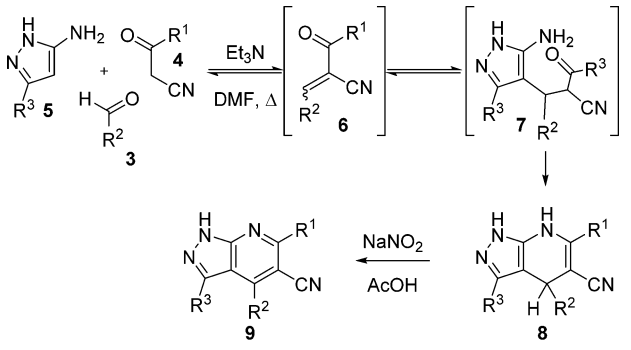


Figure 1. Examples of mGluR5 allosteric modulators.[9−14]

quickly generated functional SAR aimed at the identification of a suitable analogue to progress into the novel object recognition (NOR) cognition assay. Herein, we report these data in addition to studies designed to elucidate the allosteric site to which this chemotype binds.

1*H*-Pyrazolo[3,4-*b*]pyridines were prepared using several methods, but the most practical procedure proved to be a two-step, one-pot dehydrative cyclization process developed for this program (Scheme 1).[16,17] In a typical reaction, components **3**, **4**, and **5** were heated together with triethylamine in DMF to afford 4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine **8**. Presumably, this sequence progressed first through a Knoevenagel condensation between aldehyde **3** and 3-oxopropanenitrile **4** to form an acrylonitrile intermediate **6**. Subsequent Michael addition of 1*H*-pyrazol-5-amine **5** to the acrylonitrile followed by dehydrative cyclization completed the molecular framework. Removal of volatiles *in situ*, followed by oxidation with sodium nitrite in acetic acid provided final compound **9**. Notably, this procedure was used to synthesize more than one gram of our HTS hit **2** in 80% yield.

Scheme 1. Two-Step, One-Pot Synthetic Route to 1*H*-Pyrazolo[3,4-*b*]pyridines 9



Our primary program goals included increasing the potency and mouse (Ms) liver microsomal (LM) stability relative to HTS hit **2** in order to facilitate *in vivo* efficacy studies. In parallel with this effort, we sought opportunities to reduce planarity (measured as fraction of sp^3-hybridized carbons, Fsp^3) of the series using alkyl replacements for aryl substituents (R^1, R^2, and R^3; Scheme 1).[18] We targeted compounds with mGluR5 EC_{50}s under 50 nM (Table 1) as measured in a Ca^{2+}-based FLIPR (Fluorescence Image Plate Reader) assay.[17] HTS hit **2** demonstrated a PAM EC_{50} of 120 nM and moderate human (H) LM and poor MsLM stability (75% and 17% remaining, respectively). *N*-Methylation (**10**) led to a 19-fold loss in potency, which suggested the free pyrazole −NH− might participate in a hydrogen-bond interaction with the allosteric pocket. In an effort to define the minimum pharmacophore, core nitrogens were deleted. Removal of N2 gave 7-azaindole **11**, which was about twice as potent as HTS hit **2** but with reduced LM stability. Elimination of N7 (indazole **12**) led to a 10-fold loss in potency. This suggested that N7 might engage the binding pocket through a hydrogen bond. Omitting the C5 nitrile (**13**) or replacing it with substituents such as fluorine (**14**) offered no advantage.

Changes to R^1 in **9** demonstrated very dramatic mGluR5 PAM SAR. Aryl elaboration (e.g., 4-chlorophenyl **15**) reduced potency while alkyl substitution (e.g., ^{c}Pr **17**) eliminated PAM activity all together, but LM data suggested this group could be used to affect MsLM stability (Table 1). Simple R^2 alkyl groups (e.g., analogues **18** and **19**) provided a potency advantage over aryl substituents, a finding that was critical to our goal of reducing planarity (**18** and **19**, Fsp^3 = 0.14); however, this came at the cost of LM stability. Importantly, we found that R^3 aryl modifications could be used to modulate oxidative stability and offered potential to offset the loss associated with R^2 alkyl moieties. Specifically, 4-chloro- (**20**) and 3-chloro-analogues (**21**) provided a significant MsLM stability advantage compared to unsubstituted phenyl analogue **2**. Moreover, these examples demonstrated the effect of substitution pattern on potency. R^3 alkyl groups were also tolerated (examples **22**−**25**), although potency and Fsp^3 appeared inversely related.

