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Discovery of VU0431316: A negative allosteric modulator of mGlu₅ with activity in a mouse model of anxiety



Brittney S. Bates^{a,b}, Alice L. Rodriguez^{a,b}, Andrew S. Felts^{a,b}, Ryan D. Morrison^{a,b}, Daryl F. Venable^{a,b}, Anna L. Blobaum^{a,b}, Frank W. Byers^{a,b}, Kera P. Lawson^{a,b}, J. Scott Daniels^{a,b}, Colleen M. Niswender^{a,b}, Carrie K. Jones^{a,b,d}, P. Jeffrey Conn^{a,b}, Craig W. Lindsley^{a,b,c}, Kyle A. Emmitte^{a,b,c,*}

^a Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

^b Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

^c Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA

^d Tennessee Valley Healthcare System, U.S. Department of Veterans Affairs, Nashville, TN 37212, USA

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ABSTRACT

Development of SAR in an aryl ether series of mGlu₅ NAMs leading to the identification of pyrazine analog VU0431316 is described in this Letter. VU0431316 is a potent and selective non-competitive antagonist of mGlu₅ that binds at a known allosteric binding site. VU0431316 demonstrates an attractive DMPK profile, including moderate clearance and good bioavailability in rats. Intraperitoneal (IP) dosing of VU0431316 in a mouse marble burying model of anxiety, an assay known to be sensitive to mGlu₅ antagonists and other anxiolytics, produced dose proportional effects.

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The metabotropic glutamate receptors (mGlu₅) comprise a family of eight related G-protein-coupled receptors (GPCRs) wherein each receptor acts through binding glutamate, the major excitatory transmitter in the mammalian central nervous system (CNS). In these seven transmembrane spanning (7TM) receptors, the orthosteric binding sites are located in the extracellular domain while known allosteric binding sites are contained in the transmembrane domain.¹ Design of highly selective orthosteric ligands has continually proven difficult due to the extensive homology of the binding sites across the mGlu family. In many instances, the development of allosteric modulators of mGlu₅ has been established as a viable solution to enhancing selectivity among family members.² Among the individual mGlu₅ investigated as potential drug targets, a substantial portion of that attention has been devoted to the design of small molecule negative allosteric modulators (NAMs), or non-competitive antagonists, of mGlu₅.³

The vast majority of the preclinical behavioral work with mGlu₅ NAMs has been conducted using one of two structurally related disubstituted alkyne tool compounds, MPEP⁴ and MTEP⁵ (Fig. 1).

Efficacy has been reported with these compounds across a number of different disease models. Examples include pain,⁶ anxiety,⁷ gastroesophageal reflux disease (GERD),⁸ Parkinson's disease levodopa induced dyskinesia (PD-LID),⁹ fragile X syndrome (FXS),¹⁰ and other autism spectrum disorders.¹¹ Furthermore, both MPEP and MTEP have been used effectively in various animal models of addictive behavior with well-known drugs of abuse, such as cocaine,¹² nicotine,^{12,13} methamphetamine,¹⁴ morphine,¹⁵ and ethanol.¹⁶

Currently, several mGlu₅ NAMs have progressed to human trials, and results from studies in patients with GERD,¹⁷ FXS,¹⁸ and PD-LID¹⁹ have been encouraging. Though structural diversity among mGlu₅ NAMs in the literature has expanded considerably in recent years, the majority of clinical compounds have been from the disubstituted alkyne structure class.^{3a} Furthermore, the most advanced clinical compounds, mavoglurant (AFQ056) and basimglurant (RG7090, RO4917523), each contain the alkyne moiety (Fig. 1).²⁰ Recently, concerns that such alkyne compounds might be prone to metabolic activation and resultant toxicities have proven warranted, at least in one instance. Pfizer has now disclosed their observation of biliary epithelial hyperplasia in non-human primate regulatory toxicology studies with the disubstituted alkyne compound known as GRN-529. Glutathione

* Corresponding author. Tel.: +1 615 936 8401; fax: +1 615 322 8577.

E-mail address: kyle.a.emmitte@vanderbilt.edu (K.A. Emmitte).

