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Discovery of VU0409106: A negative allosteric modulator of $mGlu_5$ with activity in a mouse model of anxiety

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ABSTRACT

Development of SAR in an aryl ether series of $mGlu_5$ NAMs leading to the identification of tool compound VU0409106 is described in this Letter. VU0409106 is a potent and selective negative allosteric modulator of $mGlu_5$ that binds at the known allosteric binding site and demonstrates good CNS exposure following intraperitoneal dosing in mice. VU0409106 also proved efficacious in a mouse marble burying model of anxiety, an assay known to be sensitive to $mGlu_5$ antagonists as well as clinically efficacious anxiolytics.
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The metabotropic glutamate receptors (mGlus) are a family of eight related G-protein-coupled receptors (GPCRs) that act through binding glutamate (L-glutamic acid), the major excitatory transmitter in the mammalian central nervous system (CNS). The orthosteric binding sites of these seven transmembrane (7TM) receptors are located in the extracellular domain while allosteric binding sites identified to date are located in the transmembrane domain.[1] Due to a highly conserved orthosteric binding site across the members of the mGlu family, the design of selective orthosteric ligands has been challenging. A solution to this problem that has garnered much attention and proven effective in many instances has been the development of allosteric modulators of mGlus.[2] One of the more developed areas within the mGlu allosteric modulator field has been the design of small molecule negative allosteric modulators (NAMs), also known as noncompetitive antagonists, of $mGlu_5$.[3]

Extensive preclinical in vivo work has been published with two structurally related disubstituted alkyne tool compounds, MPEP[4] and MTEP[5] (Fig. 1). Efficacy in numerous animal models has been noted with these compounds, including pain,[6] anxiety,[7] gastroesophageal reflux disease (GERD),[8] Parkinson's disease levodopa induced dyskinesia (PD-LID),[9] and fragile X syndrome (FXS).[10] Furthermore, another alkyne tool compound known as CTEP[11] was recently shown to reverse an already established FXS phenotype in adult *Fmr1* knockout mice following chronic dosing.[12] Recent studies in mice with MPEP and yet another alkyne tool

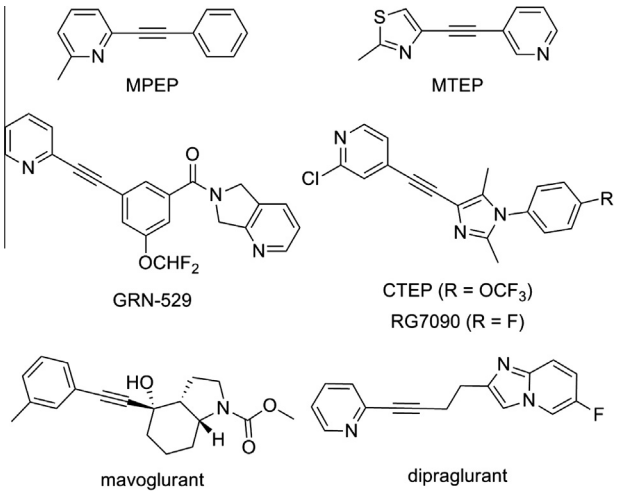

Figure 1. $mGlu_5$ NAM tool and clinical compounds.

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compound known as GRN-529 point to a potential role for $mGlu_5$ NAMs in the treatment of other autism spectrum disorders.[13] GRN-529 has also recently proven efficacious in rodent models of treatment resistant depression (TRD).[14] Finally, both MPEP and MTEP have produced encouraging results in animal models of addiction with various drugs of abuse, including cocaine,[15] nicotine,[15g,16] methamphetamine,[17] morphine,[18] and ethanol.[19]

Multiple $mGlu_5$ NAMs have advanced to clinical trials, with the most encouraging results thus far observed in GERD,[20] FXS,[21] and PD-LID.[22] The majority of clinical compounds are from within the disubstituted alkyne structure class, including each of the three molecules with confirmed ongoing clinical activity: dipraglurant (ADX48621), mavoglurant (AFQ056), and RG7090 (RO4917523) (Fig. 1).[3a] We have been interested in the identification and optimization of $mGlu_5$ NAMs within chemotypes that do not contain a disubstituted alkyne motif. One approach that we have successfully employed in this endeavor was based on the development of hits identified using a functional cell-based high-throughput screen (HTS) of a collection of 160,000 compounds.[23] We have also used both rational design approaches[24] as well as virtual screening methods to identify new $mGlu_5$ NAM tool compounds.[25]

Among the confirmed hits from our functional HTS was aryl ether benzamide **1** (Fig. 2), which demonstrated good potency in our functional assay. This assay also serves as our primary assay for lead optimization by measuring the ability of the compound to block the mobilization of calcium induced by an EC_{80} concentration of glutamate in HEK293A cells expressing rat $mGlu_5$.[26] As part of our initial hit evaluation process, the primary amine functional group was removed to afford analog **2**, which was approximately four-fold more potent than hit **1**. Another early structural modification involved preparation of the compound with the alternative orientation of the amide bond of **2** to produce analog **3**. Though compound **3** was more than 20-fold less potent than **2**, our anticipation was that optimization within this series might restore lost potency and yield interesting analogs. Indeed, such an effort was fruitful and is described in detail herein.

