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Discovery of a Selective and CNS Penetrant Negative Allosteric Modulator of Metabotropic Glutamate Receptor Subtype 3 with Antidepressant and Anxiolytic Activity in Rodents

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Supporting Information

ABSTRACT: Previous preclinical work has demonstrated the therapeutic potential of antagonists of the group II metabotropic glutamate receptors (mGlus). Still, compounds that are selective for the individual group II mGlus (mGlu₂ and mGlu₃) have been scarce. There remains a need for such compounds with the balance of properties suitable for convenient use in a wide array of rodent behavioral studies. We describe here the discovery of a selective mGlu₃ NAM **106** (VU0650786) suitable for in vivo work. Compound **106** is a



member of a series of 5-aryl-6,7-dihydropyrazolo[1,5-*a*]pyrazine-4(5*H*)-one compounds originally identified as a $mGlu_5$ positive allosteric modulator (PAM) chemotype. Its suitability for use in rodent behavioral models has been established by extensive in vivo PK studies, and the behavioral experiments presented here with compound **106** represent the first examples in which an $mGlu_3$ NAM has demonstrated efficacy in models where prior efficacy had previously been noted with nonselective group II antagonists.

INTRODUCTION

Glutamate (L-glutamic acid) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS) and acts on both ionotropic and metabotropic glutamate receptors (mGlus). While ionotropic glutamate receptors are ligand-gated ion channels, mGlus are a family of eight Gprotein coupled receptors (GPCRs). Belonging to family C of the GPCRs, the mGlus possess a seven transmembrane (7TM) α -helical domain connected via a cysteine-rich region to a large bilobed extracellular amino-terminal domain containing the orthosteric binding site. The mGlus have been further categorized into three groups according to their homology, preferred signal transduction mechanisms, and pharmacology: group I (mGlu₁ and mGlu₅), group II (mGlu₂ and mGlu₃), and group III (mGlu₄, mGlu₆, mGlu₇, and mGlu₈).¹⁻³ Both mGlu₂ and mGlu₃ are primarily located presynaptically in neurons and coupled to $G_{i/o}$ and the inhibition of adenylyl cyclase activity; in addition, mGlu3 is also expressed in glial cells. The group II mGlus are widely expressed throughout the CNS, including regions of the brain associated with emotional states such as the amygdala, hippocampus, and prefrontal cortex.^{4,5}

Researchers have been successful in designing both orthosteric antagonists and negative allosteric modulators

(NAMs), also known as noncompetitive antagonists, of the group II mGlus. Although such compounds are generally selective versus the other six members of the mGlu family, selectivity between mGlu₂ and mGlu₃ is negligible.⁶ Still, the use of such compounds in animal models has established a potential role for mGlu_{2/3} antagonists in a variety of CNS disorders. Many of these studies have been carried out with two orthosteric antagonists, both of which are highly functionalized glutamate analogues, 1 $(LY341495)^7$ and 2 $(MGS0039)^8$ (Figure 1). For example, work with these compounds has helped establish mGlu_{2/3} inhibition as a potential therapeutic application for obsessive-compulsive disorder (OCD),^{9,10} anxiety,¹¹ cognition,¹² and Alzheimer's disease.^{13–15} Additionally, antidepressant efficacy has been demonstrated in numerous rodent models of depression with these compounds,^{8,9,11,16-19} including those meant to assess treatmentresistant depression (TRD),²⁰ anhedonia,²¹ and depression associated with withdrawal from addictive substances.^{22,23} Compound 1 has also been used as a tool to establish a

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Figure 1. $mGlu_{2/3}$ orthosteric antagonist tools 1 and 2, $mGlu_{2/3}$ NAM tools 3, 4, and 6, and Roche $mGlu_{2/3}$ NAM clinical compound 5.

potential utility for $mGlu_{2/3}$ inhibition in the treatment of glioma. $^{24-27}$

Research with mGlu_{2/3} NAMs has been less extensive than with orthosteric antagonists; however, closely related tools 3 (RO4491533)²⁸ and 4 (RO4432717)^{29,30} (Figure 1) have been employed in multiple in vivo assays. In particular, these compounds have demonstrated efficacy in multiple models of depression³¹ and cognition.^{30,32,33} Furthermore, recent studies in genetically modified mice with 1^{34} and mGlu_{2/3} NAM 6^{35} (Figure 1) point toward a potential application for group II antagonists in the treatment of certain autism spectrum disorders. Finally, one mGlu_{2/3} NAM, 5 (decoglurant, RO4995819)³⁶ (Figure 1), has advanced into human clinical trials, including a phase II trial in patients with major depressive disorder (MDD) and resistant to ongoing treatment with antidepressants (NCT01457677).³⁷ In spite of the wealth of preclinical evidence for the potential utility of $mGlu_{2/3}$ antagonists, identification of compounds with ample selectivity between the two group II mGlus and the balance of pharmacology and drug metabolism and pharmacokinetics (DMPK) properties required for use in vivo has been elusive. Such tools are essential for further validation of the precise roles of each receptor in the etiology of disease.

Our first foray into this arena of research began by an observation of occasional weak $mGlu_3$ NAM activity in a series of 1,2-diphenylethyne $mGlu_5$ positive allosteric modulators (PAMs).³⁸ An optimization plan for $mGlu_3$ activity that centered on modification of the functional groups appended to the two phenyl rings within this scaffold delivered first-generation tool 7 (VU0463597, ML289)³⁹ (Figure 2).



Figure 2. mGlu₃ NAMs from the 1,2-diphenylethyne chemotype.

Importantly, not only was 7 selective versus mGlu₅ but selectivity versus mGlu, was also notable (>15-fold). Further optimization within this chemotype identified a secondgeneration compound 8 (VU0469942, ML337)⁴⁰ (Figure 2) that was devoid of both mGlu₂ and mGlu₅ activity. While 8 has proven quite useful as an in vitro tool compound and can be used in mice at high doses (100 mg/kg),⁴¹ lower CNS penetration and higher protein binding in rats prevent its utility in that species. Furthermore, 1,2-diarylethyne chemotypes similar to this one are prone to bioactivation at the alkyne moiety and subsequent formation of reactive metabolites that can lead to toxicity.^{42,43} Thus, the discovery of a superior mGlu₃ NAM from outside the 1,2-diphenylethyne chemotype, with the balance of pharmacology and DMPK properties for convenient use in both rats and mice, remained a worthy goal and is the subject of this manuscript.

RESULTS AND DISCUSSION

Lead Identification. The previous success in identification of an mGlu₃ NAM lead with inherently good selectivity versus mGlu₂ from a mGlu₅ PAM chemotype^{39,40} prompted the mining of our internal collection of mGlus PAM chemotypes lacking an alkyne moiety. Utilization of this resource provided some examples of compounds with evidence of mGlu₃ NAM activity amid the available associated cross-screening data versus the mGlu family.44,45 We selected many of these interesting compounds as well as additional closely related analogues to arrive at approximately 160 compounds for full concentration response curve (CRC) measurements in our cell-based functional assay for mGlu₃. This fluorescence-based assay measures calcium mobilization induced by mGlu₃ activation in a cell line stably expressing rat mGlu₃ and the promiscuous Gprotein G_{a15} and is capable of detecting agonists, PAMs, and NAMs of mGlu₃. We have developed similar assays and cell lines for rat mGlu₂ and rat mGlu₅, and both were used throughout this project for assessing selectivity.

One of the most interesting series to emerge from the full CRC $mGlu_3$ screen outlined above is exemplified by compounds 9–11 (Figure 3). These compounds are from



Figure 3. Representative compounds from a new mGlu₃ lead series.

within a series of 5-aryl-6,7-dihydropyrazolo[1,5-*a*]pyrazine-4(5H)-ones that includes many potent mGlu₅ PAMs.⁴⁶ An interesting piece of SAR was noted with respect to the presence of a chiral methyl group at the seven position of the pyrazine-4(5H)-one ring in the (*R*)-configuration (10). Whereas the potency of (*R*)-methyl analogue 10 at mGlu₅ was only 2-fold less than unsubstituted analogue 9, the efficacy of 10 (Glu Max = 26.5%) was much weaker than that observed with 9 (Glu

Scheme 1. Synthesis of Primary Alcohol Intermediate 20^a



"Reagents and conditions: (a) Na, EtOH, $(EtO_2C)_2$, 0 °C to rt, 27%; (b) NH₂NH₂·H₂O, EtOH, 80 °C, 98%; (c) PPh₃, D'BAD, THF, μ wave, 120 °C, 20 min; (d) 4 M HCl in dioxane, then saturated aq NaHCO₃, 74%, 2 steps; (e) CuI, K₂CO₃, 4-fluorobromobenzene, *N*,*N*'-dimethylethylenediamine, PhMe, 120 °C, 83%; (f) BBr₃, CH₂Cl₂, 0 °C to rt; (g) KOAc, DMF, 60 °C, 100%, 2 steps; (h) 1 M aq LiOH, MeOH, THF, 94%.

Scheme 2. Synthesis of New Western Ether Analogues 22-39 and 41-51^a



"Reagents and conditions: (a) PS-PPh₃, THF, DIAD, 7–37%; (b) NEt₃, MsCl, CH₂Cl₂, 63%; (c) Cs₂CO₃, DMF, 90 °C, 35–52%; (d) NaH, DMF, 8–37%.

Table 1. mGlu ₃	NAM and	mGlu _s	SAR of	Western	Phenyl	Ethers	18	, 22-	-39
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no.	R	${ m mGlu_3\ pIC_{50}\ (\pm\ SEM)^a}$	${f mGlu_3\ IC_{50}\ (nM)^a}$	% Glu Max $(\pm \text{ SEM})^{a,b}$	mGlu ₅ activity	$mGlu_5 pEC_{50}$ (± SEM) ^c	${\operatorname{mGlu}}_5 \operatorname{EC}_{50}$ (nM) ^c	% Glu Max (± SEM) ^{b,c}
18	Н	6.57 ± 0.15	267	2.21 ± 0.80	PAM	7.34 ± 0.02	46	87.7 ± 1.4
22	2-F	6.28 ± 0.20	530	2.90 ± 1.05	PAM	6.71 ± 0.04	195	89.3 ± 3.0
23	3-F	6.11 ± 0.03	773	1.19 ± 0.75	PAM	7.14 ± 0.02	73	86.1 ± 2.9
24	4-F	6.34 ± 0.04	462	2.09 ± 0.57	PAM	7.03 ± 0.05	92	87.0 ± 1.0
25	2,4-di-F	6.38 ± 0.22	417	1.70 ± 0.35	PAM	6.51 ± 0.05	307	82.3 ± 2.7
26	2-Me	6.14 ± 0.14	721	1.56 ± 0.37	PAM	5.83 ± 0.04	1500	83.7 ± 2.5
27	3-Me	5.84 ± 0.05	1440	2.35 ± 0.18	NAM ^d	<5.0	>10000	39.7 ± 7.7
28	4-Me	5.92 ± 0.02	1190	1.72 ± 0.55	PAM	5.97 ± 0.04	1080	92.5 ± 2.6
29	2-Cl	6.27 ± 0.18	532	1.63 ± 0.33	PAM	6.38 ± 0.01	419	84.4 ± 2.1
30	3-Cl	5.79 ± 0.02	1620	1.96 ± 0.11	NAM^d	<5.0	>10000	32.8 ± 6.1
31	4-Cl	5.93 ± 0.01	1170	1.86 ± 0.52	PAM	6.22 ± 0.04	604	81.8 ± 0.3
32	2-OMe	5.59 ± 0.18	2540	2.32 ± 1.39	PAM^d	<5.0	>10000	63.7 ± 3.1
33	3-OMe	5.89 ± 0.02	1290	1.78 ± 0.53	PAM	5.74 ± 0.04	1840	32.4 ± 3.0
34	4-OMe	5.98 ± 0.02	1040	1.76 ± 0.39	PAM	5.87 ± 0.16	1340	40.2 ± 4.8
35	4-CF ₃	5.73 ± 0.01	1850	4.13 ± 0.09		<4.5	>30000	
36	4-Et	6.08 ± 0.05	830	2.29 ± 0.84	PAM	5.49 ± 0.03	3210	50.8 ± 3.7
37	4-CN	6.11 ± 0.12	784	2.53 ± 0.70	PAM	5.71 ± 0.06	1940	38.6 ± 4.8
38	4-OEt	5.94 ± 0.06	1160	3.39 ± 0.87		<4.5	>30000	
39	4-OCF ₃	5.39 ± 0.04	4070	2.66 ± 0.34		<4.5	>30000	

^{*a*}Calcium mobilization mGlu₃ assay; values are average of $n \ge 3$. ^{*b*}Amplitude of response in the presence of 30 μ M test compound as a percentage of maximal response (100 μ M glutamate); average of $n \ge 3$. ^{*c*}Calcium mobilization mGlu₅ assay; values are average of $n \ge 3$. ^{*d*}Weak activity; concentration response curve (CRC) does not plateau.