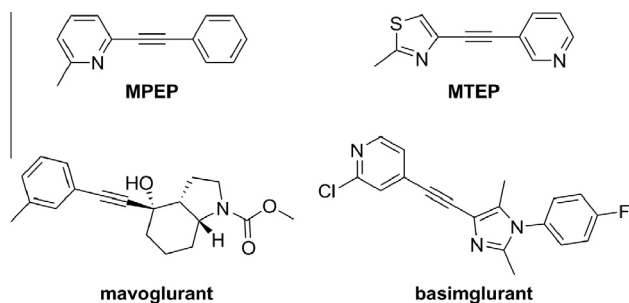


Figure 1. mGlu₅ NAM tool and advanced clinical compounds.

conjugation at the alkyne moiety was believed to be related to these adverse findings.²¹

Our mGlu₅ NAM program has long been centered on the identification and optimization of compounds from chemotypes that do not contain a disubstituted alkyne motif. The majority of this effort has been spent on the optimization of hits identified from a functional cell-based high-throughput screen (HTS) of a collection of 160,000 compounds;²² however, rational design approaches²³ and a virtual screening approach also produced new non-alkyne based mGlu₅ NAM tool compounds.²⁴ We recently reported on a lead optimization effort based around hit compound **1** from our functional HTS (Fig. 2).^{22a} This particular optimization effort, based on **1**, culminated in discovery of the in vivo tool compound VU0409106.

Concomitant to the recently described work that led to the discovery of VU0409106, we were also pursuing additional analogs of **1**. Reasoning that a potential route of metabolism for analogs of **1** might include amide bond cleavage, we immediately sought to identify compounds that would not produce electron rich anilines should the amide bond indeed be cleaved in vivo. In the case of VU0409106 and associated analogs, we achieved this goal by reversing the orientation of the amide bond; however, the work described herein centers on the replacement of the phenyl core with heteroaryl rings. Preparation of the initial heteroaryl ether analogs of **1** was executed according to one of the two general methods outlined here (Scheme 1).²⁵

Certain pyridine (**10–12**) and pyrimidine (**13**) analogs were prepared by first coupling the commercial amines **2–5** with 3-chlorobenzoic acid to afford the corresponding amides **6–9** (Route I). Reaction with 3-hydroxypyridine in the presence of copper (I) iodide and dimethylglycine afforded the desired compounds **10–13**. Alternatively, pyridine **20**, pyrazine **21**, and pyrimidine **22** were prepared via a route relying on initial installation of the aryl ether (Route II). Reaction of the commercial monomers **14–16** with 3-hydroxypyridine in a microwave assisted nucleophilic aromatic substitution (S_NAr) reaction provided heteroaryl halide intermediates **17–19**. The final analogs were prepared directly through a Buchwald–Hartwig coupling with 3-chlorobenzamide in moderate to high yields.²⁶

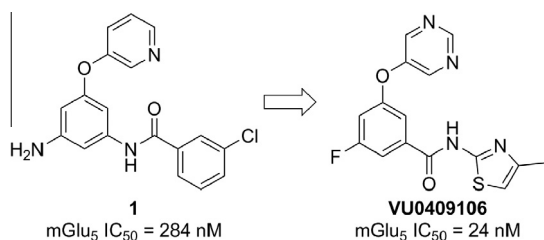
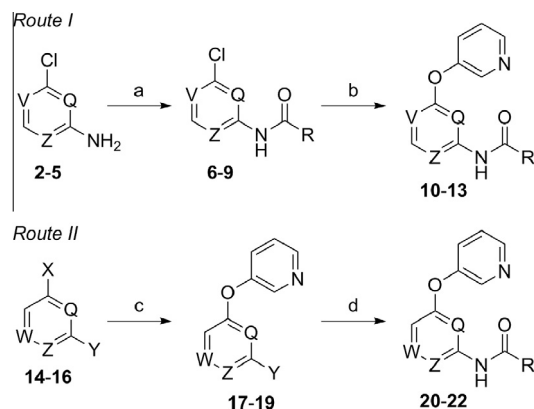


Figure 2. HTS hit **1** and mGlu₅ NAM in vivo tool VU0409106.