Preparation of aryl ether analogs of **3** was straightforward and followed the general methods outlined here (Scheme 1).[27] Though certain reactions proceeded in poor to moderate yield, these reactions were not optimized and were sufficient for analog generation. For selected compounds of interest, scalable routes with improved yields were developed.[28] A nucleophilic aromatic substitution reaction between pyridine or pyrimidine alcohols **4** and suitable 3-halobenzonitrile compounds **5** afforded ether intermediates **6**. Basic hydrolysis of the nitrile functional group provided the carboxylic acid intermediates **7**. In certain cases acids **7** were coupled with primary amines under standard conditions to give the desired amide compounds directly. Alternatively, acids **7** were first converted to the corresponding methyl esters **8**. Subsequent treatment of **8** with primary amines in the presence of potassium bis(trimethylsilyl)amide yielded the desired amide compounds. This alternative route was especially useful for the incorporation of nitrile groups into the scaffold at a late stage.

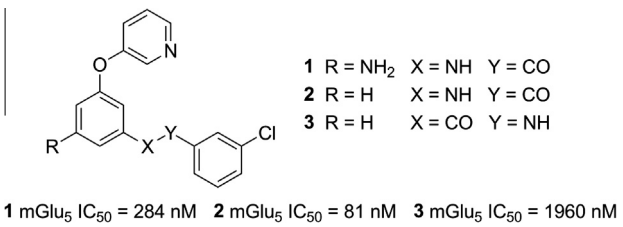
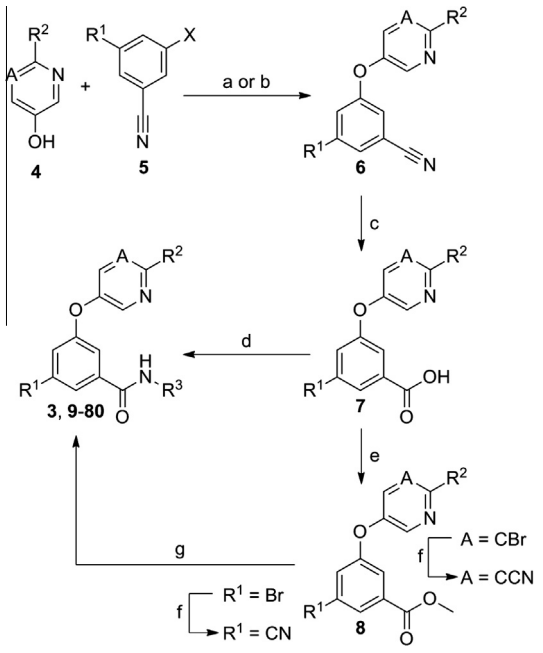


Figure 2. $mGlu_5$ NAM HTS hit and early analogs.

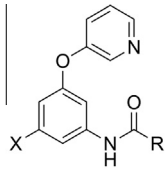


Scheme 1. Reagents and conditions: (a) For X = Br; CuO, K_2CO_3, pyridine, 80 °C (50–73%) or CuI, KO^{t}Bu, DMG, DMF, μw, 190 °C (35–50%); (b) for X = F; K_2CO_3, DMF, μw, 150–180 °C (48–72%); (c) aq NaOH, EtOH or dioxane, sealed tube, 100 °C (77–99%); (d) R^3NH_2, DIEA, HATU, DMF, CH_2Cl_2 (15–58%) or R^3NH_2, $POCl_3$, pyridine, −15 °C (36–65%); (e) H_2SO_4, MeOH, reflux (84–92%); (f) $Pd(OAc)_2$, PS–PPh_3 or $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, μw, 140 °C (32–81%); (g) R^3NH_2, $KN(SiMe_3)_2$, THF (10–65%).