Max = 93.0%). The (S)-methyl analogue 11 proved highly preferential for mGlu₅ PAM activity and was only weakly active at mGlu₃, inhibiting the glutamate response only at the top concentration tested (30 μ M). Gratifyingly, compound 10 was

also inactive up to the top concentration tested (30 μ M) in our mGlu₂ calcium mobilization assay. Given the potential benefits engendered by this (*R*)-methyl group, we incorporated this functional group into the design of future compounds.

Table 2. mGlu₃ NAM and mGlu₅ SAR of Western 2-Pyridyl Ethers 41-51

no.	R	$mGlu_3 pIC_{50}$ (± SEM) ^a	${f mGlu_3\ IC_{50}\ (nM)^a}$	% Glu Max $(\pm \text{ SEM})^{a,b}$	mGlu ₅ activity ^c	$mGlu_5 pEC_{50} $ $(\pm SEM)^c$	${ m mGlu_5\ EC_{50}\ (nM)^c}$	% Glu Max $(\pm \text{ SEM})^{b,c}$
41	3-F	5.96 ± 0.02	1100	2.22 ± 0.84	PAM	5.37 ± 0.06	4300	78.5 ± 6.1
42	3-CF ₃	5.53 ± 0.02	2940	2.11 ± 0.96		<4.5	>30000	
43	3-OMe	5.17 ± 0.02	6760	-1.14 ± 2.54		<4.5	>30000	
44	4-Me	5.68 ± 0.01	2090	0.86 ± 0.47	NAM ^d	<5.0	>10000	46.9 ± 5.9
45	4-CF ₃	5.78 ± 0.01	1670	1.62 ± 0.42	NAM ^d	<5.0	>10000	12.9 ± 2.9
46	4-OMe	5.43 ± 0.00	3690	-0.18 ± 1.39	NAM ^d	<5.0	>10000	54.7 ± 5.8
47	5-F	6.27 ± 0.02	539	1.71 ± 0.55	NAM ^d	<5.0	>10000	46.5 ± 3.4
48	5-Cl	6.22 ± 0.04	605	1.84 ± 0.51	PAM	5.53 ± 0.06	2920	81.4 ± 2.7
49	6-Me	5.62 ± 0.02	2370	1.12 ± 1.08	NAM	5.65 ± 0.07	2250	2.47 ± 0.17
50	6-CF ₃	5.63 ± 0.02	2360	2.06 ± 0.79		<4.5	>30000	
51	6-OMe	5.80 ± 0.02	1590	1.94 ± 0.62	PAM^d	<5.0	>10000	31.2 ± 3.1

"Calcium mobilization mGlu₃ assay; values are average of $n \ge 3$. "Amplitude of response in the presence of 30 μ M test compound as a percentage of maximal response (100 μ M glutamate); average of $n \ge 3$. "Calcium mobilization mGlu₅ assay; values are average of $n \ge 3$." Weak activity; CRC does not plateau.

Development of SAR in the Western Region. Our initial plan centered on the development of structure-activity relationships (SAR) in the area occupied by the 2-pyridyl ether of 10, termed the western region of the scaffold. Intermediate primary alcohol 20 was envisioned as a valuable intermediate for late stage diversification of this area (Scheme 1). The synthesis of 20 began with commercially available phenoxyacetone 12. The sodium enolate of 12 was prepared and treated in situ with diethyl carbonate to afford 2,4-diketone intermediate 13. Reaction of 13 with hydrazine readily provided 14, which could be N-alkylated via a Mitsunobu reaction⁴⁷ with commercially available chiral alcohol 15. This Mitsunobu reaction was carried out with microwave heating and resulted in inversion of stereochemistry as expected. Treatment of intermediate 16 with acid resulted in cleavage of the tert-butylcarbamate protecting group, and subsequent exposure to aqueous base provided the lactam 17. Chiral HPLC analysis of 17 showed 98.6% ee for this key intermediate. N-Arylation of 17 was accomplished by a copper mediated coupling⁴⁸ with 4-fluorobromobenzene to yield 18. Treatment of 18 with boron tribromide cleaved the phenoxy ether, affording the corresponding primary bromide, which was then reacted with potassium acetate under mild heating to afford acetate ester 19. Hydrolysis of the acetate group was accomplished with aqueous lithium hydroxide to give the desired intermediate alcohol 20.

Preliminary mGlu₃ NAM SAR obtained from the testing of analogues of lead **10** that were included in the original set of 160 compounds indicated a preference for phenyl and 2-pyridyl ethers in the western portion of the scaffold. Late stage conversion of intermediate alcohol **20** to new analogues was accomplished through one of three methods (Scheme 2). For the synthesis of substituted phenyl ether analogues **22–39**, alcohol **20** was converted to the corresponding mesylate, which was then reacted with the desired phenols **21** and cesium carbonate to afford **22–39**. Alternatively, the phenols **21** and alcohol **20** were coupled directly via a Mitsunobu reaction.⁴⁷ For synthesis of 2-pyridyl ether targets **41–51**, the sodium alkoxide of **20** was prepared in situ and treated with substituted

2-fluoropyridines 40 to facilitate a S_NAr reaction and provide 41–51.

The results obtained from preparing and testing unsubstituted phenyl ether intermediate 18 revealed a near 6-fold preference for mGlu₅ vs mGlu₃ activity, which ultimately proved a substantial hurdle with this subset of compounds (Table 1). To determine if substitution of the phenyl group could engender preference for mGlu₃ activity, we systematically installed a variety of functional groups at all positions (22-34). Most substituents at the 2-position (22, 26, 29) only modestly affected mGlu₃ NAM activity relative to 18; however, 2methoxy analogue 32 was nearly 10-fold less potent than 18. The mGlu₅ PAM activity was improved relative to 18 but remained suboptimal with these 2-substituted analogues. 2,4-Difluoro analogue 25 demonstrated modestly enhanced selectivity versus mGlu5 relative to its monosubstituted comparators 22 and 24. Although substitution of the 3-position with fluorine (23) provided little impact on activity at either receptor, installation of larger substituents (27, 30, 33) resulted in reductions in potency at both receptors and two instances of pharmacology mode switching at $mGlu_5$ (27 and 30). Such "molecular switches" have been noted previously in other mGlu₅ chemotypes.^{49,50} Substitution of the 4-position with fluorine (24) only minimally impacted potency at either receptor; larger groups (28, 31, 34) reduced potency at both receptors, although the effect on mGlu₅ was more pronounced. Thus, desiring to further examine the effects of substitution at the 4-position, additional analogues were prepared (35-39). The trend toward mGlu₃-preferring compounds continued with the 4-ethoxyphenyl analogue 38 and 4-trifluoromethoxy analogue 39, demonstrating no activity versus mGlu₅ up to the highest concentration tested (30 μ M).

Given the promising profile of initial lead 10, our hope for identifying more attractive compounds within the 2-pyridyl analogues (41-51) remained high; however, this region proved relatively intolerant of substitution (Table 2). Although a 3-fluoro substituent (41) only modestly impacted potency, larger substituents (42, 43) had more deleterious effects. Similarly modest results were observed with 4-position (44-46) and 6-

Table 3. DMPK Profiling of Early Analogues

			R	0 N 24 R = F 38 R = OEt	x-\o	0 N-N 47 X 48 X	= F = Cl	
no.	cLogP ^a	LLE ^b	$mGlu_3 \ IC_{50} \ (nM)$	fold vs mGlu ₅	rat plasma $F_{\rm u}{}^c$	$t_{1/2} (\min)^d$	$\mathrm{CL}_{\mathrm{plasma}}~(\mathrm{mL}/\mathrm{min}/\mathrm{kg})^d$	$V_{\rm SS}~({\rm L/kg})^d$
24	3.96	2.38	462	0.20	0.060	95	40	3.5
38	4.24	1.70	1160	>25	0.005	32	82	2.2
47	3.06	3.21	539	>18	0.135	88	82	6.6
48	3.57	2.65	605	4.8	0.034	141	29	5.2

^{*a*}Calculated using Dotmatics Elemental (www.dotmatics.com/products/elemental/). ^{*b*}LLE (ligand-lipophilicity efficiency) = pIC_{50} – cLogP. ^{*c*} F_u = fraction unbound. ^{*d*}Rat IV PK results (n = 2); dose = 0.2 mg/kg; solution in 9% EtOH, 37% PEG 400, 54% DMSO (2 mg/mL).

Scheme 3. Synthesis of 5-Fluoropyridin-2-yl Ether Analogues 55-72^a



"Reagents and conditions: (a) BBr₃, CH₂Cl₂, 0 °C to rt; (b) KOAc, DMF, 60 °C, 54%, 2 steps; (c) 1 M aq LiOH, MeOH, THF, 37%; (d) NaH, DMF, 2,5-difluoropyridine, 76%; (e) CuI, K₂CO₃, aryl or heteroaryl halide, *N*,*N*'-dimethylethylenediamine, PhMe, 120 °C, 17–70%.



"Reagents and conditions: (a) PPh₃, D^tBAD, THF, μ wave, 120 °C, 20 min; (b) 4 M HCl in dioxane, then saturated aq NaHCO₃, 89%, 2 steps; (c) NaH, 4-methoxybenzyl chloride, DMF, 0 °C to rt, 73%; (d) NaBH₄, THF, MeOH, 0–60 °C, 84%; (e) NaH, DMF, 5-chloro-2-fluoropyridine, 0 °C to rt, 84%; (f) (NH₄)₂Ce(NO₃)₆, MeCN, H₂O, 66%; (g) CuI, K₂CO₃, aryl or heteroaryl halide, *N*,*N*'-dimethylethylenediamine, PhMe, 120 °C, 16–99%.

position (49–51) analogues. Still, it was encouraging to identify additional compounds (42, 43, and 50) that displayed no activity versus mGlu₅ up to the highest concentration tested (30 μ M) as well as several analogues with only weak activity at mGlu₅. Fortunately, 5-halo analogues 47 and 48 proved an exception to the general trend of modest mGlu₃ potency with these analogues. Furthermore, 5-fluoro analogue 47 demonstrated enhanced selectivity approaching 20-fold versus mGlu₅, while 5-chloro analogue 48 was more modest with regard to selectivity at approximately 5-fold.

With some initial SAR in hand, we further profiled some of the more promising early analogues (**38**, **47**, **48**) to assess other properties important for the development of a useful in vivo tool compound (Table 3). In addition to assessing the degree to which the compounds were bound to rat plasma,⁵¹ the compounds were evaluated in a rat cassette pharmacokinetics (PK) study using intravenous (IV) dosing⁵² to assess their metabolic stability in vivo. In spite of its poor selectivity profile versus mGlu₅, 4-fluorophenyl ether **24** was also included with these compounds as a comparator compound. In fact, analogue

24 exhibited moderate clearance, with a half-life of over 1.5 h. Not surprisingly, replacement of the fluorine atom (24) with the more metabolically labile ethoxy group (38) increased clearance to approximately hepatic blood flow and reduced halflife. The results obtained with the 2-pyridyl analogues 47 and 48 revealed a profound metabolic difference. Whereas 5-fluoro analogue 47 was rapidly cleared, 5-chloro analogue 48 had a moderate clearance and a half-life of approximately 2 h. As indicated by their ligand-lipophilicity efficiency (LLE) values,⁵³ the 2-pyridyl ethers possessed a better balance of potency and lipophilicity than the phenyl ether compounds. Notably, 4ethoxyphenyl ether 38 also exhibited exceedingly high protein binding to rat plasma. These factors led to the conclusion that optimization should continue in the context of the 2-pyridyl ethers. Superior LLE, selectivity versus mGlu₅, and plasma unbound fraction relative to 48, made 5-fluoro analogue 47 an attractive starting point for further optimization; however, its high plasma clearance remained an issue. To avoid focusing future optimization efforts exclusively in the context of a