Combined, these SAR lessons led to compounds **26**−**32** with potencies within our desired range; however, the identification of very potent analogues with high levels of LM stability was still challenging. Metabolite identification studies conducted on **29** revealed extensive oxidation on R^1 and R^3 phenyl groups (data not shown), consistent with the finding of increased LM stability upon substitution of these moieties.[17] Various aryl substitutions (R^3) were used to tune LM stability. This exercise afforded 1*H*-pyrazolo[3,4-*b*]pyridine **31** (BMT-145027), a compound with high MsLM stability (85% remaining), acceptable potency (EC_{50} = 47 nM), and a modest decrease in planarity (Fsp^3 = 0.17). Importantly, **31** lacked inherent mGluR5 agonist activity when tested at concentrations up to 16 μM.[17,19]

PAMs function by increasing the affinity and/or functional efficacy of an endogenous ligand (e.g., glutamate) for the orthosteric binding site, a process that affords a leftward shift of the ligand concentration−response curve. Most reported highly potent mGluR5 PAMs provide concentration-dependent fold shift values ≥7;[20] however, in the context of the oxazolidinone chemotype (e.g., BMS-955829), we found that compounds with low glutamate fold shift were devoid of mechanism-based convulsions,[14] a phenomenon we observed with higher fold shift PAMs.[21,22] Ideally, we wanted a 1*H*-pyrazolo[3,4-*b*]pyridine with a low glutamate fold shift, a goal that was

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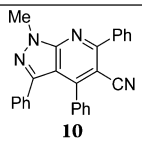
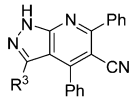
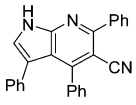
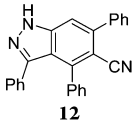
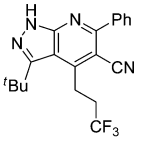
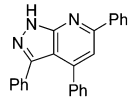
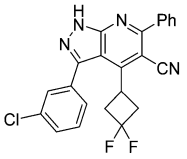
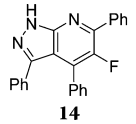
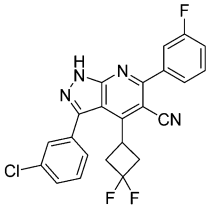
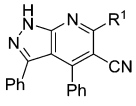
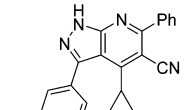
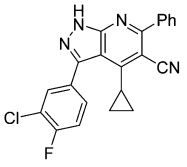
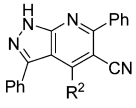
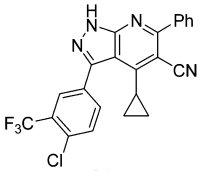
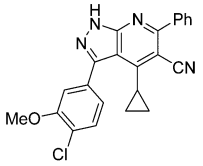
Table 1. mGluR5 PAM Potency, Fold Shift, Metabolic Stability, and Fsp^3 Data for Selected 1*H*-Pyrazolo[3,4-*b*]pyridines

Compound	PAM EC_{50} ± SEM (nM)[a]	Fold Shift	MetStab (H/Ms)[b]	Fsp^3	Compound	PAM EC_{50} ± SEM (nM)[a]	Fold Shift	MetStab (H/Ms)[b]	Fsp^3
 **10**	2,360 ± 70	n/a	73 / 47	0.04					
 **11**	49 ± 18	n/a	54 / 40	0	**20**, R^3 = 4-ClPh **21**, R^3 = 3-ClPh **22**, R^3 = ^{t}Bu **23**, R^3 = ^{i}Pr **24**, R^3 = ^{c}Pr **25**, R^3 = Me	190 ± 30 69 ± 24 83 ± 4 240 ± 40 220 ± 90 530 ± 100	5.9 9.7 4.3 6.0 3.0 n/a	84 / 83 85 / 63 37 / 1 39 / 5 43 / 12 26 / 4	0 0 0.17 0.14 0.14 0.05
 **12**	1,300 ± 500	3.6	38 / 1	0	 **26**	51 ± 12	4.5[a]	62 / 36	0.35
 **13**	330 ± 100	7.1	n/a / 7	0	 **27**	31 ± 15	9.1	59 / 38	0.17
 **14**	140 ± 60	11[a]	93 / 2	0	 **28**	71 ± 11	n/a	54 / 73	0.17
					 **29**	30 ± 5	3.3[a]	40 / 34	0.14
15, R^1 = 4-ClPh **16**, R^1 = 3-ClPh **17**, R^1 = ^{c}Pr	1400 ± 100 150 ± 40 >3000	2.8 3.8 n/a	91 / 70 76 / 27 70 / 46	0 0 0.14	 **30**	21 ± 1	4.4	45 / 11	0.14
					 **31**	47 ± 4	3.4[a]	73 / 85	0.17
18, R^2 = ^{c}Pr **19**, R^2 = ^{n}Pr	45 ± 21 53 ± 6	3.2 8.2	19 / 12 35 / 4	0.14 0.14	 **32**	110 ± 30	3.9	57 / 1	0.17

