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Discovery of VU6005649, a CNS Penetrant $mGlu_{7/8}$ Receptor PAM Derived from a Series of Pyrazolo[1,5-*a*]pyrimidines

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KEYWORDS: Positive allosteric modulator (PAM), metabotropic glutamate receptor 7 ($mGlu_7$), cognition, VU6005649, Rett syndrome

ABSTRACT: Herein, we report the structure-activity relationships within a series of $mGlu_7$ PAMs based on a pyrazolo[1,5-*a*]pyrimidine core with excellent CNS penetration (K_ps >1 and $K_{p,uu}$s >1). Analogs in this series proved to display a range of Group III mGlu receptor selectivity, but VU6005649 emerged as the first dual $mGlu_{7/8}$ PAM, filling a void in the Group III mGlu receptor PAM toolbox, and demonstrating *in vivo* efficacy in a mouse contextual fear conditioning model.

Of the eight metabotropic glutamate receptors (mGlus) and their associated three groups (Group I: $mGlu_{1,5}$, Group II: $mGlu_{2,3}$, Group III: $mGlu_{4,6,7,8}$), $mGlu_7$ and $mGlu_8$ remain the least explored due to a lack of selective small molecule tools.[1,2] Of these, $mGlu_7$ has emerged as an attractive therapeutic target for anxiety, depression, epilepsy and schizophrenia based on data from $mGlu_7$ knock-out (KO) mice.[3-11] Human genetics has further strengthened these associations, with *GRM7* polymorphisms linked to schizophrenia, depression, ADHD, and autism.[12-23] Additionally, whole exome sequencing approaches have recently identified mutations in the *GMR7* gene in patients with previously diagnosed neurodevelopmental disorders.[8,23-25] Finally, we have recently described dramatic reductions in $mGlu_7$ protein expression in the brains of patients diagnosed with Rett syndrome (RTT),[26] as well as mice modeling the disorder, and that nonselective positive allosteric modulators (PAMs) with $mGlu_7$ activity can correct apneas as well as numerous impaired cognitive and social domains in mice modeling RTT.[26]

$mGlu_7$ is broadly expressed in the CNS where it critically modulates synaptic transmission and neuronal function.[1]

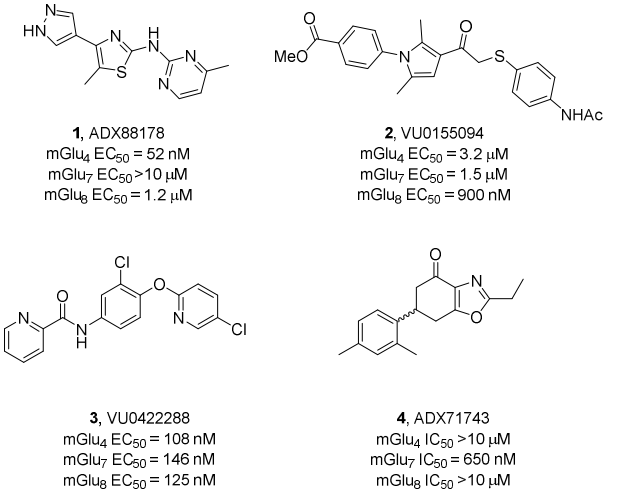


Figure 1. Structures of reported Group III mGlu receptor allosteric ligands. ADX88178 (1) is an $mGlu_{4/8}$ PAM, VU0155094 (2) is a *pan*-Group III ($mGlu_{4/7/8}$) PAM, VU0422288 (3) is a potent *pan*-Group III ($mGlu_{4/7/8}$) PAM, and ADX717743 (4) is a selective $mGlu_7$ NAM. No $mGlu_7$ selective or preferring PAMs have been disclosed to date. Potencies were determined in-house.