Table 4. mGlu₃ NAM and mGlu₅ SAR of 5-Fluoropyridin-2-yl Ether Analogues 55-72

			F→		N_R			
no.	R	$mGlu_3 pIC_{50}$ (± SEM) ^a	${ m mGlu_3\ IC_{50}}{ m (nM)}^a$	% Glu Max (± SEM) ^{a,b}	mGlu ₅ activity	$\begin{array}{c} \mathrm{mGlu_5\ pEC_{50}} \\ (\pm\ \mathrm{SEM})^c \end{array}$	${f mGlu_5\ EC_{50}\ (nM)^c}$	% Glu Max $(\pm \text{ SEM})^{b,c}$
55	2-fluorophenyl	6.79 ± 0.17	162	1.53 ± 0.12	NAM	<5.0	>10000	29.1 ± 7.3
56	3-fluorophenyl	6.72 ± 0.08	192	1.44 ± 0.38	NAM	5.56 ± 0.24	2720	9.71 ± 5.78
57	2-chlorophenyl	6.85 ± 0.16	141	1.37 ± 0.72	NAM	6.00 ± 0.25	1010	8.78 ± 5.29
58	3-chlorophenyl	6.84 ± 0.10	145	2.30 ± 0.17	NAM ^e	5.73 ± 0.24	1850	19.2 ± 10.0
59	4-chlorophenyl	6.71 ± 0.13	197	2.17 ± 0.40	PAM	6.44 ± 0.03	364	51.6 ± 7.7
60	2-methylphenyl	6.46 ± 0.06	346	1.72 ± 0.16	NAM ^f	<5.0 ^d	>10000 ^d	35.8 ^d
61	3-methylphenyl	6.67 ± 0.15	216	1.93 ± 0.56	NAM	<5.0	>10000	36.6 ± 8.7
62	4-methylphenyl	6.57 ± 0.03	269	1.04 ± 0.45	NAM	<5.0	>10000	54.8 ± 9.4
63	2-methoxyphenyl	6.47 ± 0.10	339	1.49 ± 0.60	NAM	6.52 ± 0.17	301	3.28 ± 0.45
64	3-methoxyphenyl	6.70 ± 0.06	198	1.02 ± 0.12	NAM	<5.0	>10000	59.7 ± 6.1
65	4-methoxyphenyl	6.49 ± 0.10	320	1.23 ± 0.38	PAM	5.86 ± 0.05	1380	32.3 ± 5.2
66	2,3-difluorophenyl	6.73 ± 0.16	184	2.15 ± 0.33	NAM ^e	5.68 ± 0.20	2110	21.0 ± 10.6
67	2,5-difluorophenyl	6.91 ± 0.11	123	2.62 ± 0.47	NAM ^f	<5.0	>10000	18.8 ± 6.1
68	2,6-difluorophenyl	6.74 ± 0.08	181	1.49 ± 0.17	NAM ^e	5.91 ^d	1230 ^d	50.8 ^d
69	pyridin-2-yl	6.04 ± 0.05	915	0.96 ± 0.63	NAM	5.45 ± 0.04	3520	3.71 ± 0.47
70	pyridin-3-yl	6.36 ± 0.13	436	0.64 ± 0.70	NAM	<5.0	>10000	46.3 ± 2.9
71	pyridin-4-yl	6.05 ± 0.04	881	0.77 ± 0.44		<4.5	>30000	
72	5-fluoropyridin-2-yl	6.46 ± 0.07	349	1.31 ± 0.11	NAM	5.01 ± 0.13	9740	7.0 ± 2.5
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^{*a*}Calcium mobilization mGlu₃ assay; values are average of $n \ge 3$. ^{*b*}Amplitude of response in the presence of 30 μ M test compound as a percentage of maximal response (100 μ M glutamate); average of $n \ge 3$. ^{*c*}Calcium mobilization mGlu₅ assay; values are average of $n \ge 3$. ^{*d*}Average of n = 2. ^{*e*}Partial NAM; CRC plateaus above 10% glutamate maximum. ^{*f*}Weak activity; CRC does not plateau.

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no.	R	cLogP ^a	LLE ^b	$mGlu_3 \ IC_{50} \ (nM)$	fold vs mGlu ₅	rat plasma $F_{\rm u}^{\ c}$	rat $\operatorname{CL}_{\operatorname{hep}}\left(\operatorname{mL}/\operatorname{min}/\operatorname{kg}\right)^{d}$
55	2-fluorophenyl	3.06	3.73	162	>61	0.098	64.7
56	3-fluorophenyl	3.06	3.66	192	14	0.094	53.0
57	2-chlorophenyl	3.57	3.28	141	7.2	0.054	66.5
58	3-chlorophenyl	3.57	3.27	145	13	0.034	51.9
61	3-methylphenyl	3.23	3.44	216	17	0.051	58.4
66	2,3-difluorophenyl	3.16	3.57	184	11	0.092	63.6
67	2,5-difluorophenyl	3.16	3.75	123	>81	0.089	49.6
68	2,6-difluorophenyl	3.16	3.58	181	6.8	0.064	64.6

^{*a*}Calculated using Dotmatics Elemental (www.dotmatics.com/products/elemental/). ^{*b*}LLE (ligand-lipophilicity efficiency) = pIC_{50} – cLogP. ^{*c*} F_u = fraction unbound. ^{*d*}Predicted hepatic clearance based on intrinsic clearance in rat liver microsomes.

potential PK liability, the decision was made to prepare analogues of both 47 and 48 in parallel.

Development of SAR in the Eastern Region. To enable final step diversification and the rapid synthesis of new analogues, modified syntheses were required for development of SAR in the eastern portion of the chemotype. Synthesis of new 5-fluoropyridin-2-yl ether analogues 55–72 was accomplished via a reordering of the previously outlined reactions (Scheme 3). Intermediate 17 was treated with boron tribromide and subsequently potassium acetate to afford acetate ester 52. Hydrolysis of 52 was accomplished with lithium hydroxide; however, the yield of alcohol 53 suffered due to difficulty in isolation of this polar and water-soluble intermediate. Still, preparation of the 5-fluoropyridin-2-yl ether 54 via S_NAr chemistry was readily accomplished, which

enabled a final stage installation of the aryl or heteroaryl eastern ring according to methods described previously to yield the desired products 55–72.

Because of the poor yield encountered in the synthesis of intermediate **53**, we employed what proved to be a more scalable and shorter synthesis in the preparation of the 5-chloropyridin-2-yl ether analogues (Scheme 4). Synthesis of intermediate **75** from commercially available **73** was accomplished via an analogous two-step Mitsunobu coupling⁴⁷ and deprotection/cyclization sequence as described previously. The lactam nitrogen was protected with a 4-methoxybenzyl group to afford **76**. Reduction of the ethyl ester was accomplished with sodium borohydride to yield **77**. Formation of the 5-chloropyridin-2-yl ether **78** was carried out via S_NAr chemistry as before. Oxidative removal of the 4-methoxybenzyl protecting

Table 6. mGlu₃ NAM and mGlu₅ SAR of 5-Chloropyridin-2-yl Ether Analogues 80-101



no.	R	$\begin{array}{c} \text{mGlu}_3 \text{ pIC}_{50} \\ (\pm \text{ SEM})^a \end{array}$	${ m mGlu_3\ IC_{50}}{ m (nM)}^a$	% Glu Max $(\pm \text{ SEM})^{a,b}$	mGlu ₅ activity ^c	$mGlu_5 pEC_{50}$ (± SEM) ^c	${f mGlu_5\ EC_{50}\ (nM)^c}$	% Glu Max $(\pm \text{ SEM})^{b,c}$
80	2-fluorophenyl	6.65 ± 0.04	226	1.74 ± 0.10	PAM	5.49 ± 0.06	3240	57.3 ± 4.6
81	3-fluorophenyl	6.69 ± 0.04	206	1.38 ± 0.47	PAM	5.22 ± 0.05	5970	63.5 ± 0.8
82	2-chlorophenyl	6.48 ± 0.08	328	1.87 ± 0.29	PAM	5.26 ± 0.03	5450	51.0 ± 3.9
83	3-chlorophenyl	6.25 ± 0.07	558	1.63 ± 0.20	PAM	5.54 ± 0.02	2870	65.8 ± 8.8
84	4-chlorophenyl	5.91 ± 0.25	1230	0.94 ± 1.46	PAM	5.48 ± 0.15	3310	51.2 ± 10.3
85	2-methylphenyl	6.24 ± 0.18	571	1.95 ± 0.70	PAM	5.64 ± 0.03	2280	80.7 ± 0.5
86	3-methylphenyl	6.41 ± 0.05	392	1.71 ± 0.44	PAM	5.44 ± 0.11	3600	69.9 ± 6.5
87	4-methylphenyl	6.39 ± 0.21	410	1.39 ± 0.25	PAM ^e	<5.0	>10000	70.8 ± 3.1
88	2-methoxyphenyl	6.16 ± 0.15	690	1.29 ± 0.18	NAM ^e	<5.0	>10000	58.5 ± 1.2
89	3-methoxyphenyl	6.50 ± 0.05	313	2.00 ± 0.32	PAM	5.29 ± 0.08	5070	78.0 ± 1.8
90	4-methoxyphenyl	6.36 ± 0.11	439	2.07 ± 0.68	PAM	5.36 ± 0.06	4330	59.4 ± 7.3
91	2-cyanophenyl	6.37 ± 0.05	429	1.64 ± 0.04	PAM	5.30 ± 0.10	4970	38.1 ± 5.0
92	3-cyanophenyl	6.08 ± 0.26	825	1.75 ± 0.41	PAM ^e	<5.0	>10000	32.2 ± 3.7
93	4-cyanophenyl	6.27 ± 0.05	539	1.84 ± 0.19	PAM	5.48 ± 0.08	3320	23.1 ± 6.8
94	2,3-difluorophenyl	6.07 ± 0.23	852	0.33 ± 0.71	PAM	5.17 ± 0.29	6680	36.1 ± 8.4
95	2,4-difluorophenyl	6.55 ± 0.08	280	1.34 ± 0.25	PAM	5.59 ± 0.07	2580	70.8 ± 0.8
96	2,6-difluorophenyl	6.65 ± 0.04	225	2.06 ± 0.31	PAM	5.85 ± 0.09	1420	40.3 ± 5.6
9 7	3,5-difluorophenyl	6.38 ± 0.07	420	2.18 ± 0.19	PAM ^e	<5.0	>10000	33.1 ± 2.3
98	2-cyano-5-fluorophenyl	6.28 ± 0.06	529	1.55 ± 0.31		<4.5	>30000	
99	3-cyano-5-fluorophenyl	6.50 ± 0.07	315	1.92 ± 0.12	PAM	5.98 ^d	1044 ^{<i>d</i>}	28.0 ^d
100	pyridin-2-yl	6.48 ± 0.07	328	1.07 ± 0.04		<4.5	>30000	
101	pyridin-3-yl	6.22 ± 0.10	608	1.73 ± 0.53		<4.5	>30000	

^{*a*}Calcium mobilization mGlu₃ assay; values are average of $n \ge 3$. ^{*b*}Amplitude of response in the presence of 30 μ M test compound as a percentage of maximal response (100 μ M glutamate); average of $n \ge 3$. ^{*c*}Calcium mobilization mGlu₅ assay; values are average of $n \ge 3$. ^{*c*}Average of n = 2. ^{*c*}Weak activity; CRC does not plateau.

group was carried out with ceric ammonium nitrate to provide penultimate intermediate **79**. Conversion of **79** into analogues **80–110** utilized the copper mediate *N*-arylation methods employed previously.

Testing of new 5-fluoropyridin-2-yl ether analogues 55-72 showed eastern ring modification was a useful strategy for enhancing mGlu₃ NAM activity (Table 4). In the case of monosubstituted phenyl ethers (55-65), several analogues exhibited mGlu₃ IC₅₀ values less than 200 nM. Specifically, at the 2-position, fluorine (55) and chlorine (57) substitution was preferred to methyl (60) and methoxy (63) substituents, while little difference in mGlu₃ activity was observed with variation of the same substituents at the 3-position (56, 58, 61, 64). Substitution at the 4-position (59, 62, 65) was slightly less favorable. Encouragingly, several of these analogues also demonstrated only weak activity at mGlu₅. Some difluorophenyl analogues (66-68) were also prepared and found to exhibit good potency. Simple pyridyl analogues (69-71) were less potent versus mGlu₃ than the majority of the phenyl analogues; however, selectivity versus mGlu₅ was notable in the case of 70 and 71. Finally, simple fluorine substitution of the pyridine ring (72) enhanced mGlu₃ activity relative to unsubstituted comparator 69.