[a]Values represent mean of $n \geq 2$ replicates. [b]Percentage remaining after 10 min incubation with human or mouse liver microsomes.[17]

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achieved when R^2 = ^{c}Pr (e.g., analogue **31** fold shift = 3.4, Table 1).

1*H*-Pyrazolo[3,4-*b*]pyridine **31** was chosen to advance into a mouse NOR behavioral study.[17] This model measures visual recognition memory, which is an impaired cognitive domain in schizophrenia patients.[23,24] In this model, drug-treated and control mice are shown two identical objects. After a 24-h natural forgetting period, the mice were reintroduced to a familiar object while simultaneously presented with a novel object. Since mice spend more time exploring unfamiliar objects, improved memory was measured as time spent exploring the novel object. 1*H*-Pyrazolo[3,4-*b*]pyridine **31** led to a significant ($p < 0.05$) increase in time spent with the novel object when dosed at 30 mg/kg, with an apparent trend in novel object preference at 10 mg/kg. Satellite animals indicated a total plasma concentration = 2800 nM at 30 mg/kg (B/P = 0.5, Figure 2).[25]

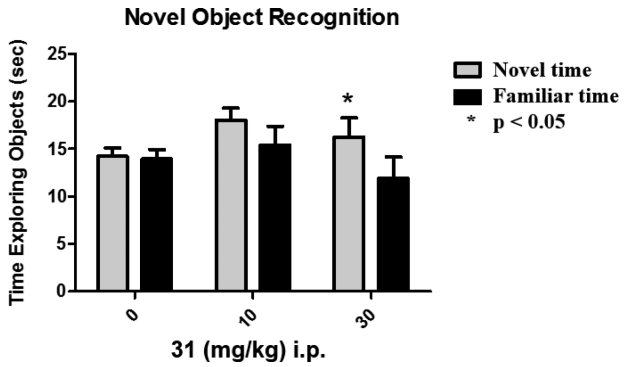

Figure 2. Pretreatment with 30 mg/kg 1*H*-pyrazolo[3,4-*b*]pyridine **31** 60 min prior to training significantly increased the time spent exploring the novel object compared to the familiar object when tested 24 h later.

We evaluated representative analogues in a competition-binding assay using a known mGluR5 ligand, ^{3}H-MPEPy, which binds to the MPEP allosteric modulator site on the receptor.[9] Data presented in Table 2 show that compounds

Table 2. Competition Binding Studies That Demonstrated Partial MPEPy Displacement

	HEK-mGluR5[a]		primary astrocytes[a]	
compd	IC_{50} ± SEM (nM)	Y_{max} ± SEM (%)	IC_{50} ± SEM (nM)	Y_{max} ± SEM (%)
BMS-955829	3.0 ± 0.6	99 ± 0.3	2.0 ± 0.1	100 ± 2
CPPHA	>10,000[b]	<20	930 ± 40	63 ± 9
28	87[b]	44	19 ± 2	63 ± 4
29	66 ± 26	45 ± 5	27 ± 6	52 ± 3
32	77[b]	60	240 ± 110	65 ± 2

[a]Values represent mean of $n \geq 2$ replicates. [b]Single run.