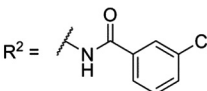
Scheme 1. Reagents and conditions: (a) For **2** (Z = N; V = Q = CH), **3** (V = N; Q = Z = CH), **4** (Q = N; V = Z = CH), and **5** (V = Z = N; Q = CH); RCO₂H (R = 3-chlorophenyl), EDC, DMAP, CH₂Cl₂, (83–94%); (b) 3-hydroxypyridine, CuI, Cs₂CO₃, Me₂NCH₂CO₂·H·Cl (35–55%); (c) For **14** (W = N; Q = Z = CH; X = F; Y = Br), **15** (Q = W = N; Z = CH; X = Y = Cl), and **16** (Q = Z = N; W = CH; X = Y = Cl); 3-hydroxypyridine, K₂CO₃, DMF, microwave, 150 °C (60–93%); (d) RCONH₂ (R = 3-chlorophenyl), NaO^tBu, Pd(OAc)₂, Xantphos, PhMe, 100 °C (50–78%).

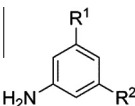
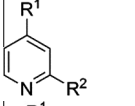
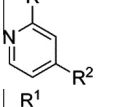
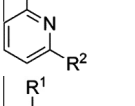
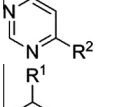
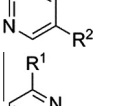
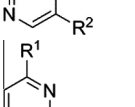
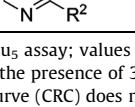
Evaluation of these initial analogs against mGlu₅ yielded clear SAR (Table 1). Our functional assay measures the ability of the compound to block the mobilization of calcium induced by an EC₈₀ concentration of glutamate in HEK293A cells expressing rat mGlu₅.²⁷ Among the pyridine analogs, compounds **12** and **20** were superior to compounds **10** and **11**. In fact both **12** and **20** exhibited potency at a level near hit **1**. Pyrimidine analogs **13** and **22** were weak antagonists; however, pyrazine **21** exhibited the best potency in this set of analogs. Having established the pyrazine core as a favorable group for further SAR development, lead optimization continued in that area.

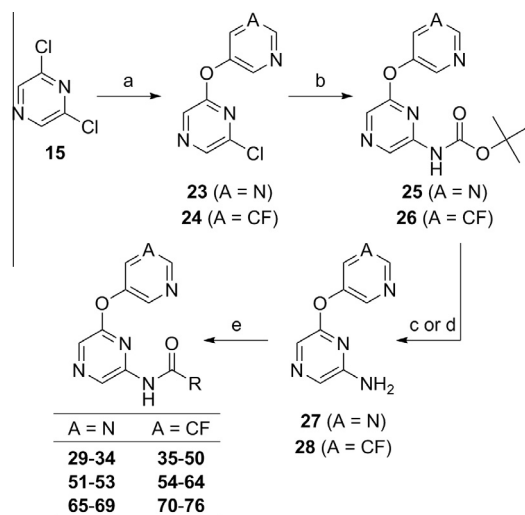
Ongoing research has identified the 5-fluoropyridin-3-yl and pyrimidin-5-yl ethers as optimal groups in the northern portion of the chemotype.^{22a} Thus, much of the SAR was developed in the context of one or both of these moieties. Compounds containing the pyrimidine moiety are less lipophilic than their 5-fluoropyridine counterparts,²⁸ a feature that can often provide advantages with respect to drug-like properties. Though the synthesis outlined in Scheme 1 (Route II) was utilized to prepare some new pyrazine analogs, a new synthetic route allowing for the preparation of a broader diversity of amides was utilized in most cases (Scheme 2).²⁹ This route also begins with a similar S_NAr reaction, providing ethers **23–24**. A Buchwald–Hartwig coupling with *t*-butyl carbamate was employed to afford intermediates **25–26**.³⁰ Cleavage of the protecting group was carried out under acidic conditions to yield amines **27–28**. Conversion to the desired amide products was accomplished using standard coupling conditions.