Although several of these new 5-fluoropyridin-2-yl ether analogues demonstrated improved mGlu₃ NAM potency, modest levels of mGlu₅ selectivity, and improved LLE values⁵³ (Table 5), their DMPK profiles remained a critical unanswered question. Thus, selected analogues were profiled in our aforementioned rat protein binding assay,⁵¹ and metabolic stability was also assessed in vitro by measuring the intrinsic clearance of the compound when incubated with rat liver microsomes (RLM).⁵⁴ While the fraction unbound in rat plasma was encouraging for most compounds, metabolic stability was uniformly poor. Unfortunately, on the basis of their intrinsic clearance in RLM, the compounds were predicted to exhibit hepatic clearance near blood flow.⁵⁵

Fortunately, testing of new 5-chloropyridin-2-yl ether analogues 80-101 ultimately provided another path forward for the design of both potent and highly selective mGlu₃ NAMs (Table 6). Monosubstituted phenyl analogues (80-93) generally exhibited similar activity at mGlu₃ (IC₅₀ = 200-600nM) regardless of the position of the substituent on the ring. 4-Chlorophenyl analogue 84, 2-methoxyphenyl analogue 88, and 3-cyanophenyl analogue 92 were exceptions to this trend with each exhibiting reduced activity at mGlu₃. Although most of these monosubstituted phenyl analogues (80-93) demonstrated only modest selectivity versus mGlu₅, 4-methylphenyl analogue 87, 2-methoxyphenyl analogue 88, and 3-cyanophenyl analogue 92 exhibited weak activity at that receptor. Preparation of disubstituted phenyl analogues (94-99) yielded additional compounds with improved selectivity versus mGlu₅. 3,5-Difluorophenyl analogue 97 was a weak mGlu₅ PAM, and 2-cyano-5-fluorophenyl analogue 98 was inactive versus mGlu₅ up to the highest concentration tested (30 μ M). Finally, encouraging results were observed with unsubstituted pyridyl analogues 100 and 101, where both proved inactive versus mGlu₅ while maintaining good mGlu₃ NAM activity.

Table 7. mGlu₃ NAM and mGlu₅ SAR of Additional 5-Chloropyridin-2-yl Ether Analogues 102-110

			CI—	N Series I		O N Serie			
no.	series	R	$mGlu_3 pIC_{50}$ (± SEM) ^a	${ m mGlu_3\ IC_{50}\ (nM)^a}$	% Glu Max $(\pm \text{ SEM})^{a,b}$	mGlu ₅ activity ^c	$mGlu_5 pEC_{50}$ (± SEM) ^c	${f mGlu_5\ EC_{50}\ (nM)^c}$	% Glu Max $(\pm \text{ SEM})^{b,c}$
102	Ι	3-F	6.44 ± 0.03	362	1.64 ± 0.36	PAM ^d	<5.0	>10000	32.4 ± 4.4
103	Ι	3-CN	6.17 ± 0.06	677	1.35 ± 0.55		<4.5	>30000	
104	Ι	5-F	6.59 ± 0.09	260	2.44 ± 0.32	PAM ^d	<5.0	>10000	47.9 ± 5.1
105	Ι	6-F	6.53 ± 0.08	294	1.75 ± 0.19	NAM ^d	<5.0	>10000	24.8 ± 5.5
106	II	2-F	6.41 ± 0.05	392	1.71 ± 0.41		<4.5	>30000	
107	II	4-F	6.32 ± 0.03	482	1.45 ± 0.22		<4.5	>30000	
108	II	5-F	6.32 ± 0.03	481	1.85 ± 0.32		<4.5	>30000	
109	II	5-CN	6.22 ± 0.01	605	1.08 ± 0.50		<4.5	>30000	
110	П	6-F	6.39 ± 0.06	408	1.45 ± 0.29	PAM^d	< 5.0	>10000	30.6 ± 2.4

"Calcium mobilization mGlu₃ assay; values are average of $n \ge 3$. ^bAmplitude of response in the presence of 30 μ M test compound as a percentage of maximal response (100 μ M glutamate); average of $n \ge 3$. ^cCalcium mobilization mGlu₅ assay; values are average of $n \ge 3$. ^dWeak activity; CRC does not plateau.

Table 8. In Vitro DMPK Profiling of Select 5-Chloropyridin-2-yl Ether Analogues



no.	R	cLogP ^a	LLE ^b	$mGlu_3 \ IC_{50} \ (nM)$	fold vs mGlu ₅	rat plasma $F_{\rm u}^{\ c}$	rat CL _{hep} (mL/min/kg) ^d
99	2-cyano-5-fluorophenyl	3.29	2.99	529	>56	0.045	47.7
100	pyridin-2-yl	2.57	3.91	328	>91	0.051	69.5
101	pyridin-3-yl	2.16	4.06	608	>49	0.118	42.0
106	2-fluoropyridin-3-yl	2.67	3.74	392	>76	0.083	36.9
107	4-fluoropyridin-3-yl	2.26	4.06	482	>62	0.085	36.0
108	5-fluoropyridin-3-yl	2.26	4.06	481	>62	0.092	26.6
109	5-cyanopyridin-3-yl	1.88	4.34	605	>49	0.078	22.8

^{*a*}Calculated using Dotmatics Elemental (www.dotmatics.com/products/elemental/). ^{*b*}LLE (ligand-lipophilicity efficiency) = pIC_{50} – cLogP. ^{*c*} F_u = fraction unbound. ^{*d*}Predicted hepatic clearance based on intrinsic clearance in rat liver microsomes.

Table 9. In Vivo DMPK Profiling of Select 5-Chloropyridin-2-yl Ether Analogues



			rat IV PK results ^b			rat IP tissue distribution results ^{c,d,e}				
no.	rat plasma F_u^a	rat brain F_u^a	$t_{1/2}$ (min)	CL _{plasma} (mL/min/kg)	V _{SS} (L/kg)	plasma conc (µM)	brain conc (μM)	$K_{\rm p}^{f}$	$K_{p,uu}^{g}$	
99	0.045	0.032	194	13	2.2	1.68	2.41	1.4	1.0	
101	0.118		21	50	1.2					
106	0.083	0.052	42	37	1.6	9.49	15.85	1.7	1.0	
108	0.092		30	50	1.9					
109	0.078	0.033	59	22	1.4	4.52	5.28	1.2	0.49	

^{*a*} F_u = fraction unbound. ^{*b*}n = 2; dose = 0.2 mg/kg; solution in 10% EtOH, 38–40% PEG 400, 50–52% DMSO (2 mg/mL). ^{*c*}n = 2; time point = 15 min; dose = 10 mg/kg. ^{*d*}For **99** and **109**, fine homogeneous suspension in 0.1% Tween 80 and 0.5% methyl cellulose in H₂O (4 mg/mL). ^{*c*}For **106**, fine homogeneous suspension in 10% EtOH and 90% PEG400 (4 mg/mL). ^{*f*} K_p = total brain to total plasma ratio. ^{*g*} $K_{p,uu}$ = unbound brain (brain F_u × total brain) to unbound plasma (plasma F_u × total plasma) ratio

Encouraged by the selectivity profiles seen with pyridyl analogues **100** and **101** but suspecting that these unsubstituted compounds may be prone to rapid metabolism, we immediately prepared several substituted analogues of each (Table 7).

Unfortunately, in the pyridin-2-yl set (102-105), the majority of these modifications enhanced mGlu₅ activity, albeit only slightly. Cyano analogue 103 was the lone exception; however, this compound was approximately 2-fold less potent than



Figure 4. Progressive fold-shift of the glutamate CRC by mGlu₃ NAM 106 (A) and mGlu_{2/3} NAM 111 (B); compound concentrations shown in M.

unsubstituted analogue 100. Results with the pyridin-3-yl set (106-110) were more encouraging as all new compounds except 6-fluoro analogue 110 maintained the excellent selectivity profile versus mGlu₅ observed with 101 without a loss of activity at mGlu₃. At this point, several compounds with good mGlu₃ NAM activity and devoid of mGlu₅ activity were in hand, which set the stage for further profiling in pursuit of compounds meriting extensive in vivo evaluation.

As before, we moved several promising compounds into assays to assess protein binding⁵¹ in rat plasma as well as metabolic stability in RLM^{54} (Table 8). The lone eastern phenyl analogue 99 was slightly more protein bound than its eastern pyridyl comparators, which was not surprising given its higher lipophilicity. A range of predicted hepatic clearance values based on the intrinsic clearance of the compound in RLM were observed including one analogue of less than onethird hepatic blood flow (109) and one analogue near hepatic blood flow (100). The remaining analogues were predicted to have moderate clearance in vivo. Somewhat surprisingly, unsubstituted pyridine-3-yl analogue 101 was more metabolically stable than its regioisomeric comparator 100. Still, substituted versions of 101 (106–109) did exhibit increased stability relative to 101.

Several interesting analogues were next advanced into rat cassette PK studies using IV dosing⁵² to assess their metabolic stability in vivo (Table 9). As the most promising analogue with an eastern phenyl group, 99 was selected for these studies. Unsubstituted eastern pyridyl analogue 101 and several substituted comparators (106, 108, and 109) were also chosen based on the totality of data collected to that point. Distinguishing between analogues 108 and 107 was difficult; however, compound 108 was ultimately selected as it had a marginally better in vitro DMPK profile. 5-Cyanopyridin-3-yl analogue 109 was selected as it was predicted to have the lowest clearance, and 2-fluoropyridin-3-yl analogue 106 was chosen as it was the most potent analogue with a moderate predicted clearance. Compound 99 exhibited a lower clearance in vivo than expected and had a long half-life in excess of 3 h. The in vivo clearance for the pyridine-3-yl analogues was generally well predicted by the RLM experiments, with analogue 108 being the lone exception. Analogues 99, 106, and 109 were thus selected for further study in single time point (15 min) tissue distribution studies at a higher dose (10 mg/kg).⁵⁶ Intraperitoneal (IP) dosing was chosen for these studies as this route is convenient for use in our planned behavioral studies. The same three compounds were also examined in protein binding assays with rat brain homogenates.⁵¹ CNS penetration with each compound was excellent, with compound 106 exhibiting the highest plasma and brain levels. Both 99 and 106 exhibited unbound brain to unbound plasma ratios $(K_{p,uu})$ of one, indicating distribution equilibrium

between the compartments and a low probability of the compounds being substrates for transporters.⁵⁷ Compound **109** had a $K_{p,uu}$ value of 0.49, indicating possible efflux; however, additional experiments would be required to determine such conclusively. Compound **106** (VU0650786) was deemed the most attractive and targeted for extensive profiling.

Profiling of Compound 106. Profiling of compound 106 began with determination of its full selectivity versus other members of the mGlu family. In addition to mGlu₅, selectivity versus fellow group II receptor subtype, mGlu₂, was critical to assess. We evaluated the selectivity of 106 versus rat mGlu2 using full CRC analysis, and the compound was inactive up to the highest concentration tested (30 μ M). Thus, compound 106 has been established to have no functional activity at either mGlu₂ or mGlu₅ up to a concentration that is more than 75fold over the functional potency at mGlu₃. The effect of 10 μ M 106 on the orthosteric agonist CRC was measured in fold-shift experiments to evaluate selectivity versus the other mGlus.^{44,45} No significant effect was found, indicating the compound was inactive at those receptors as well. We further evaluated the nature of the interaction between 106 and mGlu₃ by examining the effects of increasing concentrations of 106 on the glutamate CRC in a progressive fold-shift experiment. If an antagonist acts via a noncompetitive mechanism, we anticipate increasing concentrations of antagonist would shift the glutamate curve to the right and decrease the maximal signal of glutamate. Figure 4 depicts the effects of multiple concentrations of 106 and known $mGlu_{2/3}$ NAM 111 (MNI-137)⁵⁸ on the glutamate CRC. As expected, both compounds exhibited the characteristic rightward shift and depressed glutamate maximum typically observed with NAMs, suggesting that 106 does not bind to the orthosteric glutamate binding site but instead acts via an allosteric mechanism.

To evaluate the ancillary pharmacology of the compound, a commercially available radioligand binding assay panel of 68 clinically relevant GPCRs, ion channels, kinases, and transporters was employed,⁵⁹ and only a single significant response (5-HT_{2B}, 65% inhibition) was found at 10 μ M 106.⁶⁰ Because 5-hydroxytryptamine receptor 2B $(5-HT_{2B})$ agonists are associated with cardiotoxicity,⁶¹ we followed this result up with a functional cell-based assay to assess potential agonist or antagonist activity of the compound at 5-HT_{2B}.⁶² Fortunately, testing up to 10 μ M in this assay revealed no functional activity for 106 at 5-HT_{2B}. Potential for drug-drug interactions was negligible as assessed in a human liver microsomes (HLM) cocktail assay with probe substrates for four common P450s (Table 10).⁶³ Finally, permeability and potential for Pglycoprotein (P-gp) mediated efflux was assessed in Madin-Darby canine kidney (MDCK) cells transfected with the human MDR1 gene.⁶⁴ Not surprisingly, compound **106** was highly permeable with no evidence of efflux in this assay.