from the 1*H*-pyrazolo[3,4-*b*]pyridine series (**9**) displaced MPEPy with nanomolar potency in membranes prepared from both HEK cells expressing mGluR5 and primary rat astrocytes, indicating that the overexpressing system mimicked endogenously expressed mGlu5 receptor binding.[17,26] Interestingly, the displacement of MPEPy was partial and only reached about 50% for all 1*H*-pyrazolo[3,4-*b*]pyridines tested. This observation suggested that this PAM chemotype did not bind at the MPEP binding site and instead disrupted MPEPy binding through an allosteric interaction with a non-MPEP binding site. These results were consistent with those described for several non-MPEP site PAMs including CPPHA.[27] To confirm this, we performed a dissociation binding assay, which indicated that both **21** (K_{off} = 114% of control) and **28** (K_{off} = 116% of control) increased the dissociation rate of ^{3}H-MPEPy relative to the known MPEP site ligand ($p < 0.05$), BMS-955829 (K_{off} = 100% of control), using HEK cell membrane expressing mGluR5.[17] The altered dissociation rates of **21** and **28** unequivocally demonstrated binding to a non-MPEP site[28] Additionally, compound **21** was inactive up to 10 μM in PAM mode against group I receptor, mGluR1, and group II receptors up to 30 μM.[29]

To further understand how this chemotype (**9**) binds to the mGlu5 receptor, we screened compounds in a functional assay that measured potentiated glutamate-induced intracellular calcium increases using HEK cells that expressed mGluR5 with mutations in the MPEP and CPPHA[30] allosteric binding sites. Binding results demonstrated that mutation of either site failed to impact PAM activity of our 1*H*-pyrazolo[3,4-*b*]pyridines (**16**, **20**, and **24**) relative to wild type (Table 3),[17] suggesting that the 1*H*-pyrazolo[3,4-*b*]pyridine chemotype (**9**) did not bind at either the MPEP or CPPHA sites. As expected, BMS-955829 and MPEP are inactive in the mutant MPEP site cells, whereas both compounds retain efficacy in the CPPHA mutant cells. Conversely, CPPHA retains efficacy in the mutated MPEP site cells, but not the mutated CPPHA site cells (Table 3).

Table 3. Impact of Point Mutations on FLIPR Activity

	mutant/wild type EC_{50} ratio	
compd	MPEP mutant	CPPHA mutant
BMS-955829	**>10**	1.1
MPEP	**>10**	0.5
CPPHA	1.0	**7.2**
16	1.4	n.d.
20	0.66	1.9
24	0.73	1.4

In summary, a structurally distinct and potent 1*H*-pyrazolo-[3,4-*b*]pyridine-based series (**9**) of mGluR5 PAMs was developed for the potential treatment of schizophrenia. Analogues were prepared using a convergent synthesis that provided significant structural diversity and permitted rapid SAR investigations, which focused on allosteric potency and microsomal stability. 1*H*-Pyrazolo[3,4-*b*]pyridine **31** emerged as a low fold-shift and potent mGluR5 PAM with no inherent mGluR5 agonist activity. This compound advanced into the NOR behavioral model in which it demonstrated an improvement in memory retention. Data from parallel binding studies suggests that members of this chemotype do not engage the receptor at the MPEP and CPPHA allosteric sites. To the best of our knowledge, **31** is the first example of a non-MPEP site PAM to demonstrate *in vivo* efficacy.[31] Ultimately, our company's strategic shift away from neuroscience research prevented further advancement of this promising series.

■ ASSOCIATED CONTENT

Supporting Information

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Experimental procedures and characterization data for key compounds, and details of *in vitro* and *in vivo* assays (PDF)

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Saha, S.; McGrath, J. A systematic review of mortality in schizophrenia: is the differential mortality gap worsening over time? *Arch. Gen. Psychiatry* **2007**, *64*, 1123−1131.

(2) Van Os, J.; Kapur, S. Schizophrenia. *Lancet* **2009**, *374*, 635−645.

(3) Conn, P. J.; Pin, J.-P. Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205−237.

(4) Vanejevs, M.; Jatzke, C.; Renner, S.; Müller, S.; Hechenberger, M.; Bauer, T.; Klochkova, A.; Pyatkin, I.; Kazyulkin, D.; Aksenova, E.; Shulepin, S.; Timonina, O.; Haasis, A.; Gutcaits, A.; Parsons, C. G.; Kauss, V.; Weill, T. Positive and Negative Modulation of Group I Metabotropic Glutamate Receptors. *J. Med. Chem.* **2008**, *51*, 634−647.

(5) Anikszteyn, L.; Bregestovski, P.; Ben-Ari, Y. Selective activation of quisqualate metabotropic receptor potentiates NMDA but not AMPA responses. *Eur. J. Pharmacol.* **1991**, *205*, 327−328.