Evaluation of various substituted benzamides revealed some additional potent compounds (Table 2). As anticipated, both the 5-fluoropyridin-3-yl (**35**) and pyrimidin-5-yl (**29**) ethers proved competent replacements for the simple pyridine-3-yl (**21**) ether. Furthermore, the importance of the 3-chloro substituent on the benzamide was established through preparation of unsubstituted analogs **30** and **36**. Many additional 3-substituted analogs were prepared and tested (**31–34** and **36–41**); however, only the 3-methyl analogs **32** and **38** demonstrated potency comparable to **29** and **35**. 3-Cyanobenzamides **33** and **39** were approximately six fold less potent than 3-chlorobenzamide comparators **29** and **35**. Additional monosubstituted benzamides demonstrated moderate to weak antagonist activity. Several disubstituted benzamides were also evaluated (**42–50**), with only 2-fluoro-5-chlorobenzamide **45** demonstrating an IC₅₀ less than one micromolar.

Table 1
Core SAR

$R^1 =$

 $R^2 =$


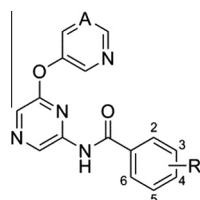
Compd	Core	mGlu ₅ pIC ₅₀ ^a (± SEM)	mGlu ₅ IC ₅₀ (nM)	% Glu Max ^{a,b} (± SEM)
1		6.55 ± 0.19	284	1.3 ± 0.2
10		6.00 ± 0.21	994	1.8 ± 0.7
11		5.43 ± 0.26	3700	−4.9 ± 4.7
12		6.44 ± 0.19	359	2.0 ± 0.7
13		5.53 ± 0.23	2930	1.1 ± 0.5
20		6.39 ± 0.07	403	1.1 ± 0.4
21		6.89 ± 0.04	129	1.0 ± 0.3
22		<5.0 ^c	>10,000	44 ± 8

^a Calcium mobilization mGlu₅ assay; values are average of $n \geq 3$.^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 $n \geq 3$.^c Concentration response curve (CRC) does not plateau.

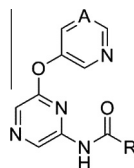
Scheme 2. Reagents and conditions: (a) 3-fluoro-5-hydroxypyridine or 5-hydroxypyrimidine, K₂CO₃, DMF, microwave, 120 °C (51% for **23**; 62% for **24**); (b) H₂NCO₂^tBu, NaO^tBu, Pd₂(dba)₃·CHCl₃, ^tBuXPhos, PhMe (46% for **25**; 64% for **26**); (c) For **25** → **27**, TFA, CH₂Cl₂ (96%); (d) For **26** → **28**, 4 N HCl in dioxane (100%); (e) RCO₂H, EDC, DMAP, CH₂Cl₂ or RCO₂H, HATU, DIEA, CH₂Cl₂, DMF or RCO₂H, POCl₃, pyridine or RCOCl, DMAP, CH₂Cl₂ (30–80%).

Turning our attention from benzamide analogs to analogs with a heteroaryl amide moiety identified a mixture of weak antagonists and compounds that were inactive up to the top concentration tested of 30 μ M (Table 3). Recognizing the importance of substitution that was observed in the case of the benzamide analogs, particularly at the 3-position, we decided to prepare some additional analogs of the weak antagonists identified from this initial set (Table 4). Methyl substitution resulted in modest potency enhancement in the case of thiophene **70** relative to **55**. More dramatic potency improvement was noted with picolinamides **67** and **75** when compared to **59**. Preparation of additional 4-substituted picolinamides (**68**, **69**, and **76**) identified 4-chloropicolinamide **68** (VU0431316) as the most potent mGlu₅ NAM in this series.

Further in vitro characterization of **68** (VU0431316) was subsequently initiated. Competitive displacement of the established radioligand [³H]3-methoxy-5-(pyridin-2-ylethynyl)pyridine³¹ confirmed the interaction of the compound with a known mGlu₅ allosteric binding site (mGlu₅ K_i = 37 nM ($n = 1$)).³² Evaluation of **68** (VU0431316) in cell based functional assays against the other seven mGlu₅ showed no detectable activity at 10 μ M.³³ Additionally, the functional activity of **68** (VU0431316) at human mGlu₅ was determined and was essentially identical to that at the rat receptor (human mGlu₅ IC₅₀ = 85 nM ($n = 1$)).³⁴ Finally, **68** (VU0431316)