Due to a lack of $mGlu_7$-selective small molecule tools, recent efforts, including our own, have employed non-selective, *pan*-Group III mGlu receptor (PAMs) in combination with synaptic

localization studies, $mGlu_7$ negative allosteric modulators (NAM), and/or *Grm7*$^{-/-}$ mice to 'isolate' selective $mGlu_7$ activation.[23,27] As shown in Figure 1, all PAM ligands reported to date are not selective for $mGlu_7$ (e.g., **1** is an $mGlu_{4/8}$ PAM and both **2** and **3** are *pan*-$mGlu_{4/7/8}$ PAMs), while **4** is a selective $mGlu_7$ NAM.[1,2,26,27,28, 29 and in house data] Thus, our lab initiated a campaign to discover and optimize selective and CNS penetrant $mGlu_7$ PAMs for target validation studies. Here, we detail a new high-throughput screening (HTS) campaign that identified a novel series of pyrazolo[1,5-*a*]pyrimidines as $mGlu_7$-preferring and dual $mGlu_{7/8}$ PAMs with excellent CNS penetration and *in vivo* efficacy in a mouse contextual fear conditioning model.

We performed an HTS campaign on a collection of 63,000 small molecules and identified 438 hits as $mGlu_7$ PAMs in a single point screen.[2] After single-point hit confirmation, counter-screening against untransfected HEK cells and full concentration-response curve confirmation, 98 compounds were confirmed as $mGlu_7$ PAMs. Hits with attractive chemotypes were then evaluated against $mGlu_4$ and $mGlu_8$; HTS hit **5** (**Figure 2**), based on a pyrazolo[1,5-*a*]pyrimidine core, proved worthy of further attention. Hit **5** was selective for $mGlu_7$ ($mGlu_7$ EC_{50} = 3.3 μM, pEC_{50} = 5.48±0.14, 104±5 Glu Max, $mGlu_4$ EC_{50} >10 μM, $mGlu_8$ EC_{50} >10 μM) and displayed exceptional CNS penetration (rat plasma:brain K_p = 1.4, $K_{p,uu}$ = 1.1). From an optimization standpoint, **5** was also attractive in that multiple domains could be surveyed in parallel.

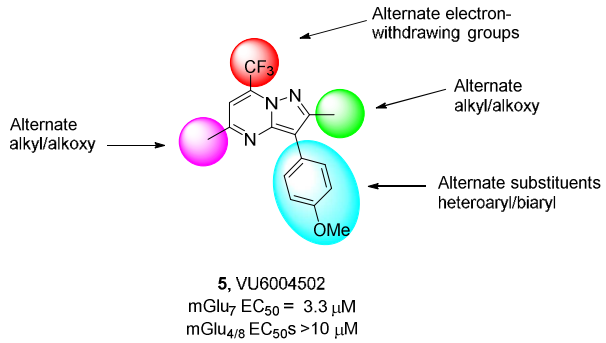
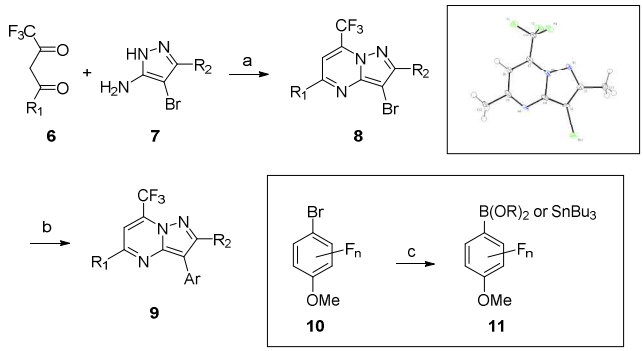


Figure 2. Structure of HTS hit **5** (VU6004502), and the four domains to be investigated in the course of the lead optimization campaign.

The synthesis of **5** and related analogs **9** was straightforward (retaining the CF_3 moiety) and required only two steps from known materials (**Scheme 1**) when the requisite boronic acid was commercial.[30] In other cases, the desired coupling partner (either aryl boronic acid or aryl stannane **11**) was prepared from the corresponding commercial aryl bromides **10**. However, this streamlined strategy hinged on a successful condensation of **6** and **7** to form **8** as a single regioisomer. Fortunately, the condensation afforded a single product **8**, and single X-ray crystallography[30] confirmed the correct regioisomer was obtained (insert). Subsequent Suzuki or Stille cross-