Table 10. P450 Inhibition and Permeability Profile of Compound 106



Confident that 106 possessed a favorable profile with respect to its pharmacology, in vitro DMPK properties, and preliminary in vivo DMPK properties, we moved the compound into several definitive in vivo DMPK studies in rats and mice (Table 11).⁵⁶ Time course studies using IP dosing revealed nearly identical and rapidly reached C_{max} values in both rats and mice. A single time point (30 min) tissue distribution study in mice analogous to the one previously conducted in rats showed excellent CNS penetration in that species as well. Again, the $K_{\rm p,uu}$ value was near one as would be expected for a highly permeable compound devoid of efflux issues.⁵⁷ A definitive rat IV PK study (1.0 mg/kg) was conducted, and results were essentially identical to those collected with the prior cassette study (0.2 mg/kg). Finally, a rat oral PK study (3.0 mg/kg) was carried out to assess bioavailability, which proved quite good (60%). Taking into account the data summarized herein, it was estimated that the unbound C_{max} in the CNS following the 10 mg/kg IP studies in both rats and mice was at or beyond the measured functional mGlu₃ NAM activity. With that in mind, evaluation of compound **106** in rodent behavioral models known to be sensitive to mGlu_{2/3} antagonists was initiated.

Behavioral Pharmacology of Compound 106. It is wellknown that naïve mice will bury foreign objects, such as glass marbles, in deep bedding. This behavior can be inhibited by pretreatment with low doses of certain benzodiazepines, such as diazepam,⁶⁵ as well as certain selective serotonin reuptake inhibitors (SSRIs), such as fluvoxamine.⁶⁶ Likewise, this behavior has proven sensitive to a variety of $mGlu_5$ NAM compounds from diverse chemotypes.^{38,67,68} As such, the marble burying assay has often been used as a convenient method for assessing anxiolytic activity. It is worth noting that recent reports have argued that the assay reflects a repetitive and perseverative behavior such as OCD as opposed to noveltyinduced anxiety.⁶⁹ For our purposes, the most important fact was that the $mGlu_{2/3}$ orthosteric antagonists 1 and 2 have both been previously shown to inhibit marble burying.^{9,10} Thus, examination of the selective mGlu₂ NAM 106 in this assay was warranted to determine the contribution of mGlu₂ versus mGlu₃ to this effect (Figure 5). Gratifyingly, dose dependent efficacy was observed in this assay, with statistically significant effects at all three doses and essentially complete inhibition at the highest dose (56.6 mg/kg). The positive control for this assay was the well characterized mGlu₅ NAM 3-[(2-methyl-1,3thiazol-4-yl)ethynyl]-pyridine (112).⁷⁰ On the basis of the available DMPK data, it is estimated that CNS unbound exposure of compound 106 at the 10 mg/kg dose in this study reached a peak of 564 nM or approximately 1.5-fold the functional IC₅₀.



^{*a*} F_u = fraction unbound. ^{*b*}n = 3; fine microsuspension in 0.1% Tween 80 and 0.5% methyl cellulose in H₂O (4 mg/mL). ^{*c*}n = 2; dose = 1.0 mg/kg; solution in 10% EtOH, 50% PEG 400, 40% saline (1 mg/mL). ^{*d*}n = 2; dose = 3.0 mg/kg; fine microsuspension in 0.1% Tween 80 and 0.5% methyl cellulose in H₂O (0.3 mg/mL). ^{*e*}n = 3; time point = 30 min post dose; fine microsuspension in 0.1% Tween 80 and 0.5% methyl cellulose in H₂O (1 mg/mL). ^{*f*} K_p = total brain to total plasma ratio. ^{*g*} $K_{p,uu}$ = unbound brain (brain $F_u \times$ total brain) to unbound plasma (plasma $F_u \times$ total plasma) ratio. ^{*h*}n = 3 per time point; fine microsuspension in 0.1% Tween 80 and 0.5% methyl cellulose in H₂O (1 mg/mL).



Figure 5. Inhibition of marble burying in mice by compound **106**. n = 8-10 male CD-1 mice per treatment group; vehicle = 10% Tween 80 in H₂O; 15 min pretreatment with compound or vehicle; 30 min burying time; *, p < 0.05 vs vehicle control group. Compound **112** is the mGlu₅ NAM 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine.

Having established its efficacy in an anxiolytic/OCD mouse model, profiling of compound **106** in a rat model of depression was considered valuable for illustrating the utility of the compound. The forced swim test (FST) measures immobility time in rats placed in a tank of water from which they cannot escape and is sensitive to many antidepressants including several SSRIs.⁷¹ Importantly, efficacy with the mGlu_{2/3} orthosteric antagonists **1** and **2** has been demonstrated in this assay,⁸ making the examination of **106** compelling (Figure 6).



Figure 6. Decrease in immobility in the FST in rats by compound **106**. n = 8-10 male Sprague–Dawley rats per treatment group; **106** vehicle = 10% Tween 80 in H₂O; ketamine vehicle = saline; 30 min pretreatment with compound or vehicle; 6 min testing session; *, p < 0.05 vs vehicle control group.

In this case, significant effects in decreasing immobility time were observed at the highest dose (56.6 mg/kg). On the basis of the available DMPK data and an assumption of dose linearity, it is estimated that CNS unbound exposure of **106** at the high dose in these studies reached a peak of 2.3 μ M or approximately 6-fold over the functional IC₅₀. The positive control in this assay was the *N*-methyl-D-aspartate (NMDA) receptor antagonist drug ketamine,⁷² which was introduced to the market over 50 years ago.⁷³ Ketamine has recently demonstrated rapid acting antidepressant efficacy in TRD patients.^{74–76} Unfortunately, ketamine produces several undesirable side effects including psychotomimetic effects.⁷⁷ Preclinical studies implicate common downstream signaling

pathways in the antidepressant effects of ketamine and mGlu_{2/3} antagonists, including activation of the α -amino-3-hydroxy-5methyl-4-isoxazolepropionic acid (AMPA) receptor and the mammalian target of rapamycin (mTOR) pathway.^{18,19,78–82} Such studies have raised the possibility that mGlu_{2/3} antagonists or selective antagonists of each individual group II mGlu might represent novel approaches to rapid acting antidepressants without the side effect profile of ketamine. A selective and CNS penetrant mGlu₃ NAM compound such as **106** will be a valuable tool in shedding light on such questions.

CONCLUSION

A cross screening hit from a nonalkyne $mGlu_5$ PAM chemotype served as a successful launching point for the discovery of compound **106**, a highly selective $mGlu_3$ NAM with DMPK properties that enable its convenient use in rodent models of psychiatric disorders. In addition to these features, the compound displays moderate clearance and good bioavailability in rats. Furthermore, the compound is highly permeable, not a substrate for P-gp mediated efflux, and possesses an attractive P450-inhibition profile. The compound has demonstrated efficacy in two rodent models previously shown to be sensitive to $mGlu_{2/3}$ inhibition. This highly selective $mGlu_3$ NAM can thus serve as a useful tool for elucidating the role of selective inhibition of $mGlu_3$ and its potential utility as a novel therapeutic target. Such studies will constitute the subject of future communications.

EXPERIMENTAL SECTION

Diethyl (R)-1-(1-((tert-Butoxycarbonyl)amino)propan-2-yl)-1H-pyrazole-3,5-dicarboxylate (74). Diethyl 3,5-pyrazoledicarboxylate 73 (4.24 g, 20 mmol, 1.0 equiv) and tert-butyl (S)-(2hydroxypropyl) carbamate 15 (7.01 g, 40 mmol, 2.0 equiv) were dissolved in THF (100 mL. 0.2 M), and triphenyl phosphine (9.44 g, 36 mmol, 1.8 equiv) was added. After 5 min, the mixture was cooled to 0 °C and di-tert-butyl azodicarboxylate (8.29 g, 36 mmol, 1.8 equiv) was added. The reaction mixture was then subjected to microwave irradiation for 25 min at 120 °C. The mixture was cooled to room temperature, and the solvent was removed in vacuo. Purification via flash chromatography on silica gel provided the title compound as a semisolid (8.2 g, yield was not determined due to contamination of D^tBAD byproduct, di-*tert*-butyl hydrazine-1,2-dicarboxylate). ¹H NMR (400 MHz, MeOD) δ 7.29 (s, 1H), 5.69-5.01 (m, 1H), 4.41-4.35 (m, 4H), 3.51-3.39 (m, 2H), 1.51 (d, J = 6.8 Hz, 3H), 1.41-1.38 (m, 2H), 1.41-1.41-1.38 (m, 2H), 1.41-1.38 (m, 2H), 1.41-1.3815H). ¹³C NMR (100 MHz, CDCl₃) δ 161.7, 159.0, 155.8, 142.6, 134.2, 114.0, 79.4, 61.4, 61.1, 53.4, 45.1, 28.2 (3C), 18.5, 14.3, 14.2. LCMS (method A): $R_{\rm T} = 1.052$ min, m/z = 314.2 [M + H]⁺. HRMS, calcd for C17H27N3O6 [M], 369.1900; found, 369.1899.

Ethyl (R)-7-Methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-2-carboxylate (75). Compound 74 (8.2 g, 22.2 mmol, 1.0 equiv) was treated with a solution of 4 N HCl in 1,4-dioxane (78 mL). Deprotection of the tert-butyl carbamate protecting group was monitored by LCMS. Once deprotection was complete, the reaction mixture was carefully basified with saturated aqueous NaHCO3 (verified by pH paper) and was allowed to stir at room temperature overnight. The mixture was diluted with dichloromethane, and the aqueous layer was extracted with dichloromethane $(3\times)$. The combined organic layers were washed with brine, dried over Na2SO4, and concentrated in vacuo to provide the title compound as a white solid (4.4 g, 89% yield over two steps), which was used without further purification. ¹H NMR (400 MHz, CDCl₃) δ 7.35 (s, 1H), 7.13 (bs, 1H), 4.68–4.62 (m, 1H), 4.41 (q, J = 7.1 Hz, 2H), 3.86 (ddd, J = 15.8, 10.2, 1.4 Hz, 1H), 3.51 (ddd, J = 9.4, 6.4, 3.0 Hz, 1H),1.65 (d, J = 6.6 Hz, 3H), 1.39 (t, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 161.7, 159.3, 143.8, 134.4, 110.7, 61.3, 53.2, 46.0, 17.4, 14.3. LCMS (method A): $R_T = 0.546 \text{ min}, m/z = 224.2 \text{ [M + H]}.$ HRMS, calcd for $C_{10}H_{13}N_3O_3$ [M], 223.0957; found, 223.0957. [α]²⁵_D = -29.9° (*c* 0.500, CHCl₃).

Ethyl (R)-5-(4-Methoxybenzyl)-7-methyl-4-oxo-4,5,6,7tetrahydropyrazolo[1,5-a]pyrazine-2-carboxylate (76). Compound 75 (2.23 g, 10 mmol, 1.0 equiv) was dissolved in DMF (50 mL, 0.2 M), cooled to 0 °C, and treated with 60% sodium hydride in mineral oil (480 mg, 12 mmol, 1.2 equiv) in five portions. The reaction mixture was stirred for 15 min, and 4-methoxybenzyl chloride (1.63 mL, 12 mmol, 1.2 equiv) was added. After 16 h, the reaction mixture was diluted with water and extracted with EtOAc (3×). The combined extracts were washed with water and brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude material was purified by flash chromatography on silica gel to provide the title compound (2.51 g, 73% yield) as a pale-yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 7.37 (s, 1H), 7.23 (d, *J* = 14.1, 2H), 6.87 (d, *J* = 14.1, 2H), 4.76 (d, J = 14.5, 1H), 4.59–4.50 (m, 2H), 4.44–4.36 (m, 2H), 3.79 (s, 3H), 3.69 (dd, J = 13.1, 4.6 Hz, 1H), 3.35 (dd, J = 13.1, 6.3 Hz, 1H), 1.47 (d, J = 6.6 Hz, 3H), 1.38 (t, J = 7.0 Hz, 3H). ¹³C NMR $(100 \text{ MHz}, \text{CDCl}_3) \delta 161.7, 159.5, 137.1, 143.9, 134.8, 129.9 (2C),$ 127.9, 114.3, 110.9 (2C), 61.2, 55.3, 52.9, 50.4, 48.8, 17.5, 14.3. LCMS (method A): $R_{\rm T} = 0.955$ min, m/z = 344.2 [M + H]⁺. HRMS, calcd for $C_{18}H_{21}N_3O_4$ [M], 343.1532; found, 343.1533. [α]²⁵_D = -8.1° (*c* 0.157, CHCl₂).