Table 2
Benzamide SAR

Compd	A	R	mGlu ₅ pIC ₅₀ ^a (± SEM)	mGlu ₅ IC ₅₀ (nM)	% Glu Max ^{a,b} (± SEM)
29	N	3-Cl	6.94 ± 0.10	116	1.2 ± 0.0
30	N	H	<5.0 ^c	>10,000	12 ± 11
31	N	3-F	5.29 ± 0.17	5140	1.5 ± 2.7
32	N	3-Me	6.67 ± 0.16	212	1.5 ± 0.5
33	N	3-CN	6.15 ± 0.17	709	1.5 ± 0.2
34	N	3-OMe	5.39 ± 0.15	4110	−4.3 ± 2.7
35	CF	3-Cl	6.93 ± 0.10	118	2.0 ± 0.3
36	CF	H	5.06 ± 0.09	8650	−9.4 ± 2.3
37	CF	3-F	5.42 ± 0.09	3820	−0.4 ± 1.7
38	CF	3-Me	6.70 ± 0.18	199	2.1 ± 0.4
39	CF	3-CN	6.18 ± 0.16	662	1.2 ± 0.3
40	CF	3-OMe	5.36 ± 0.17	4400	−5.6 ± 5.1
41	CF	3-NMe ₂	<5.0 ^c	>10,000	52 ± 11
42	CF	2-F, 3-Cl	<4.5	>30,000	—
43	CF	3-Cl, 4-F	5.34 ± 0.15	4590	−2.8 ± 6.2
44	CF	3-Cl, 5-F	<5.0 ^c	>10,000	48 ± 10
45	CF	2-F, 5-Cl	6.05 ± 0.13	894	1.0 ± 0.8
46	CF	3,5-Di-F	<4.5	>30,000	—
47	CF	3,5-Di-Cl	<4.5	>30,000	—
48	CF	3,5-Di-Me	5.18 ± 0.18	6600	6.6 ± 2.0
49	CF	3,5-Di-OMe	<4.5	>30,000	—
50	CF	3-CN, 5-F	<4.5	>30,000	—

^a Calcium mobilization mGlu₅ assay; values are average of $n \geq 3$.^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 $n \geq 3$.^c CRC does not plateau.**Table 3**
Heteroaryl amide SAR

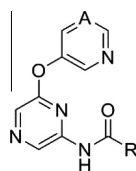
Compd	A	R	mGlu ₅ pIC ₅₀ ^a (± SEM)	mGlu ₅ IC ₅₀ (nM)	% Glu Max ^{a,b} (± SEM)
51	N		<5.0 ^c	>10,000	20 ^d
52	N		<5.0 ^c	>10,000	15 ± 8
53	N		<5.0 ^c	>10,000	35 ± 6
54	CF		5.2 ± 0.11	6370	7.8 ± 2.8
55	CF		5.5 ± 0.15	3450	0.1 ± 2.4
56	CF		<4.5	>30,000	—
57	CF		<5.0 ^c	>10,000	40 ± 9

Table 3 (continued)

Compd	A	R	mGlu ₅ pIC ₅₀ ^a (± SEM)	mGlu ₅ IC ₅₀ (nM)	% Glu Max ^{a,b} (± SEM)
58	CF		5.07 ± 0.05	8430	22 ± 11
59	CF		<5.0 ^c	>10,000	40 ± 15
60	CF		<4.5	>30,000	—
61	CF		<4.5	>30,000	—
62	CF		<4.5	>30,000	—
63	CF		<4.5	>30,000	—
64	CF		<4.5	>30,000	—

^a Calcium mobilization mGlu₅ assay; values are average of $n \geq 3$.^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 $n \geq 3$.^c CRC does not plateau.^d Average of $n = 2$.**Table 4**

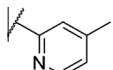
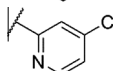
Substituted heteroaryl amide SAR