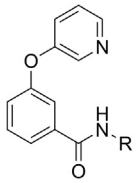
The first area of the chemotype that was targeted for SAR exploration was the eastern secondary amide group (Table 1). Potency data in the primary assay is presented here as both pIC_{50} and IC_{50} values for convenient evaluation of SAR. Not surprisingly, the 3-chlorophenyl group (**3**) can be replaced with a 3-methylphenyl group (**9**) with little effect on potency. More interesting was the enhanced potency observed with 2-pyridyl derivative **10**. While compound **3** was considered relatively lipophilic ($cLogP = 3.86$), the more polar analog **10** was considerably less lipophilic ($cLogP = 2.70$).[29] Lipophilicity can be an important parameter to monitor during the course of a CNS drug discovery program.[30] For this reason, the flexibility to install a heteroaryl ring at this position of the chemotype was considered attractive, and SAR development continued along those lines. Further modification of this ring in the form of pyrimidine **11** was not well tolerated. Efforts to evaluate substituted analogs of **10** produced 6-methylpyridine **12**, which was equipotent to the original hit **1**. Other substituted analogs of **10** (**14**–**16**) were two to five-fold less potent than **10**. 4-Pyridyl analog **13** was only weakly active and more than 25-fold less active than **12**, highlighting the importance of the location of the nitrogen atom in the pyridine ring.

In addition to these six-membered ring heteroaryl analogs, several five-membered ring heteroaryl analogs were also prepared (Table 2). Although unsubstituted thiazole **17** was only weakly active, simple modification of the thiazole ring by installation of a 4-methly group (**18**) resulted in substantially enhanced potency. As can often be the case in allosteric modulator chemotypes, large changes in potency were observed with quite minor structural modifications. For example, both thiadiazole **19** and 4-trifluoromethylthiazole **20** were inactive up to the highest concentration tested (30 μM) in spite of the fact that each compound was quite closely related to **18** from a structural standpoint. Triazole derivatives **21** and **22** were also both inactive up to the highest concentration tested.

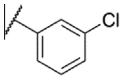
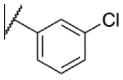
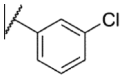
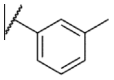
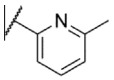
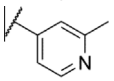
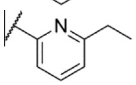
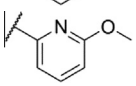
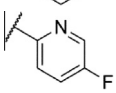
Table 1
Initial amide SAR



1 X = NH_2 **2** X = H



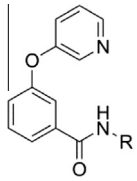
3, **9**–**16**

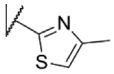
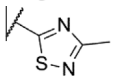
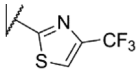
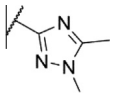
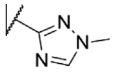
Compd	R	$mGlu_5$ pIC_{50}[a] (±SEM)	$mGlu_5$ IC_{50} (nM)	% Glu Max[a,b] (±SEM)
1		6.55 ± 0.19	284	1.3 ± 0.2
2		7.09 ± 0.07	81	1.0 ± 0.2
3		5.71 ± 0.14	1960	1.6 ± 0.4
9		5.66 ± 0.11	2190	1.4 ± 0.1
10		6.07 ± 0.13	844	1.6 ± 0.6
11		<5.0[c]	>10,000	49.9 ± 16.4
12		6.45 ± 0.16	354	1.3 ± 0.4
13		<5.0[c]	>10,000	27.8 ± 7.3
14		5.73 ± 0.13	1860	1.9 ± 0.2
15		5.44 ± 0.11	3610	3.2 ± 0.9
16		5.17 ± 0.05	3780	6.8 ± 0.9

[a] Calcium mobilization $mGlu_5$ assay; values are average of $n \geqslant 3$.
[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geqslant 3$.
[c] Concentration–response curve (CRC) does not plateau.

One of the early modifications made within this chemotype was evaluation of the pyrimidine ether (**23**) as an alternative to the pyridine ether in the northern portion of the chemotype (Table 3). This modification not only reduced lipophilicity (*c*Log*P* **23** = 1.27; *c*Log*P* **18** = 2.32)[29] but also enhanced potency. As such, substantial SAR was developed in the context of the pyrimidine ether group. One area of interest that was investigated was substitution of the phenyl core. A number of substituents were tolerated at the position *meta* to both the ether oxygen and the benzamide group, and representative examples are pictured here (Table 3). Installation of fluoro (**24**) and chloro (**25**) groups on the phenyl core provided analogs with enhanced potency relative to **23**, while trifluoromethyl derivative **26** was essentially equipotent to **23**. Additional texture in the SAR can be observed by moving to the 5-fluoropyridyl amide, which was only moderately potent in an earlier analog (**16**). In this case, the chlorophenyl analog **28** was approximately nine-fold more potent than its fluorophenyl counterpart (**27**). Interestingly, methyl analog **29** was more than 250-fold more potent than trifluoromethyl analog **30**. The cyano derivative **31** was similarly potent to fluorophenyl analog **27**.