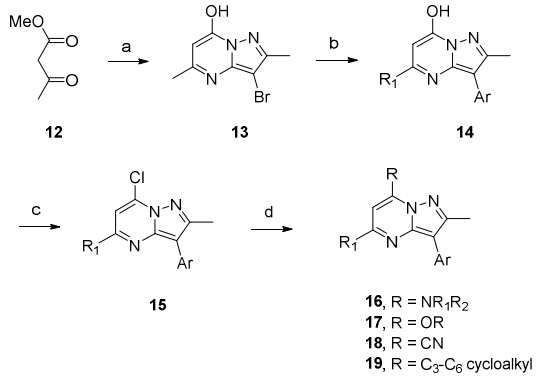
Scheme 1. Synthesis of Analogues **9**[a]



[a]Reagents and conditions: (a) EtOH, mw, 110 °C, 30 min, 56-76%; (b) $ArB(OR)_2$, 10 mol% $Pd(dppf)Cl_2$, Cs_2CO_3, dioxane:water, mw, 150 °C, 30 min, 44-55% or $ArSnBu_3$, 5 mol% $Pd(PPh_3)_4$, PPh_3, CuBr, LiCL, dioxane, mw, 120 °C, 3h, 38-42%; (c) *bis*-pinacolborate, 5 mol% $PdCl_2(PPh_3)_2$, KOAc, dioxane, 100 °C, 16 h, 50-58% or *n*-BuLi, Bu_3SnCl, THF, 0 °C, 30 min, 84-93%.

coupling of **8**, with partners **11**, generated analogs **9** in moderate yields. To explore alternatives for the CF_3 moiety, additional chemistry was required (**Scheme 2**).[30] Here, a related condensation between **12** and **7** provided a single regioisomer **13**, which smoothly underwent cross-coupling reactions to deliver **14**. Treatment with $POCl_3$ gave the key chloro derivative **15** that could be diversified via either S_NAr reactions to introduce amines **16** and ethers **17** or palladium-catalyzed

Scheme 2. Synthesis of non-CF_3 Analogues **16**, **17**, **18** and **19**[a]



[a]Reagents and conditions: (a) 4-bromo-3-methyl-1*H*-pyrazol-5-amine, AcOH, mw, 110 °C, 30 min, 68%; (b) $ArB(OR)_2$, 10 mol% $Pd(dppf)Cl_2$, Cs_2CO_3, dioxane:water, mw, 150 °C, 30 min, 26-38% (c) $POCl_3$, reflux, 30 min, 61-70%; (d) HNR_1R_2, EtOH, rt, 2h, 46-56%, or NaOR, ROH, rt, 30 min, 86-97%, or $Zn(CN)_2$, 5 mol% $Pd(PPh_3)_4$, NMP, 110 °C, 5h, 53-75%, or (cycloalkyl)ZnCl, 5 mol% $Pd(PPh_3)_4$, THF, reflux, 3h 74-83%.

organozinc chemistry to introduce either a nitrile **17** or cycloalkyl moieties **18** in good yields. All final compounds were >98% pure.

Evaluation of all of these analogs in our functional $mGlu_7$ assay highlighted extremely steep SAR, with the vast majority of the 100 analogues prepared devoid of $mGlu_7$ PAM activity

(**Figure 3**). Both the 7-CF_3 and 5-CH_3 moieties in **5** proved essential for $mGlu_7$ PAM activity, as all other substituents were inactive ($mGlu_7$ EC_{50}s >10 μM). The 2-CH_3 could be replaced with an ethyl moiety in **5**, but all other substituents lost $mGlu_7$ PAM activity. Thus, the only productive SAR resulted from functionalized aryl moieties in analogues **9**, where once again, the "fluorine walk" strategy[2] for allosteric modulator optimization proved fruitful (**Table 1**). Moreover, all analogs **9** evaluated for CNS penetration in the context of the broader SAR displayed favorable brain penetration (K_ps > 1, $K_{p,uu}$s >0.6) in our high throughput rat plasma:brain level (PBL) cassette paradigm.[28]