Compd	A	R	mGlu ₅ pIC ₅₀ ^a (± SEM)	mGlu ₅ IC ₅₀ (nM)	% Glu Max ^{a,b} (± SEM)
65	N		<4.5	>30,000	—
66	N		<4.5	>30,000	—
67	N		6.72 ± 0.22	193	2.1 ± 0.8
68	N		7.20 ± 0.06	62.4	1.6 ± 0.1
69	N		7.00 ± 0.29	100	2.3 ± 0.6
70	CF		6.00 ± 0.04	990	1.1 ± 0.75
71	CF		<5.0 ^c	>10,000	20 ± 10
72	CF		5.33 ± 0.17	4640	19 ± 6
73	CF		<4.5	>30,000	—
74	CF		<4.5	>30,000	—

(continued on next page)

Table 4 (continued)

Compd	A	R	mGlu ₅ pIC ₅₀ ^a (± SEM)	mGlu ₅ IC ₅₀ (nM)	% Glu Max ^{a,b} (± SEM)
75	CF		6.51 ± 0.15	311	1.7 ± 0.4
76	CF		6.95 ± 0.29	111	2.0 ± 0.3

^a Calcium mobilization mGlu₅ assay; values are average of $n \geq 3$.^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 $n \geq 3$.^c CRC does not plateau.

Table 5

In vitro DMPK Profile for **68** (VU0431316)

Protein binding ^{a,b} (<i>F</i> _u)				MDCK-WT permeability	
Rat PPB	0.179			<i>P</i> _{app} (A-B)	50.4 × 10 ⁻⁶ cm/s
Cynomolgus PPB	0.167			<i>P</i> _{app} (B-A)	45.0 × 10 ⁻⁶ cm/s
Human PPB	0.120			Efflux ratio	0.89
Mouse BHB	0.054				
CYP inhibition IC ₅₀ ^c (μM)				Caco-2 permeability	
3A4	2D6	2C9	1A2	<i>P</i> _{app} (A-B)	66.8 × 10 ⁻⁶ cm/s
>30	>30	>30	0.6	<i>P</i> _{app} (B-A)	71.4 × 10 ⁻⁶ cm/s
				Efflux ratio	1.07

^a F_u = fraction unbound.^b PPB = plasma protein binding; BHB = brain homogenate binding.^c Assayed in pooled HLM in the presence of NADPH.

Table 6

Rat PK Results for **68** (VU0431316)

IV ^{a,b}		PO ^{a,c}		
Half-life	193 min	Plasma C _{max}	1.12 μM	
Clearance	30.2 mL/min/kg	Plasma T _{max}	240 min	
V _{ss}	2.7 L/kg	AUC	8.37 μM h	
AUC	1.73 μM h	F	48%	
PO tissue distribution study ^{a,c}				
Time	Plasma	HPV	Brain	B/P ratio
60 min	0.92 μM	1.31 μM	1.45 μM	1.6

^a Male Sprague-Dawley rats ($n = 2$ per time point).^b 1 mg/kg; vehicle = 10% ethanol, 90% PEG400.^c 10 mg/kg; vehicle = 10% Tween 80 in 0.5% MC.

was tested in a commercially available radioligand binding assay panel of **68** clinically relevant GPCRs, ion channels, kinases, and transporters,³⁵ and no significant responses were found at 10 μ M compound.³⁶

The pharmacological profile of **68** (VU0431316) warranted further evaluation of its drug-likeness and potential utility as an *in vivo* tool (Table 5). Evaluation of the compound's propensity to bind to plasma proteins revealed similar results across multiple species. Nonspecific binding to mouse brain homogenates was also evaluated to enable the estimation of the fraction unbound in the CNS.³⁷ Bidirectional permeability was assessed in both Madin Darby canine kidney (MDCK) and human intestinal epithelial (Caco-2) cells, and permeability was high with no evidence of efflux.³⁸ Determination of the cytochrome P450 (CYP) inhibition profile of **68** (VU0431316) indicated potent inhibition of CYP1A2 with no inhibition of other isoforms up to the top concentration tested of 30 μ M.³⁹ As was the case with the related tool compound VU0409106, the common pyrimidine ether moiety resulted in a major non-P450 mediated metabolic pathway for **68** (VU0431316).⁴⁰

The *in vitro* DMPK profile of **68** (VU0431316) was deemed supportive of *in vivo* evaluation (Table 6). Pharmacokinetic parameters