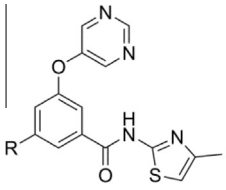
Table 2
Initial amide SAR



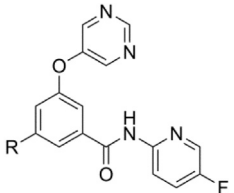
Compd	R	$mGlu_5$ pIC_{50}[a] (±SEM)	$mGlu_5$ IC_{50} (nM)	% Glu Max[a,b] (±SEM)
17		<5.0[c]	>10,000	14.4 ± 6.7
18		6.11 ± 0.12	772	1.7 ± 0.2
19		<4.5	>30,000	–
20		<4.5	>30,000	–
21		<4.5	>30,000	–
22		<4.5	>30,000	–

[a] Calcium mobilization $mGlu_5$ assay; values are average of $n \geqslant 3$.
[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geqslant 3$.
[c] CRC does not plateau.

Table 3
Phenyl ring SAR



Series I



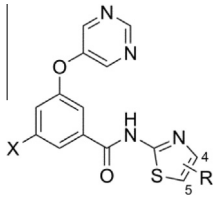
Series II

Compd	Series	R	$mGlu_5$ pIC_{50}[a] (±SEM)	$mGlu_5$ IC_{50} (nM)	% Glu Max[a,b] (±SEM)
23	I	H	6.69 ± 0.25	205	1.7 ± 0.3
24	I	F	7.62 ± 0.03	24	1.2 ± 0.1
25	I	Cl	7.94 ± 0.19	11	1.4 ± 0.2
26	I	CF_3	6.55 ± 0.10	283	1.7 ± 0.2
27	II	F	6.52 ± 0.07	305	1.3 ± 0.3
28	II	Cl	7.49 ± 0.02	33	1.2 ± 0.2
29	II	CH_3	7.72 ± 0.06	19	0.8 ± 0.2
30	II	CF_3	5.26 ± 0.01	5530	3.2 ± 0.3
31	II	CN	6.50 ± 0.08	318	1.1 ± 0.4

[a] Calcium mobilization $mGlu_5$ assay; values are average of $n \geqslant 3$.
[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geqslant 3$.

A second generation library of thiazole amides was prepared in the context of both fluorophenyl and chlorophenyl cores (Table 4). The importance of the optimized functional group modifications presented thus far is evidenced by moderately potent unsubstituted thiazole analog **32** (compare to **17**). Unfortunately, efforts to

Table 4
Second generation amide SAR—thiazoles



Compd	X	R	$mGlu_5$ pIC_{50}[a] (±SEM)	$mGlu_5$ IC_{50} (nM)	% Glu Max[a,b] (±SEM)
32	F	H	5.75 ± 0.25	1790	3.2 ± 1.2
33	F	4-(C_3H_5)	6.62 ± 0.08	238	1.7 ± 0.3
34	F	4-CH_3, 5-F	7.27 ± 0.03	54	1.5 ± 0.1
35	F	4-CH_2F	6.66 ± 0.03	217	1.6 ± 0.4
36	F	4-CHF_2	5.66 ± 0.06	2200	1.7 ± 0.4
37	F	4-CF_3	5.49 ± 0.05	3230	1.5 ± 0.5
38	Cl	4-(C_3H_5)	7.09 ± 0.19	82	1.7 ± 0.2
39	Cl	4-CH_3, 5-F	7.44 ± 0.09	37	1.4 ± 0.2
40	Cl	4-CH_2F	7.01 ± 0.08	99	1.3 ± 0.2
41	Cl	4-CHF_2	6.51 ± 0.07	310	1.2 ± 0.2
42	Cl	4-CF_3	6.68 ± 0.04	210	1.2 ± 0.4

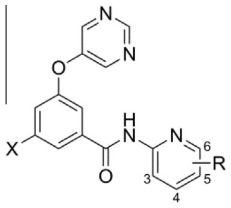
[a] Calcium mobilization $mGlu_5$ assay; values are average of $n \geqslant 3$.
[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geqslant 3$.

further enhance potency by modification or replacement of the 4-methyl group on the thiazole ring proved unsuccessful. For example, the cyclopropyl derivates **33** and **38** reduced potency by 10 and seven-fold, respectively (compare to **24** and **25**). Likewise progressive fluorination of the methyl group (**35**–**37** and **40**–**42**) was deleterious for $mGlu_5$ activity, though monofluorinated derivatives **35** and **40** were more potent than their difluoromethyl (**36** and **41**) and trifluoromethyl (**37** and **42**) counterparts.

A more successful modification was fluorination at the 5-position of thiazole ring, where only a two (**34** vs **24**) to three-fold (**39** vs **25**) loss of potency was observed.