(R)-2-(Hydroxymethyl)-5-(4-methoxybenzyl)-7-methyl-6,7dihydropyrazolo[1,5-a]pyrazin-4(5H)-one (77). Sodium borohydride (1.16 g, 30.6 mmol, 5.0 equiv) was added slowly to a solution of compound 76 (2.1 g, 6.11 mmol, 1.0 equiv) in THF (20 mL) and MeOH (5.0 mL) at 0 °C. The reaction was heated to 60 °C, and after 30 min at that temperature, the reaction mixture was diluted with water and extracted with dichloromethane. The aqueous layer was acidified with a 1 M aqueous HCl solution and extracted with dichloromethane (2×). The combined extracts were dried over Na₂SO₄ and concentrated in vacuo. Purification by flash chromatography on silica gel provided the title compound as a viscous oil (1.55 g, 84% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.23-7.21 (m, 2H), 6.87-6.84 (m, 3H), 4.72 (d, J = 14.5 Hz, 1H), 4.68 (s, 2H), 4.59 (d, J = 14.4 Hz, 1H), 4.43-4.35 (m, 1H), 3.78 (s, 3H), 3.60 (dd, J = 13.0, 4.6 Hz, 1H), 3.31 (dd, J = 13.0, 7.4 Hz, 1H), 1.42 (d, J = 6.5 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 159.4, 157.8, 152.6, 134.5, 129.8 (2C), 128.2, 114.3 (2C), 106.5, 58.7, 55.3, 52.0, 50.8, 48.7, 17.2. LCMS (method A): $R_T = 0.680 \text{ min}, m/z = 302.2 [M + H]^+$. HRMS, calcd for $C_{16}H_{19}N_3O_3$ [M], 301.1426; found, 301.1428. $[\alpha]^{25}_{D} = -5.3^{\circ}$ (c 0.98, CHCl₂).

(R)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(4-methoxybenzyl)-7-methyl-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one (78). To a solution of compound 77 (1.5 g, 4.98 mmol, 1.0 equiv) in DMF (25 mL, 0.2 M) at 0 °C was added NaH (300 mg, 12.44 mmol, 2.5 equiv). The resulting mixture was stirred for 15 min, and 5-chloro-2-fluoropyridine (1.25 mL, 12.44 mmol, 2.5 equiv) was added. The mixture was stirred overnight and extracted with EtOAc $(3\times)$. The combined extracts were concentrated in vacuo. Purification by flash chromatography on silica gel afforded the title compound (1.72 g, 84% yield) as a viscous oil. ¹H NMR (400 MHz, CDCl₃) δ 8.13 (d, J = 2.6 Hz, 1H), 7.54 (dd, J = 8.8, 2.6 Hz, 1H), 7.25 (d, J = 8.5 Hz, 2H), 6.98 (s, 1H), 6.88 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.8 Hz, 1H), 5.39 (s, 2H), 4.75 (d, J = 14.5 Hz, 1H), 4.63 (d, J = 14.5 Hz, 1H), 4.50–4.42 (m, 1H), 3.81 (s, 3H), 3.64 (dd, J = 13.0, 4.6 Hz, 1H), 3.35 (dd, J = 13.0, 7.4 Hz, 1H), 1.48 (d, J = 6.5 Hz, 3H). ¹³C NMR (100 MHz, $CDCl_3$) δ 161.6, 159.4, 157.7, 148.9, 145.1, 138.6, 134.5, 129.8 (2C), 128.2, 124.4, 114.2 (2C), 112.3, 108.2, 61.6, 55.3, 52.1, 50.8, 48.8, 17.2. LCMS (method A): $R_{\rm T} = 1.080$ min, m/z = 413.2 [M + H]⁺. HRMS, calcd for $C_{21}H_{21}CIN_4O_3$ [M], 412.1302; found, 412.1305. $[\alpha]^{25}_{D}$ = -10.3° (c 1.512, CHCl₃).

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-6,7dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (79). Compound 78 (1.65 mg, 4.0 mmol, 1.0 equiv) was dissolved in MeCN (40 mL, 0.1 M). and a solution of ceric ammonium nitrate (6.57 g, 12 mmol, 4.0 equiv) in water (12 mL) was added. After 30 min at room temperature, solvents were removed in vacuo. Purification using flash chromatography on silica gel provided the title compound (764 mg, 65% yield) as a pale-yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.26 (d, *J* = 2.7 Hz, 1H), 8.21 (s, 1H), 7.83 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.92 (dd, *J* = 8.8, 0.5 Hz, 1H), 6.77 (s, 1H), 5.29 (s, 2H), 4.51–4.46 (m, 1H), 3.66 (ddd, *J* = 13.0, 8.7, 8.7 Hz, 1H), 3.34 (ddd, *J* = 13.1, 7.9, 2.2 Hz, 1H), 1.45 (d, *J* = 6.5 Hz, 3H). ¹³C NMR (100 MHz, DMSO*d*₆) δ 161.9, 158.7, 147.7, 145.3, 139.7, 135.1, 124.1, 112.9, 107.4, 61.7, 52.3, 45.6, 17.0. LCMS (method A): $R_{\rm T}$ = 0.804 min, *m*/*z* = 293.2 [M + H]⁺. HRMS, calcd for C₁₃H₁₃ClN₄O₂ [M], 292.0727; found, 292.0727. [*α*]²⁵_D = -38.3° (*c* 0.442, CHCl₃). (*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(2-fluoropyridin-

3-yl)-7-methyl-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one (106). Copper(I) iodide (13.7 mg, 0.072 mmol, 2.1 equiv) was added to a suspension of compound 79 (10 mg, 0.035 mmol, 1.0 equiv), 3bromo-2-fluoropyridine (7.40 µL, 0.072 mmol, 2.1 equiv), potassium carbonate (10 mg, 0.072 mmol, 2.1 equiv), and N,N'-dimethylethylenediamine (20.7 μ L, 0.19 mmol, 5.5 equiv) in toluene (0.44 mL) in a sealed reaction vial. The reaction mixture was stirred at 120 °C. After 16 h, the mixture was diluted with EtOAc, filtered through a Celite pad which was rinsed with EtOAc $(2\times)$, and concentrated in vacuo. Purification using reserve phase HPLC method 1 with 39-71% CH_3CN in $H_2O(0.1\%$ TFA) over 4 min provided the title compound (7.2 mg, 53% yield) as a white powder. ¹H NMR (400 MHz, DMSO d_6) δ 8.27 (dd, J = 2.7, 0.5 Hz, 1H), 8.23 (ddd, J = 4.8, 2.7, 2.7 Hz, 1H), 8.09 (ddd, J = 9.6, 7.7, 1.8 Hz, 1H), 7.84 (dd, J = 8.8, 2.7 Hz, 1H), 7.50 (ddd, J = 9.0, 4.9, 1.3 Hz, 1H) 6.95 (s, 1H), 6.92 (d, J = 0.5 Hz, 1H), 5.35 (s, 2H), 4.82-4.75 (m, 1H), 4.27 (dd, J = 12.8, 4.3 Hz, 1H), 3.98 (dd, J = 12.8, 7.2 Hz, 1H) 1.55 (d, J = 6.5 Hz, 3H). ¹³C NMR (100 MHz, DMSO- d_6) δ 161.9, 158.2 (d, J_{CF} = 239 Hz), 156.6, 148.4, 146.4 (d, $J_{C,F}$ = 14 Hz), 145.3, 133.9 (d, $J_{C,F}$ = 13 Hz), 139.8, 134.0, 124.4 (d, $J_{C,F}$ = 28 Hz), 124.2, 123.2 (d, $J_{C,F}$ = 4 Hz), 112.9, 108.7, 61.6, 54.0, 52.7, 17.1. LCMS (method A): $R_T = 0.944 \text{ min}, m/z$ = 388.2 $[M + H]^+$. HRMS, calcd for $C_{18}H_{15}CIFN_5O_2$ [M], 387.0898; found, 387.0899. $[\alpha]^{25}_{D} = -23.6^{\circ}$ (*c* 0.100, DMSO).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmed-chem.5b01005.

Experimental procedures and spectroscopic data for additional compounds, molecular pharmacology methods, DMPK methods, behavioral pharmacology methods, and the ancillary pharmacology profile details of **106** (PDF)

Molecular formula strings (CSV)

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Author Contributions

Drs. Emmitte and Lindsley directed and designed the chemistry. Drs. Engers and Konkol performed the medicinal chemistry. Drs. Conn and Niswender directed and designed the molecular pharmacology experiments. Dr. Rodriguez directed and performed molecular pharmacology experiments. Mr. Venable and Mr. Loch performed molecular pharmacology experiments. Dr. Daniels directed and designed the DMPK experiments. Dr. Blobaum directed DMPK experiments and performed bioanalytical work. Mr. Morrison performed bioanalytical work. Mr. Morrison performed bioanalytical work. Mr. DMPK work. Mr. Byers performed in vitro DMPK work. Dr. Jones directed and designed the behavioral experiments. Dr. Thompson performed the behavioral experiments.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

Ac, acetate; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AUC, area under the curve; C, concentration; CL, clearance; CNS, central nervous system; CRC, concentration response curve; DIAD, di-iso-propyl azodicarboxylate; DMF, N,N-dimethylformamide; DMPK, drug metabolism and pharmacokinetics; DMSO, dimethyl sulfoxide; D^tBAD, di-tertbutyl azodicarboxylate; Et, ethyl; FST, forced swim test; F, bioavailability; F_{w} fraction unbound; GPCR, G-protein-coupled receptors; HLM, human liver microsomes; IP, intraperitoneal; IV, intravenous; K_p , brain to plasma ratio; $K_{p,uu}$, unbound brain to unbound plasma ratio; LLE, ligand-lipophilicity efficiency; max, maximum; MDCK, Madin-Darby canine kidney; MDD, major depressive disorder; Me, methyl; mGlu, metabotropic glutamate receptor; Ms, methanesulfonyl; mTOR, mammalian target of rapamycin; NAM, negative allosteric modulator; NMDA, N-methyl-D-aspartate; OCD, obsessive-compulsive disorder; PAM, positive allosteric modulator; PEG, polyethylene glycol; Ph, phenyl; PK, pharmacokinetics; PO, oral; PS, polystyrene; RLM, rat liver microsomes; SAR, structureactivity relationships; THF, tetrahydrofuran; TRD, treatmentresistant depression; T, time; $t_{1/2}$, half-life; V_{SS} , volume of distribution at steady-state; 5-HT_{2B}, 5-hydroxytryptamine receptor 2B; 7TM, seven transmembrane

REFERENCES

(1) Niswender, C. M.; Conn, P. J. Metabotropic glutamate receptors: Physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 295–322.

(2) Schoepp, D. D.; Jane, D. E.; Monn, J. A. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. *Neuropharmacology* **1999**, 38, 1431–1476.

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

(4) Chaki, S.; Ago, Y.; Palucha-Paniewiera, A.; Matrisciano, F.; Pilc, A. mGlu2/3 and mGlu5 receptors: Potential targets for novel antidepressants. *Neuropharmacology* **2013**, *66*, 40–52.

(5) Palucha, A.; Pilc, A. Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. *Pharmacol. Ther.* **200**7, *115*, 116–147.

(6) Célanire, S.; Sebhat, I.; Wichmann, J.; Mayer, S.; Schann, S.; Gatti, S. Novel metabotropic glutamate receptor 2/3 antagonists and their therapeutic applications: a patent review (2005 – present). *Expert Opin. Ther. Patents* **2015**, *25*, 69–90.

(7) Ornstein, P. L.; Bleisch, T. J.; Arnold, M. B.; Kennedy, J. H.; Wright, R. A.; Johnson, B. G.; Tizzano, J. P.; Helton, D. R.; Kallman, M. J.; Schoepp, D. D.; Hérin, M. 2-Substituted (2SR)-2-amino-2-((1SR,2SR)-2-carboxycycloprop-1-yl)glycines as potent and selective antagonists of group II metabotropic glutamate receptors. 2. Effects of aromatic substitution, pharmacological characterization, and bioavailability. J. Med. Chem. **1998**, 41, 358–378.

(8) Chaki, S.; Yoshikawa, R.; Hirota, S.; Shimazaki, T.; Maeda, M.; Kawashima, N.; Yoshimizu, T.; Yasuhara, A.; Sakagami, K.; Okuyama, S.; Nakanishi, S.; Nakazato, A. MGS0039: a potent and selective group II metabotropic glutamate receptor antagonist with antidepressant-like activity. *Neuropharmacology* **2004**, *46*, 457–467.

(9) Bespalov, A. Y.; van Gaalen, M. M.; Sukhotina, I. A.; Wicke, K.; Mezler, M.; Schoemaker, H.; Gross, G. Behavioral characterization of the mGlu group II/III receptor antagonist, LY-341495, in animal models of anxiety and depression. *Eur. J. Pharmacol.* **2008**, *592*, 96– 102.