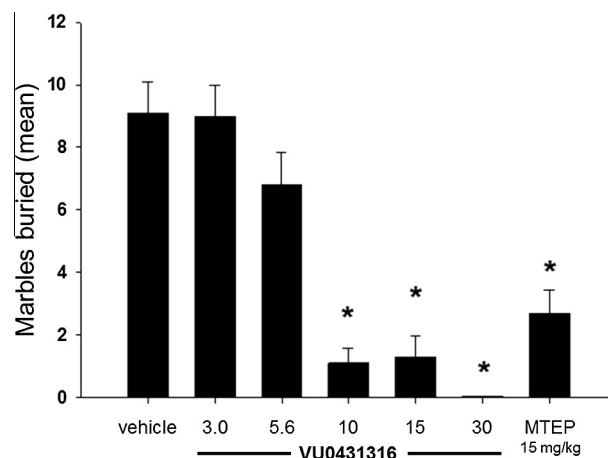


Figure 3. Dose dependent inhibition of marble burying with **68** (VU0431316); $n = 7-8$ per dose; * $P < 0.05$ versus vehicle control group, Dunnett's test. Bars denote marbles buried. Vehicle = 10% Tween 80.

were calculated from an IV dosing of **68** (VU0431316) to male Sprague-Dawley rats; hepatic clearance was moderate with an approximate three hour half-life. Bioavailability from a single oral (PO) dose was also encouraging, approaching fifty percent. A satellite tissue distribution study was conducted one hour after a 10 mg/kg PO dose. Seventy percent of the compound was detected in plasma relative to the hepatic portal vein, indicative of a low first-pass effect and consistent with the previously observed clearance. CNS penetration was also excellent with a brain to plasma ratio (B/P) of 1.6.⁴¹

It is recognized that naïve mice will bury foreign objects such as glass marbles in deep bedding. Pretreating such mice with low doses of anxiolytic benzodiazepines will consistently inhibit this behavior.⁴² Additionally, the well-known and thoroughly characterized mGlu₅ NAMs MPEP and fenobam each inhibit marble burying in this model.^{7a,d} Furthermore, we have used this model successfully to evaluate several of our own novel mGlu₅ NAM tool compounds.^{22a,23b,24} More recent reports have raised the possibility that marble burying reflects a repetitive and perseverative behavior as opposed to novelty-induced anxiety.⁴³ Still, given the convenience and reliability of the marble burying assay, it has served as the frontline behavioral assay for our mGlu₅ NAM discovery program.⁴⁴ Thus, a dose response study with **68** (VU0431316) using a 15 minute pretreatment time following intraperitoneal (IP) administration was carried out in this model (Fig. 3).⁴⁵ Statistically significant inhibition of marble burying was noted at all doses greater than or equal to 10 mg/kg. A satellite tissue distribution experiment in mice at the top dose of 30 mg/kg (IP) showed considerable concentrations of compound in the brain.⁴⁶ Using the results from this study to extrapolate exposures at 10 mg/kg

along with the aforementioned brain homogenate binding data indicates that unbound compound concentrations in the brain were likely near the functional IC₅₀ at the minimum effective dose.

In conclusion, a second in vivo tool compound has been developed from HTS hit **1**. VU0431316 offers an advantage to VU0409106 in that it is orally bioavailable in rats. VU0431316 is a potent and selective mGlu₅ NAM binding to a known allosteric site. CNS exposure in both mice and rats is quite good and efficacy has been established in a proven anxiolytic model. A host of additional behavioral models associated with mGlu₅ are either planned or already underway with VU0431316 and will be the subject of future communications.

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- According to the cLogP calculator developed by ADRIANA.Code (www.molecular-networks.com) 5-fluoropyridin-3-yl ether compounds are ~1.2 units more lipophilic than their pyrimidin-5-yl ether counterparts in this chemotype (e.g., **76** cLogP = 3.72; **68** cLogP = 2.51).
- The synthesis of VU0431316 (**68**) is representative: (i) 5-((6-chloropyrazin-2-yl)oxy)pyrimidine (**23**). A mixture of 2,6-dichloropyrazine (1.00 g, 6.71 mmol, 1.00 equiv), pyrimidin-5-ol (645 mg, 6.71 mmol, 1.00 equiv), and potassium carbonate (1.39 g, 10.1 mmol, 1.50 equiv) in DMF (20 mL) was heated via microwave irradiation at 120 °C for 10 min. The reaction was cooled and diluted with EtOAc and washed with H₂O (3×) and brine (1×). The organic layer was dried (MgSO₄), filtered, and concentrated in vacuo. Purification by flash chromatography on silica gel afforded 710 mg (51%) of the title