A parallel second generation library of 2-pyridyl amides was also prepared in the context of both fluorophenyl and chlorophenyl cores (Table 5). The unsubstituted 2-pyridyl analogs **43** and **55** were prepared in order to have a baseline for comparison, and both were potent with **55** demonstrating excellent potency. Substitution at the 3-position was not favorable as evidenced by weak antagonism seen with 3-fluoropyridine analogs **44** and **56**. SAR at the 4-position was more nuanced. In the context of the fluorophenyl core, the 4-chloropyridine **45** demonstrated enhanced potency relative to **43**, while the 4-methylpyridine **46** and 4-trifluoromethylpyridine **47** were less potent than **43**. In the case of the chlorophenyl core, each of the substituted analogs (**57**–**61**) was less potent than unsubstituted comparator **55**. 5-Fluoropyridines **27** and **28** were discussed previously and proved to be the most potent among the 5-substituted analogs (**48**–**49** and **62**–**63**). Fluorophenyl core analogs with substituents at the 6-position (**50**–**54**) generally demonstrated good to moderate potency, and 6-chloropyridine **51** exhibited enhanced potency relative to **43**. The most potent compounds were obtained through the preparation of 6-substituted pyridine analogs in the context of the chlorophenyl core (**64**–**70**). In this case, a number of substituents (**64**–**67**) were well tolerated and demonstrated potency on par with **55**. Difluoromethylpyridine **68** and methoxypyridine **70** were only two and three-fold less potent than **55**, respectively. Finally, a limited number of disubstituted pyridines (**71**–**74**) were prepared and tested. Among these analogs, only **72** demonstrated potency within three-fold of comparator **55**. Interestingly, compound **72** was slightly less potent than both 5-fluoropyridine **28** and 6-methylpyridine **66** indicating that there was no additive effect on potency with these substituents.

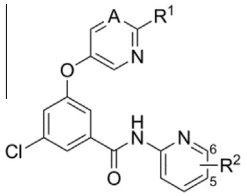
Table 5
Second generation amide SAR—pyridines



Compd	X	R	$mGlu_5$ pIC_{50}[a] (±SEM)	$mGlu_5$ IC_{50} (nM)	% Glu Max[a,b] (±SEM)	Compd	X	R	$mGlu_5$ pIC_{50}[a] (±SEM)	$mGlu_5$ IC_{50} (nM)	% Glu Max[a,b] (± SEM)
43	F	H	7.02 ± 0.09	95	1.4 ± 0.2	**59**	Cl	4-CH_3	6.85 ± 0.05	140	1.7 ± 0.2
44	F	3-F	<5.0[c]	>10,000	70.5 ± 3.2	**60**	Cl	4-CH_2CH_3	5.35 ± 0.02	4450	2.0 ± 0.7
45	F	4-Cl	7.38 ± 0.03	42	1.5 ± 0.1	**61**	Cl	4-CF_3	5.36 ± 0.12	4340	2.0 ± 0.0
46	F	4-CH_3	6.67 ± 0.01	215	1.4 ± 0.2	**28**	Cl	5-F	7.49 ± 0.02	33	1.2 ± 0.2
47	F	4-CF_3	5.98 ± 0.06	1050	1.5 ± 0.3	**62**	Cl	5-Cl	6.99 ± 0.11	103	1.3 ± 0.4
27	F	5-F	6.52 ± 0.07	305	1.3 ± 0.3	**63**	Cl	5-CF_3	<4.5	>30,000	—
48	F	5-Cl	5.97 ± 0.14	1080	2.0 ± 0.4	**64**	Cl	6-F	8.00[d]	10[d]	0.8[d]
49	F	5-CN	<4.5	>30,000	—	**65**	Cl	6-Cl	7.86 ± 0.06	14	1.2 ± 0.3
50	F	6-F	6.59 ± 0.13	258	1.6 ± 0.2	**66**	Cl	6-CH_3	7.75 ± 0.13	18	1.1 ± 0.3
51	F	6-Cl	7.35 ± 0.09	45	1.5 ± 0.5	**67**	Cl	6-CH_2F	7.74 ± 0.04	18	1.4 ± 0.4
52	F	6-OCH_3	6.18 ± 0.11	668	1.6 ± 0.3	**68**	Cl	6-CHF_2	7.44 ± 0.15	36	1.1 ± 0.4
53	F	6-CHF_2	6.83 ± 0.08	149	1.2 ± 0.4	**69**	Cl	6-CF_3	6.51 ± 0.14	309	1.5 ± 0.2
54	F	6-CF_3	5.64 ± 0.13	2320	3.7 ± 1.3	**70**	Cl	6-OCH_3	7.27 ± 0.04	53	1.3 ± 0.2
55	Cl	H	7.77 ± 0.03	17	1.2 ± 0.4	**71**	Cl	4-CH_3, 5-F	5.49 ± 0.04	3250	10.2 ± 7.7
56	Cl	3-F	<5.0[c]	>10,000	52.6 ± 5.4	**72**	Cl	5-F, 6-CH_3	7.30 ± 0.08	50	1.1 ± 0.3
57	Cl	4-F	6.47 ± 0.08	341	1.4 ± 0.2	**73**	Cl	5-F, 6-CH_2F	6.84 ± 0.10	145	1.2 ± 0.3
58	Cl	4-Cl	6.77 ± 0.02	169	1.2 ± 0.2	**74**	Cl	5-F, 6-CHF_2	6.30 ± 0.11	502	1.6 ± 0.3