(6) Coyle, J. T. NMDA Receptor and Schizophrenia: A Brief History. *Schizophrenia Bull.* **2012**, *38*, 920−926.

(7) Newell, K. A. Metabotropic glutamate receptor 5 in schizophrenia: emerging evidence for the development of anti-psychotic drugs. *Future Med. Chem.* **2013**, *5*, 1471−1474.

(8) Matosin, N.; Newell, K. A. Metabotropic glutamate receptor 5 in the pathology and treatment of schizophrenia. *Neurosci. Biobehav. Rev.* **2013**, *37*, 256−268.

(9) Gasparini, F.; Lingenhohl, K.; Stoehr, N.; Flor, P. J.; Heirich, M.; Vranesic, I.; Biollaz, M.; Allgeier, H.; Heckendorn, R.; Uwyler, S.; Varney, M. A.; Johnson, E. C.; Hess, S. D.; Rao, S. P.; Sacaan, A. I.; Santori, E. M.; Velicelebi, G.; Kuhn, R. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology* **1999**, *38*, 1493−1503.

(10) Malherbe, P.; Kratochwil, N.; Zenner, M. T.; Piussi, J.; Diener, C.; Kratzeisen, C.; Fischer, C.; Porter, R. H. Mutational Analysis and Molecular Modeling of the Binding Pocket of the Metabotropic Glutamate 5 Receptor Negative Modulator 2-Methyl-6-(phenyl-ethynyl)-pyridine. *Mol. Pharmacol.* **2003**, *64*, 823−832.

(11) Compound **1**: Jaeschke, G.; Jolidon, S.; Lindemann, L.; Ricci, A.; Rueher, D.; Stadler, H.; Vieira, E. (Hoffmann-La Roche Inc., USA). Preparation of phenyl or pyridinyl-ethynyl derivatives. US Patent 8,772,300. July 8, 2014.

(12) VU0409551: Conde-Ceide, S.; Martínez-Viturro, C. M.; Alcázar, J.; Garcia-Barrantes, P. M.; Lavreysen, H.; Mackie, C.; Vinson, P. N.; Rook, J. M.; Bridges, T. M.; Daniels, J. S.; Megens, A.; Langlois, X.; Drinkenburg, W. H.; Ahnaou, A.; Niswender, A. M.; Jones, C. K.; Macdonald, G. J.; Steckler, T.; Conn, P. J.; Stauffer, S. R.; Bartolomé-Nebreda, J. M.; Lindsley, C. W. Discovery of VU0409551/JNJ-46778212: An mGlu5 Positive Allosteric Modulator Clinical Candidate Targeting Schizophrenia. *ACS Med. Chem. Lett.* **2015**, *6*, 716−720.

(13) CPPHA: O'Brien, J. A.; Lemaire, W.; Wittmann, M.; Jacobson, M. A.; Ha, S. N.; Wisnoski, D. D.; Lindsley, C. W.; Schaffhauser, H. J.; Sur, C.; Duggan, M. E.; Pettibone, D. J.; Conn, J.; Williams, D. L. A novel selective allosteric modulator potentiates the activity of the native metabotropic glutamate receptor subtype 5 (mGluR5) in rat forebrain. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 568−577.

(14) Yang, F.; Snyder, L. B.; Balakrishnan, A.; Brown, J. M.; Sivarao, D. V.; Easton, A.; Fernandes, A.; Gulianello, M.; Hanumegowda, U. M.; Huang, H.; Huang, Y.; Jones, K. M.; Li, Y.-W.; Matchett, M.; Mattson, G.; Miller, R.; Santone, K. S.; Senapati, A.; Shields, E. E.; Simutis, F. J.; Westphal, R.; Whiterock, V. J.; Bronson, J. J.; Macor, J. E.; Degnan, A. P. Discovery and Preclinical Evaluation of BMS-955829, A Potent Positive Allosteric Modulator of mGluR5. *ACS Med. Chem. Lett.* **2016**, *7*, 289−293.

(15) Smith, G. F. Designing drugs to avoid toxicity. *Prog. Med. Chem.* **2011**, *50*, 1−47.

(16) Hill, M. D. A Multicomponent Approach to Highly Substituted 1*H*-Pyrazolo[3,4-*b*]pyridines. *Synthesis* **2016**, *48*, 2201−2204.