(10) Shimazaki, T.; Iijima, M.; Chaki, S. Anxiolytic-like activity of MGS0039, a potent group II metabotropic glutamate receptor antagonist, in a marble-burying behavior test. *Eur. J. Pharmacol.* **2004**, *501*, 121–125.

(11) Yoshimizu, T.; Shimazaki, T.; Ito, A.; Chaki, S. An mGluR2/3 antagonist, MGS0039, exerts antidepressant and anxiolytic effects in behavioral models in rats. *Psychopharmacology* **2006**, *186*, 587–593.

(12) Higgins, G. A.; Ballard, T. M.; Kew, J. N.; Richards, J. G.; Kemp, J. A.; Adam, G.; Woltering, T.; Nakanishi, S.; Mutel, V. Pharmacological manipulation of mGlu2 receptors influences cognitive performance in the rodent. *Neuropharmacology* **2004**, *46*, 907–917.

(13) Kim, S. H.; Steele, J. W.; Lee, S. W.; Clemenson, G. D.; Carter, T. A.; Treuner, K.; Gadient, R.; Wedel, P.; Glabe, C.; Barlow, C.; Ehrlich, M. E.; Gage, F. H.; Gandy, S. Proneurogenic group II mGluR antagonist improves learning and reduces anxiety in Alzheimer $A\beta$ oligomer mouse. *Mol. Psychiatry* **2014**, *19*, 1235–1242.

(14) Kim, S. H.; Fraser, P. E.; Westaway, D.; St. George-Hyslop, P. H.; Ehrlich, M. E.; Gandy, S. Group II metabotropic glutamate receptor stimulation triggers production and release of Alzheimer's amyloid β_{42} from isolated intact nerve terminals. *J. Neurosci.* **2010**, *30*, 3870–3875.

(15) Yoshimizu, T.; Chaki, S. Increased cell proliferation in the adult mouse hippocampus following chronic administration of group II metabotropic glutamate receptor antagonist, MGS0039. *Biochem. Biophys. Res. Commun.* **2004**, *315*, 493–496.

(16) Gleason, S. D.; Li, X.; Smith, I. A.; Ephlin, J. D.; Wang, X. S.; Heinz, B. A.; Carter, J. H.; Baez, M.; Yu, J.; Bender, D. M.; Witkin, J. M. mGlu2/3 agonist-induced hyperthermia: An in vivo assay for detection of mGlu2/3 receptor antagonism and its relation to antidepressant-like efficacy in mice. *CNS Neurol. Disord.: Drug Targets* **2013**, *12*, 554–566.

(17) Koike, H.; Fukumoto, K.; Iijima, M.; Chaki, S. Role of BDNF/ TrkB signaling in antidepressant-like effects of a group II metabotropic glutamate receptor antagonist in animal models of depression. *Behav. Brain Res.* **2013**, 238, 48–52.

(18) Koike, H.; Iijima, M.; Chaki, S. Involvement of the mammalian target of rapamycin signaling in the antidepressant-like effect of group II metabotropic glutamate receptor antagonists. *Neuropharmacology* **2011**, *61*, 1419–1423.

(19) Karasawa, J.; Shimazaki, T.; Kawashima, N.; Chaki, S. AMPA receptor stimulation mediates the antidepressant-like effect of a group II metabotropic glutamate receptor antagonist. *Brain Res.* **2005**, *1042*, 92–98.

(20) Ago, Y.; Yano, K.; Araki, R.; Hiramatsu, N.; Kita, Y.; Kawasaki, T.; Onoe, H.; Chaki, S.; Nakazato, A.; Hashimoto, H.; Baba, A.; Takuma, K.; Matsuda, T. Metabotropic glutamate 2/3 receptor antagonists improve behavioral and prefrontal dopaminergic alter-

ations in the chronic corticosterone-induced depression model in mice. *Neuropharmacology* **2013**, *65*, 29–38.

(21) Dwyer, J. M.; Lepack, A. E.; Duman, R. S. mGluR2/3 blockade produces rapid and long-lasting reversal of anhedonia caused by chronic stress exposure. *J. Mol. Psychiatry* **2013**, *1*, 15.

(22) Iijima, M.; Koike, H.; Chaki, S. Effect of an mGlu2/3 receptor antagonist on depressive behavior induced by withdrawal from chronic treatment with methamphetamine. *Behav. Brain Res.* **2013**, *246*, 24–28.

(23) Markou, A. Metabotropic glutamate receptor antagonists: Novel therapeutics for nicotine dependence and depression? *Biol. Psychiatry* **2007**, *61*, 17–22.

(24) Ciceroni, C.; Bonelli, M.; Mastrantoni, E.; Niccolini, C.; Laurenza, M.; Larocca, L. M.; Pallini, R.; Traficante, A.; Spinsanti, P.; Ricci-Vitiani, L.; Arcella, A.; De Maria, R.; Nicoletti, F.; Battaglia, G.; Melchiorri, D. Type-3 metabotropic glutamate receptors regulate chemoresistance in glioma stem cells, and their levels are inversely related to survival in patients with malignant gliomas. *Cell Death Differ.* **2013**, *20*, 396–407.

(25) Ciceroni, C.; Arcella, A.; Mosillo, P.; Battaglia, G.; Mastrantoni, E.; Oliva, M. A.; Carpinelli, G.; Santoro, F.; Sale, P.; Ricci-Vitiani, L.; De Maria, R.; Pallini, R.; Giangaspero, F.; Nicoletti, F.; Melchiorri, D. Type-3 metabotropic glutamate receptors negatively modulate bone morphogenetic protein receptor signaling and support the tumourigenic potential of glioma-initiating cells. *Neuropharmacology* **2008**, *55*, 568–576.

(26) Arcella, A.; Carpinelli, G.; Battaglia, G.; D'Onofrio, M.; Santoro, F.; Ngomba, R. T.; Bruno, V.; Casolini, P.; Giangaspero, F.; Nicoletti, F. Pharmacological blockade of group II metabotropic glutamate receptors reduces the growth of glioma cells in vivo. *Neuro-Oncology* **2005**, *7*, 236–245.

(27) D'Onofrio, M.; Arcella, A.; Bruno, V.; Ngomba, R. T.; Battaglia, G.; Lombari, V.; Ragona, G.; Calogero, A.; Nicoletti, F. Pharmacological blockade of mGlu2/3 metabotropic glutamate receptors reduces cell proliferation in cultured human glioma cells. *J. Neurochem.* **2003**, *84*, 1288–1295.

(28) Campo, B.; Kalinichev, M.; Lambeng, N.; Yacoubi, M. E.; Royer-Urios, I.; Schneider, M.; Legrand, C.; Parron, D.; Girard, F.; Bessif, A.; Poli, S.; Vaugeois, J.-M.; Le Poul, E.; Celanire, S. Characterization of an mGluR2/3 negative allosteric modulator in rodent models of depression. *J. Neurogenet.* **2011**, *25*, 152–166.

(29) Pritchett, D.; Jagannath, A.; Brown, L. A.; Tam, S. K. E.; Hasan, S.; Gatti, S.; Harrison, P. J.; Bannerman, D. M.; Foster, R. G.; Peirson, S. N. Deletion of metabotropic glutamate receptors 2 and 3 (mGlu2 & mGlu3) in mice disrupts sleep and wheel-running activity, and increases the sensitivity of the circadian system to light. *PLoS One* **2015**, *10*, e0125523.

(30) Woltering, T. J.; Wichmann, J.; Goetschi, E.; Knoflach, F.; Ballard, T. M.; Huwyler, J.; Gatti, S. Synthesis and characterization of 1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives: Part 4. In vivo active potent and selective non-competitive metabotropic glutamate receptor 2/3 antagonists. *Bioorg. Med. Chem. Lett.* **2010**, 20, 6969–6974.

(31) Yacoubi, M. E.; Vaugeois, J.-M.; Kalinichev, M.; Célanire, S.; Parron, D.; Le Poul, E.; Campo, B. Effects of a mGluR2/3 negative allosteric modulator and a reference mGluR2/3 orthosteric antagonist in a genetic mouse model of depression. In *Behavioral Studies of Mood Disorders*; Proceedings of the 40th Annual Meeting of the Society for Neuroscience, San Diego, CA, Nov 13–17, 2010; Society for Neuroscience: Washington, DC, 2010; 886.14/VV7.

(32) Goeldner, C.; Ballard, T. M.; Knoflach, F.; Wichmann, J.; Gatti, S.; Umbricht, D. Cognitive impairment in major depression and the mGlu2 receptor as a therapeutic target. *Neuropharmacology* **2013**, *64*, 337–346.

(33) Kalinichev, M.; Campo, B.; Lambeng, N.; Célanire, S.; Schneider, M.; Bessif, A.; Royer-Urios, I.; Parron, D.; Legrand, C.; Mahious, N.; Girard, F.; Le Poul, E. An mGluR2/3 negative allosteric modulator improves recognition memory assessed by natural forgetting in the novel object recognition test in rats. In *Memory* *Consolidation and Reconsolidation: Molecular Mechanisms II*; Proceedings of the 40th Annual Meeting of the Society for Neuroscience, San Diego, CA, Nov 13–17, 2010; Society for Neuroscience: Washington, DC, 2010; 406.9/MMM57.

(34) Choi, C. H.; Schoenfeld, B. P.; Bell, A. J.; Hinchey, P.; Kollaros, M.; Gertner, M. J.; Woo, N. H.; Tranfaglia, M. R.; Bear, M. F.; Zukin, R. S.; McDonald, T. V.; Jongens, T. A.; McBride, S. M. Pharmacological reversal of synaptic plasticity deficits in the mouse model of fragile X syndrome by group II mGluR antagonist or lithium treatment. *Brain Res.* **2011**, *1380*, 106–119.

(35) Gatti McArthur, S.; Saxe, M.; Wichmann, J.; Woltering, T. mGlu2/3 antagonists for the treatment of autistic disorders. PCT Int. Pat. Appl. WO 2014/064028 A1, May 1, 2014.

(36) Structure of decoglurant disclosed in Recommended International Nonproprietary Names (INN). *WHO Drug Information*; World Health Organization: Geneva, Switzerland, 2013; Vol. 27, no. 3, p 150.

(37) ARTDeCo Study: A study of RO4995819 in patients with major depressive disorder and inadequate response to ongoing antidepressant treatment. *ClinicalTrials.gov;* U.S. National Institutes of Health: Bethesda, MD, 2011; https://www.clinicaltrials.gov/ct2/show/NCT01457677 (accessed August 12, 2015).

(38) Rodriguez, A. L.; Grier, M. D.; Jones, C. K.; Herman, E. J.; Kane, A. S.; Smith, R. L.; Williams, R.; Zhou, Y.; Marlo, J. E.; Days, E. L.; Blatt, T. N.; Jadhav, S.; Menon, U. N.; Vinson, P. N.; Rook, J. M.; Stauffer, S. R.; Niswender, C. M.; Lindsley, C. W.; Weaver, C. D.; Conn, P. J. Discovery of novel allosteric modulators of metabotropic glutamate receptor subtype 5 reveals chemical and functional diversity and in vivo activity in rat behavioral models of anxiolytic and antipsychotic activity. *Mol. Pharmacol.* **2010**, *78*, 1105–1123.

(39) Sheffler, D. J.; Wenthur, C. J.; Bruner, J. A.; Carrington, S. J. S.; Vinson, P. N.; Gogi, K. K.; Blobaum, A. L.; Morrison, R. D.; Vamos, M.; Cosford, N. D. P.; Stauffer, S. R.; Daniels, J. S.; Niswender, C. M.; Conn, P. J.; Lindsley, C. W. Development of a novel, CNS-penetrant, metabotropic glutamate receptor 3 (mGlu₃) NAM probe (ML289) derived from a closely related mGlu₅ PAM. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3921–3925.

(40) Wenthur, C. J.; Morrison, R.; Felts, A. S.; Smith, K. A.; Engers, J. L.; Byers, F. W.; Daniels, J. S.; Emmitte, K. A.; Conn, P. J.; Lindsley, C. W. Discovery of (R)—(2-fluoro-4-((-4-methoxyphenyl)ethynyl)-phenyl(3-hydroxypiperidin-1-yl)methanone (ML337), An mGlu₃ selective and CNS penetrant negative allosteric modulator (NAM). *J. Med. Chem.* **2013**, 56, 5208–5212.

(41) Walker, A. G.; Wenthur, C. J.; Xiang, Z.; Rook, J. M.; Emmitte, K. A.; Niswender, C. M.; Lindsley, C. W.; Conn, P. J. Metabotropic glutamate receptor 3 activation is required for long-term depression in medial prefrontal cortex and fear extinction. *Proc. Natl. Acad. Sci. U. S.* A. **2015**, *112*, 1196–1201.