[a] Calcium mobilization $mGlu_5$ assay; values are average of $n \geqslant 3$.
[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geqslant 3$.
[c] CRC does not plateau.
[d] Average of $n = 2$.

Table 6
Head group SAR



Compd	A	R^1	R^2	$mGlu_5$ pIC_{50}[a] (±SEM)	$mGlu_5$ IC_{50} (nM)	% Glu Max[a,b] (±SEM)
28	N	H	5-F	7.49 ± 0.02	33	1.2 ± 0.2
66	N	H	6-CH_3	7.75 ± 0.13	18	1.1 ± 0.3
75	N	CH_3	5-F	5.35 ± 0.04	4470	2.6 ± 0.1
76	N	CH_3	6-CH_3	7.02 ± 0.06	96	1.6 ± 0.2
77	CF	H	5-F	7.61 ± 0.04	25	1.1 ± 0.1
78	CF	H	6-CH_3	8.13 ± 0.08	7.5	1.8 ± 0.4
79	CCN	H	5-F	7.57 ± 0.08	27	1.7 ± 0.2
80	CCN	H	6-CH_3	7.99 ± 0.15	10	1.6 ± 0.1

[a] Calcium mobilization $mGlu_5$ assay; values are average of $n \geqslant 3$.
[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geqslant 3$.

Table 7
CYP3A4 inhibition and brain homogenate binding

Compound	*c*Log*P*[a]	$mGlu_5$ IC_{50} (nM)	CYP3A4 IC_{50}[b] (μM)	Mouse BHB[c] (F_u)
24	1.43	24	22.3	0.082
25	1.89	11	2.2	0.025
28	2.44	33	6.2	0.080
64	2.53	10	<0.1	0.057
65	2.99	14	1.9	0.019
66	2.59	18	4.3	0.052
77	3.65	25	1.3	0.007
78	3.80	7.5	0.7	0.003
79	3.22	27	1.7	0.016

[a] Calculated using ADRIANA. *Code* (www.molecular-networks.com).
[b] Inhibition of CYP3A4 assayed in pooled HLM + NADPH.
[c] BHB = brain homogenate binding; F_u = fraction unbound.

Table 8
Mouse PK results[a]

	24 VU0409106[b]	**28** VU0415303[c]
Plasma T_{max} (h)	0.25	0.5
Plasma C_{max} (ng/mL)	1450	303
Brain T_{max} (h)	0.25	0.25
Brain C_{max} (ng/g)	1350	311
AUC_{plasma} (ng h/mL)	702	391
AUC_{brain} (ng h/g)	696	365
B/P ratio	0.99	0.93

[a] 10 mg/kg IP dose; 10% Tween 80 formulation.
[b] CD-1 mice ($n = 3$ per time point).
[c] CD-1 mice ($n = 2$ per time point).

With a number of interesting analogs in hand, attention returned to preparing a limited number of new analogs in the northern heteroaryl ether portion of the chemotype. Representative SAR is presented here in the context of the 5-fluoropyridine and 6-methylpyridine amide groups exemplified in previously discussed compounds **28** and **66** (Table 6). Substitution of the pyrimidine ether at the 2-position ($R^1 = CH_3$) resulted in a dramatic loss of potency (>100-fold) in the context of the 5-fluoropyridine amide (**75** vs **28**); however, the decrease in potency was much less severe

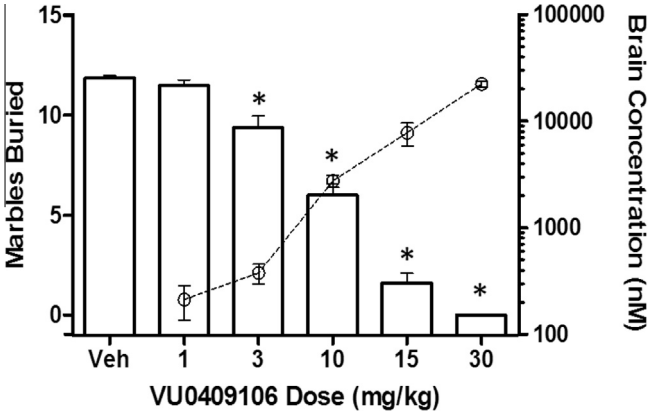


Figure 3. Dose dependent inhibition of marble burying with **24** (VU0409106) correlates with increased total brain exposure. $n = 8$ per dose; $^*P < 0.001$ versus vehicle control group, Dunnett's test. Bars denote marbles buried. Open circles denote brain exposure.