(17) For experimental details, please see Supporting Information.

(18) Lovering, F.; Bikker, J.; Humblet, C. Escape from Flatland: Increasing Saturation as an Approach to Improving Clinical Success. *J. Med. Chem.* **2009**, *52*, 6752−6756.

(19) This chemotype lacked inherent mGluR5 agonist activity up to 16 μM.

(20) Zhao, Z.; Wisnoski, D. D.; O'Brien, J. A.; Lemaire, W.; Williams, D. L., Jr.; Jacobson, M. A.; Wittman, M.; Ha, S. N.; Schaffhauser, H.; Sur, C.; Pettibone, D. J.; Duggan, M. E.; Conn, P. J.; Hartman, G. D.; Lindsley, C. W. Challenges in the development of mGluR5 positive allosteric modulators: The discovery of CPPHA. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1386−1391.

(21) Rook, J. M.; Noetzel, M. J.; Pouliot, W. A.; Bridges, T. M.; Vinson, P. N.; Cho, H. P.; Zhou, Y.; Gogliotti, R. D.; Manka, J. T.; Gregory, K. J.; Stauffer, S. R.; Dudek, F. E.; Xiang, Z.; Niswender, C. M.; Daniels, J. S.; Jones, C. K.; Lindsley, C. W.; Conn, P. J. Unique signaling profiles of positive allosteric modulators of metabotropic glutamate receptor subtype 5 determine differences in *in vivo* activity. *Biol. Psychiatry* **2013**, *73*, 501−509.

(22) Parmentier-Batteur, S.; Hutson, P. H.; Menzel, K.; Uslaner, J. M.; Mattson, B. A.; O'Brien, J. A.; Magliaro, B. C.; Forest, T.; Stump, C. A.; Tynebor, R. M.; Anthony, N. J.; Tucker, T. J.; Zhang, X.-F.; Gomez, R.; Huszar, S. L.; Lambeng, N.; Fauré, H.; Le Poul, E.; Poli, S.; Rosahl, T. W.; Rocher, J.-P.; Hargreaves, R.; Williams, T. M. Mechanism based neurotoxicity of mGlu5 positive allosteric modulators − Development challenges for a promising novel antipsychotic target. *Neuropharmacology* **2014**, *82*, 161−173.

(23) McGuire, K. A.; Blahnik, M. M.; Sponheim, S. R. Discrimination within Recognition Memory in Schizophrenia. *Behav. Sci.* **2013**, *3*, 273−297.

(24) Mathiasen, J. R.; DiCamillo, A. Novel Object Recognition in the Rat: A Facile Assay for Cognitive Function. *Curr. Protoc. Pharmacol.* **2010**, *5*, 1−15.

(25) No adverse events were observed at either dose.

(26) Binding studies were performed prior to the NOR study with **31**, so its significance to the program was as of yet unknown.

(27) Gregory, K. J.; Conn, P. J. Molecular insights into metabotropic glutamate receptor allosteric modulation. *Mol. Pharmacol.* **2015**, *88*, 188−202.

(28) Kostenis, E.; Mohr, K. Two-point kinetic experiments to quantify allosteric effects on radioligand dissociation. *Trends Pharmacol. Sci.* **1996**, *8*, 280−283.

(29) All compounds tested lacked mGluR1,2,3 PAM activity. The series was not screened against other mGlu receptors.

(30) Noetzel, M. J.; Gregory, K. J.; Vinson, P. N.; Manka, J. T.; Stauffer, S. R.; Lindsley, C. W.; Niswender, C. M.; Xiang, Z.; Conn, P. J. A Novel Metabotropic Glutamate Receptor 5 Positive Allosteric Modulator Acts at a Unique Site and Confers Stimulas Bias to mGlu5 Signaling. *Mol. Pharmacol.* **2013**, *83*, 835−847.

(31) Rodriguez, A. L.; Zhou, Y.; Williams, R.; Weaver, C. D.; Vinson, P. N.; Dawson, E. S.; Steckler, T.; Lavreysen, H.; Mackieg, C.; Bartolomé, J. M.; Macdonald, G. J.; Daniels, J. S.; Niswender, C. M.; Jones, C. K.; Conn, P. J.; Lindsley, C. W.; Stauffer, S. R. Discovery and SAR of a novel series of non-MPEP site mGlu5 PAMs based on an aryl glycine sulfonamide scaffold. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7388−7392.

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