(42) Zhang, L.; Balan, G.; Barreiro, G.; Boscoe, B. P.; Chenard, L. K.; Cianfrogna, J.; Claffey, M. M.; Chen, L.; Coffman, K. J.; Drozda, S. E.; Dunetz, J. R.; Fonseca, K. R.; Galatsis, P.; Grimwood, S.; Lazzaro, J. T.; Mancuso, J. Y.; Miller, E. L.; Reese, M. R.; Rogers, B. N.; Sakurada, I.; Skaddan, M.; Smith, D. L.; Stepan, A. F.; Trapa, P.; Tuttle, J. B.; Verhoest, P. R.; Walker, D. P.; Wright, A. S.; Zaleska, M. M.; Zasadny, K.; Shaffer, C. L. Discovery and preclinical characterization of 1methyl-3-(4-methylpyridin-3-yl)-6-(pyridin-2-ylmethoxy)-1*H*-pyrazolo-[3,4-*b*]pyrazine (PF470): a highly potent, selective, and efficacious metabotropic glutamate receptor 5 (mGluR5) negative allosteric modulator. *J. Med. Chem.* **2014**, *S7*, 861–877.

(43) Zhuo, X.; Huang, X. S.; Degnan, A. P.; Snyder, L. B.; Yang, F.; Huang, H.; Shu, Y.-Z.; Johnson, B. M. Identification of glutathione conjugates of acetylene-containing positive allosteric modulators of metabotropic glutamate receptor subtype 5. *Drug Metab. Dispos.* **2015**, 43, 578–589.

(44) Noetzel, M. J.; Rook, J. M.; Vinson, P. N.; Cho, H.; Days, E.; Zhou, Y.; Rodriguez, A. L.; Lavreysen, H.; Stauffer, S. R.; Niswender, C. M.; Xiang, Z.; Daniels, J. S.; Jones, C. K.; Lindsley, C. W.; Weaver, C. D.; Conn, P. J. Functional impact of allosteric agonist activity of selective positive allosteric modulators of metabotropic glutamate receptor subtype 5 in regulating central nervous system function. *Mol. Pharmacol.* **2012**, *81*, 120–133.

(45) Niswender, C. M.; Johnson, K. A.; Luo, Q.; Ayala, J. E.; Kim, C.; Conn, P. J.; Weaver, C. D. A novel assay of Gi/o-linked G proteincoupled receptor coupling to potassium channels provides new insights into the pharmacology of the group III metabotropic glutamate receptors. *Mol. Pharmacol.* **2008**, *73*, 1213–1224.

(46) Conn, P. J.; Lindsley, C. W.; Stauffer, S. L.; Bartolome-Nebreda, J. M.; Conde-Ceide, S.; MacDonald, G. J.; Tong, H. M.; Jones, C. K.; Alcazar-Vaca, J. M.; Andres-Gil, J. I.; Malosh, C. Bicyclic triazole and pyrazole lactams as allosteric modulators of mGluR5 receptors. PCT Int. Pat. Appl. WO 2012/083224 A1, June 21, 2012.

(47) But, T. Y. S.; Toy, P. H. The Mitsunobu reaction: Origin, mechanism, improvements, and applications. *Chem. - Asian J.* **2007**, *2*, 1340–1355.

(48) Klapars, A.; Huang, X.; Buchwald, S. L. A general and efficient copper catalyst for the amidation of aryl halides. *J. Am. Chem. Soc.* **2002**, *124*, 7421–7428.

(49) Gregory, K. J.; Nguyen, E. D.; Reiff, S. D.; Squire, E. F.; Stauffer, S. R.; Lindsley, C. W.; Meiler, J.; Conn, P. J. Probing the metabotropic glutamate receptor 5 (mGlu₅) positive allosteric modulator (PAM) binding pocket: discovery of point mutations that engender a "molecular switch" in PAM pharmacology. *Mol. Pharmacol.* **2013**, *83*, 991–1006.

(50) Lamb, J. P.; Engers, D. W.; Niswender, C. M.; Rodriguez, A. L.; Venable, D. F.; Conn, P. J.; Lindsley, C. W. Discovery of molecular switches within the ADX-47273 mGlu₅ PAM scaffold that modulate modes of pharmacology to afford potent mGlu₅ NAMs, PAMs and partial antagonists. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2711–2714.

(51) Kalvass, J. C.; Maurer, T. S. Influence of nonspecific brain and plasma binding on CNS exposure: implications for rational drug discovery. *Biopharm. Drug Dispos.* **2002**, *23*, 327–338.

(52) Bridges, T. M.; Morrison, R. D.; Byers, F. W.; Luo, S.; Daniels, J. S. Use of a novel rapid and resource-efficient cassette dosing approach to determine the pharmacokinetics and CNS distribution of small molecule 7-transmembrane receptor allosteric modulators in rat. *Pharmacol. Res. Perspect.* **2014**, *2*, e00077.

(53) Leeson, P. D.; Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discovery* **2007**, *6*, 881–890.

(54) Obach, R. S. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab. Dispos.* **1999**, *27*, 1350–1359.

(55) Davies, B.; Morris, T. Physiological parameters in laboratory animals and humans. *Pharm. Res.* **1993**, *10*, 1093–1095.

(56) Bridges, T. M.; Rook, J. M.; Noetzel, M. J.; Morrison, R. D.; Zhou, Y.; Gogliotti, R. D.; Vinson, P. N.; Xiang, Z.; Jones, C. K.; Niswender, C. M.; Lindsley, C. W.; Stauffer, S. L.; Conn, P. J.; Daniels, J. S. Biotransformation of a novel positive allosteric modulator of metabotropic glutamate receptor subtype 5 contributes to seizure-like adverse events in rats involving a receptor agonism-dependent mechanism. *Drug Metab. Dispos.* **2013**, *41*, 1703–1714.

(57) Di, L.; Rong, H.; Feng, B. Demystifying brain penetration in central nervous system drug discovery. J. Med. Chem. 2013, 56, 2–12.

(58) Hemstapat, K.; Da Costa, H.; Nong, Y.; Brady, A. E.; Luo, Q.; Niswender, C. M.; Tamagnan, G. D.; Conn, P. J. A novel family of potent negative allosteric modulators of group II metabotropic glutamate receptors. *J. Pharmacol. Exp. Ther.* **2007**, 322, 254–264.

(59) *LeadProfilingScreen;* (catalogue no. 68) Eurofins Panlabs, Inc.: Redmond, WA (www.eurofinspanlabs.com).

(60) Significant responses are defined as those that inhibited more than 50% of radioligand binding.

(61) Fitzgerald, L. W.; Burn, T. C.; Brown, B. S.; Patterson, J. P.; Corjay, M. H.; Valentine, P. A.; Sun, J. H.; Link, J. R.; Abbaszade, I.; Hollis, J. M.; Largent, B. L.; Hartig, P. R.; Hollis, G. F.; Meunier, P. C.; Robichaud, A. J.; Robertson, D. W. Possible role of valvular serotonin 5-HT(2B) receptors in the cardiopathy associated with fenfluramine. *Mol. Pharmacol.* **2000**, *57*, 75–81. (62) Serotonin (5-Hydroxytryptamine) $5-HT_{2B}$, IP_{1} ; (catalogue no. 355260); Eurofins Panlabs, Inc.: Redmond, WA, (www. eurofinspanlabs.com).

(63) Zientek, M.; Miller, H.; Smith, D.; Dunklee, M. B.; Heinle, L.; Thurston, A.; Lee, C.; Hyland, R.; Fahmi, O.; Burdette, D. Development of an in vitro drug-drug interaction assay to simultaneously monitor five cytochrome P450 isoforms and performance assessment using drug library compounds. *J. Pharmacol. Toxicol. Methods* **2008**, *58*, 206–214.

(64) Wang, Q.; Rager, J. D.; Weinstein, K.; Kardos, P. S.; Dobson, G. L.; Li, J.; Hidalgo, I. J. Evaluation of the MDR-MDCK cell line as a permeability screen for the blood-brain barrier. *Int. J. Pharm.* **2005**, 288, 349–359.

(65) Njung'e, K.; Handley, S. L. Evaluation of marble-burying behavior as a model of anxiety. *Pharmacol., Biochem. Behav.* **1991**, *38*, 63–67.

(66) Njung'e, K.; Handley, S. L. Effects of 5-HT uptake inhibitors, agonists and antagonists on the burying of harmless objects by mice; a putative test for anxiolytic agents. *Br. J. Pharmacol.* **1991**, *104*, 105–112.

(67) Nicolas, L. B.; Kolb, Y.; Prinssen, E. P. A combined marble burying-locomotor activity test in mice: a practical screening test with sensitivity to different classes of anxiolytics and antidepressants. *Eur. J. Pharmacol.* **2006**, *547*, 106–115.

(68) Felts, A. S.; Rodriguez, A. L.; Morrison, R. D.; Venable, D. F.; Manka, J. T.; Bates, B. S.; Blobaum, A. L.; Byers, F. W.; Daniels, J. S.; Niswender, C. M.; Jones, C. K.; Conn, P. J.; Lindsley, C. W.; Emmitte, K. A. Discovery of VU0409106: A negative allosteric modulator of mGlu5 with activity in a mouse model of anxiety. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5779–5785.

(69) Thomas, A.; Burant, A.; Bui, N.; Graham, D.; Yuva-Paylor, L. A.; Paylor, R. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology* **2009**, *204*, 361–373.

(70) Cosford, N. D.; Tehrani, L.; Roppe, J.; Schweiger, E.; Smith, N. D.; Anderson, J.; Bristow, L.; Brodkin, J.; Jiang, X.; McDonald, I.; Rao, S.; Washburn, M.; Varney, M. A. 3-[(2-Methyl-1,3-thiazol-4-yl)-ethynyl]-pyridine: a potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. *J. Med. Chem.* **2003**, *46*, 204–206.

(71) Detke, M. J.; Rickels, M.; Lucki, I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology (Berl)* **1995**, *121*, 66–72.

(72) Garcia, L. S.; Comim, C. M.; Valvassori, S. S.; Réus, G. Z.; Barbosa, L. M.; Andreazza, A. C.; Stertz, L.; Fries, G. R.; Gavioli, E. C.; Kapczinski, F.; Quevedo, J. Acute administration of ketamine induces antidepressant-like effects in the forced swimming test and increases BDNF levels in the rat hippocampus. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2008**, *32*, 140–144.

(73) Kohrs, R.; Durieux, M. E. Ketamine: Teaching an old drug new tricks. *Anesth. Analg.* **1998**, *87*, 1186–1193.

(74) Murrough, J. W.; Iosifescu, D. V.; Chang, L. C.; Al Jurdi, R. K.; Green, C. E.; Perez, A. M.; Iqbal, S.; Pillemer, S.; Foulkes, A.; Shah, A.; Charney, D. S.; Mathew, S. J. Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *Am. J. Psychiatry* **2013**, *170*, 1134–1142.

(75) Browne, C. A.; Lucki, I. Antidepressant effects of ketamine: mechanisms underlying fast-acting novel antidepressants. *Front. Pharmacol.* **2013**, *4*, DOI: 10.3389/fphar.2013.00161.

(76) aan het Rot, M.; Collins, K. A.; Murrough, J. W.; Perez, A. M.; Reich, D. L.; Charney, D. S.; Mathew, S. J. Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biol. Psychiatry* **2010**, *67*, 139–145.

(77) Morgan, C. J. A.; Curran, H. V. Ketamine use: A review. *Addiction* **2012**, *107*, 27–38.

(78) Zhou, W.; Wang, N.; Yang, C.; Li, X.-M.; Zhou, Z.-Q.; Yang, J.-J. Ketamine-induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus and prefrontal cortex. *Eur. Psychiatry* **2014**, *29*, 419–423.

(79) Koike, H.; Chaki, S. Requirement of AMPA receptor stimulation for the sustained antidepressant activity of ketamine and LY341495 during the forced swim test in rats. *Behav. Brain Res.* **2014**, *271*, 111–115.

(80) Duman, R. S.; Li, N.; Liu, R.-J.; Duric, V.; Aghajanian, G. Signaling pathways underlying the rapid antidepressant actions of ketamine. *Neuropharmacology* **2012**, *62*, 35–41.

(81) Dwyer, J. M.; Lepack, A. E.; Duman, R. S. mTOR activation is required for the antidepressant effects of mGluR_{2/3} blockade. *Int. J. Neuropsychopharmacol.* **2012**, *15*, 429–434.

(82) Li, N.; Lee, B.; Liu, R. J.; Banasr, M.; Dwyer, J. M.; Iwata, M.; Li, X. Y.; Aghajanian, G.; Duman, R. S. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* **2010**, *329*, 959–964.