(five-fold) in the context of the 6-methylpyrimidine (**76** vs **66**). Installation of substituted pyridine ethers (**77**–**80**) proved more advantageous for maintaining or enhancing potency as evidenced by fluoropyridine ethers **77**–**78** and cyanopyridine ethers **79**–**80**.

Throughout the course of a typical discovery project, we prefer to triage our compounds for advanced assays and to inform design of subsequent analogs using a number of important in vitro assays. Such assays can help predict future liabilities or inform us as to the drug-likeness of our analogs. Typically, measuring intrinsic clearance in rodent and human liver microsomes is a useful means of assessing metabolism that is mediated by cytochrome P450; however, internal research with compounds in this chemical series has demonstrated a major role for nonP450 mediated metabolism with compounds containing the pyrimidine ether in the northern region of the chemotype. A detailed description of this metabolic pathway has been elucidated and characterized with analog **24** (VU0409106) and has recently been published in the literature.[31] Still, other in vitro assays did prove useful, including assessment of cytochrome P450 inhibition[32] and nonspecific binding to rodent brain homogenates (BHB).[33] CYP3A4 is the major P450 present in the human liver and responsible for the metabolism of approximately half of the drugs in clinical use.[34] Inhibition of CYP3A4 by representative compounds from this series of $mGlu_5$ NAMs is presented here (Table 7). Also shown for each compound is the unbound fraction in mouse brain homogenates, which provides an indication of free drug available to interact with the receptor. In the context of the 4-methylthiazole amide, the halogen on the phenyl core proved important. The more lipophilic chlorophenyl analog **25** was both more highly protein bound and a more potent inhibitor of CYP3A4 than fluorophenyl analog **24** (VU0409106). Unbound fraction in brain homogenate was increased, and CYP3A4 inhibition was reduced by moving from the 4-methylthiazole **25** to the 5-fluoropyridine amide **28** (VU0415303). The 6-substituted pyridine analogs (**64**–**66**) demonstrated some interesting SAR. 6-Fluoropyridine **64** was an extremely potent inhibitor of CYP3A4; however, this liability could be mitigated by installation of larger chloro (**65**) and methyl (**66**) groups at the 6-position. Not surprisingly the more lipophilic analog **65** was the more highly protein bound among this group of 6-substituted analogs. The pyridyl ether compounds (**77**–**79**) were generally more highly protein bound and more potent inhibitors of CYP3A4 than their corresponding pyrimidine ether counterparts.

Consideration of the potency and in vitro data presented herein identified compounds **24** (VU0409106) and **28** (VU0415303) as promising analogs for evaluation in mouse pharmacokinetic (PK)

studies (Table 8). Prior PK studies in rats had demonstrated that VU0409106 was a moderate to high clearance compound;[31] thus, intraperitoneal (IP) dosing was chosen as a convenient route for assessing exposure in mice.[35] Both compounds demonstrated good brain to plasma ratios near unity; however, **24** (VU0409106) had nearly two-fold better overall exposure than **28** (VU0415303). Using the brain homogenate binding data for **24** (VU0409106), the unbound drug levels in the brain are calculated to be 335 nM at 15 min post dose and 38 nM at 1 h post dose. Given that these unbound brain levels are in excess of the functional potency of **24** (VU0409106), the compound was deemed an excellent candidate for evaluation in an acute behavioral study in mice. Prior to undertaking such studies, it was considered prudent to better understand the molecular pharmacology of **24** (VU0409106). The ability of the compound to compete with the equilibrium of [^{3}H]3-methoxy-5-(pyridin-2-ylethynyl)pyridine,[36] a close structural analog of MPEP, confirmed the interaction of the compound with the known $mGlu_5$ allosteric binding site ($mGlu_5$ K_i = 6.8 nM). **24** (VU0409106) was also examined in cell based functional assays at 10 μM for its selectivity versus the other seven mGlus and was determined to be inactive against each.[37] The functional activity of **24** (VU0409106) at human $mGlu_5$ was also evaluated, and little difference was found across species (human $mGlu_5$ IC_{50} = 49 nM). Finally, **24** (VU0409106) was submitted to a commercially available radioligand binding assay panel of 68 clinically relevant GPCRs, ion channels, kinases, and transporters,[38] and no significant responses were found at 10 μM compound.[39]