- compound. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.15 (s, 1H), 8.93 (s, 2H), 8.73 (s, 1H), 8.62 (s, 1H); ES-MS $[\text{M}+\text{H}]^+$: 209.1. (ii) *tert*-Butyl (6-(pyrimidin-5-yloxy)pyrazin-2-yl)carbamate (**25**). Compound **23** (760 mg, 3.64 mmol, 1.00 equiv), *tert*-butyl carbamate (117 mg, 4.37 mmol, 1.20 equiv), $\text{Pd}_2(\text{dba})_3\cdot\text{CHCl}_3$ (113 mg, 0.110 mmol, 0.030 equiv), NaO^tBu (490 mg, 5.10 mmol, 1.40 equiv), and $^t\text{BuXPhos}$ (164 mg, 0.330 mmol, 0.090 equiv) were stirred in toluene (14 mL) at rt overnight. The mixture was filtered through celite and washed with 5% MeOH in CH_2Cl_2 . The filtrate was collected and concentrated in vacuo. Purification by flash chromatography on silica gel afforded 480 mg (46%) of the title compound. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.14 (s, 1H), 9.09 (s, 1H), 8.91 (s, 2H), 8.82 (s, 1H), 8.25 (s, 1H), 1.45 (s, 9H); ES-MS $[\text{M}+\text{H}]^+$: 290.1. (iii) 6-(pyrimidin-5-yloxy)pyrazin-2-amine (**27**). Compound **25** (480 mg, 1.66 mmol, 1.00 equiv) was stirred in 4:1 CH_2Cl_2 :TFA (2 mL) at rt overnight. The reaction mixture was concentrated in vacuo and diluted in EtOAc. The organic layer was washed with saturated NaHCO_3 (3 \times) and brine (1 \times), dried (MgSO_4), filtered and concentrated in vacuo to give 300 mg (96%) of the title compound. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.04 (s, 1H), 8.79 (s, 2H), 7.65 (s, 1H), 7.60 (s, 1H), 6.64 (s, 2H); ES-MS $[\text{M}+\text{H}]^+$: 190.1. (iv) 4-Chloro-*N*-(6-(pyrimidin-5-yloxy)pyrazin-2-yl)picolinamide (**68**, VU0431316). Compound **27** (150 mg, 0.790 mmol, 1.00 equiv) and 4-chloropicolinic acid (125 mg, 0.790 mmol, 1.00 equiv) were dissolved in pyridine (10 mL) under argon and cooled to -15°C with stirring. POCl_3 (0.080 mL, 0.870 mmol, 1.10 equiv) was added dropwise. The resulting reaction mixture was stirred at -15°C for 30 min. The reaction was then warmed to rt and quenched with ice water. The mixture was extracted with CH_2Cl_2 . The organics were washed with water (3 \times) and brine (1 \times). The organic layer was dried (MgSO_4), filtered, and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel to provide 125 mg (48%) of the desired product as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.64 (s, 1H), 9.24 (s, 1H), 9.14 (s, 1H), 8.99 (s, 2H), 8.71 (d, $J = 5.3$ Hz 1H) 8.50 (s, 1H), 8.19 (d, $J = 1.8$ Hz, 1H), 7.89 (dd, $J = 5.3, 2.0$ Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 329.1.
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 45. IP dosing has proven over time to be a convenient and consistent route of administration for our behavioral studies in mice.
 46. Mice were euthanized and decapitated at predetermined time-points after dose administration and blood and brain samples were collected. Following protein precipitation, the supernatants of all plasma and brain homogenate samples were analyzed by means of LC–MS/MS. PK studies were approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee. Average plasma concentrations: 3.99 μM (15 min), 3.00 μM (30 min), and 1.69 μM (60 min). Average brain concentrations: 6.21 μM (15 min), 3.69 μM (30 min), and 2.80 μM (60 min).