It has been established that mice will bury foreign objects such as glass marbles in deep bedding. Pretreatment of mice with low doses of anxiolytic benzodiazepines have been shown to inhibit this behavior.[40] Furthermore, the known $mGlu_5$ NAMs MPEP and fenobam are also effective in this model.[7a,d] Additionally, novel tool compounds developed in our laboratory have also demonstrated efficacy in this model.[24b,25] As this is also a convenient and rapid assay that is performed with naïve mice, we have found it to be a useful in vivo screening paradigm.[41] Given these facts, a dose response marble burying study using IP dosing of **24** (VU0409106) was conducted using a 15 min pretreatment (Fig. 3). To relate the observed results to compound exposure, brain samples were collected and analyzed immediately following each experiment. Gratifyingly, dose-dependent inhibition of marble burying that correlated with increased brain concentration of drug was observed with **24** (VU0409106). Statistically significant inhibition was noted at all doses greater than or equal to 3 mg/kg.

In conclusion, substantial SAR has been developed in an aryl ether series of $mGlu_5$ NAMs leading to the identification of tool compound **24** (VU04019106). The compound is a potent and selective noncompetitive antagonist of $mGlu_5$ that binds at the known allosteric binding site and demonstrates good CNS exposure following intraperitoneal dosing in mice. Compound **24** (VU0409106) also proved efficacious in a mouse marble burying model of anxiety, an assay known to be sensitive to $mGlu_5$ NAMs, and that efficacy appears to correlate with drug exposure in the brain. Many additional in vivo experiments with this new tool compound are ongoing, including behavioral models of other $mGlu_5$ related diseases, and will be the subject of forthcoming communications.

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28. Scalable synthesis of VU0409106: (a) 3,5-difluorobenzonitrile (1.0 g, 7.2 mmol, 1.0 equiv), 5-hydroxypyrimidine (691 mg, 7.19 mmol, 1.0 equiv), K_2CO_3 (1.2 g, 8.7 mmol, 1.2 equiv) and DMF (17 mL) were added to a microwave vial and heated at 150 °C for 15 min. The reaction was filtered and concentrated on silica gel. The silica gel with absorbed compound was loaded on top a fresh pad of silica gel and washed with 50% ethyl acetate/hexane. The solvents were removed in vacuo and the crude mixture was purified by flash chromatography on silica gel to afford 750 mg (48%) of the desired compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.75 (s, 2H), 7.71 (ddd, J = 8.4, 2.2, 1.2 Hz, 1H), 7.62–7.61 (m, 1H), 7.56 (dt, J = 10.0, 2.3 Hz, 1H); $[M+H]^+$: 216.1. (b) 3-Fluoro-5-(pyrimidin-5-yloxy)benzonitrile (1.36 g, 6.32 mmol, 1.0 equiv) was dissolved in dioxane (32 mL) and 2 N NaOH (17 mL) in a sealed tube. The mixture was heated for 18 h at 100 °C. After cooling the reaction was neutralized with 2 N HCl (11 mL), and the mixture was concentrated in vacuo. The crude mixture was dissolved in 10% MeOH/CH_2Cl_2 and the undissolved salt was filtered off, and the solvents were removed in vacuo to afford 1.47 g (99%) of the desired compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.72 (s, 2H), 7.44 (ddd, J = 9.2, 2.4, 1.1 Hz, 1H), 7.34–7.32 (m, 1H), 7.22 (dt, J = 9.5, 2.4 Hz, 1H); $[M+H]^+$: 235.1. (c) 3-Fluoro-5-(pyrimidin-5-yloxy)benzoic acid (5.18 g, 22.1 mmol, 1.0 equiv) and 2-amino-4-methylpyridine (2.78 g, 24.3 mmol, 1.1 equiv) were dissolved in pyridine (147 mL) and cooled to −15 °C. $POCl_3$ (2.27 mL, 24.3 mmol, 1.1 equiv) was added dropwise keeping the temperature below −15 °C. The reaction was stirred an additional 30 min at −15 °C and quenched with water and 10% aqueous K_2CO_3 (37 mL). The reaction was extracted with EtOAc (3×), dried with $MgSO_4$ and concentrated in vacuo. Purification using reverse phase chromatography afforded 4.76 g (65%) of the desired compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.77 (s, 2H), 7.74–7.70 (m, 1H), 7.62 (t, J = 1.6 Hz, 1H), 7.43 (dt, J = 9.6, 2.3 Hz, 1H), 6.83 (d, J = 1.0 Hz, 1H), 2.28 (s, 3H). $[M+H]^+$: 331.0.
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