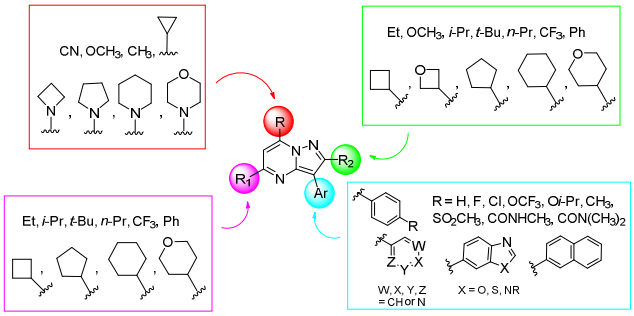
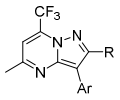
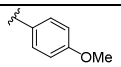
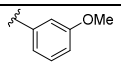
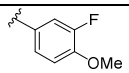
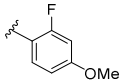
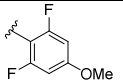
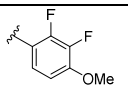
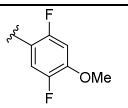
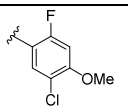
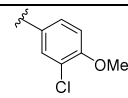
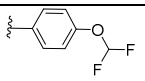
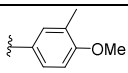
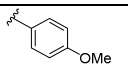
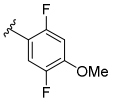
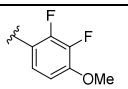
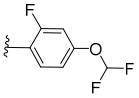


Figure 3. Substituents explored in analogs **9**, **16**, **17**, **18** and **19** that were inactive as $mGlu_7$ PAMs.

Table 1. Structures and Activities of Analogs **9**[a]



9

Entry	Ar (Het)	R	$rmGlu_7$ EC_{50} (μM)[a] [% L-AP4 Max ±SEM]	$rmGlu_7$ pEC_{50} (±SEM)	Rat K_p ($K_{p,uu}$)[b]
5		Me	3.3 [104±5]	5.48±0.14	1.4 (1.1)
9a		Me	>10 [20]	<5	ND
9b		Me	>10 [54]	<5	ND
9c		Me	1.5 [78±2]	5.82±0.06	4.3 (1.7)
9d		Me	1.7 [106±4]	5.78±0.10	1.4 (0.9)
9e		Me	0.60 [107±12]	6.22±0.11	2.2 (0.6)
9f		Me	0.65 [112±10]	6.19±0.14	4.3 (2.3)
9g		Me	1.0 [82±6]	5.99±0.06	2.7 (0.9)
9h		Me	0.48 [51±7]	6.32±0.06	ND
9i		Me	2.3 [87±6]	5.64±0.16	4.8 (1.7)
9j		Me	2.8 [91±3]	5.56±0.13	1.5 (0.8)
9k		Me	3.0 [98±7]	5.53±0.20	ND
9l		Et	1.7 [123±13]	5.76±0.16	ND
9m		Et	0.74 [102±4]	6.13±0.08	ND
9n		Et	1.05 [113±5]	5.98±0.08	ND
9o		Et	1.48 [102±6]	5.83±0.10	ND

[a]Calcium mobilization assays with rat $mGlu_7/G_{qi5}$-HEK cells performed in the presence of an EC_{20} fixed concentration of L-AP4; values represent means from three (n=3) independent experiments performed in triplicate.[b] Total and calculated unbound brain:plasma partition coefficients determined at 0.25 h post-administration of an IV cassette dose (0.20–0.25 mg/kg) to male, SD rats (n = 1), in conjunction with *in vitro* rat plasma protein and brain homogenate binding assay data. ND = not determined.

As discussed previously, HTS hit **5** exhibited improved selectivity for $mGlu_7$ over previous compounds VU0155094 and VU0422288 ($mGlu_7$ EC_{50} for **5** = 3.3 μM, pEC_{50} = 5.48±0.14, 104±5 L-AP4 Max, $mGlu_4$ EC_{50} >10 μM, $mGlu_8$ EC_{50} >10 μM) and displayed exceptional CNS penetration (rat plasma:brain K_p = 1.4, $K_{p,uu}$ = 1.1). Moving the methoxy group to either the 2- or 3-position, as in **9a** and **9b**, respectively, led to inactive analogs, as did a wide-range of substituents across multiple domains of **5** (**Figure 3**). When steep SAR has presented in other GPCR allosteric modulator programs, application of the 'fluorine walk' strategy[31] has proven beneficial, and this is true for the present series. The addition

of a single fluorine atom to either the 2- or 3-position of the 4-methoxyl phenyl moiety increased $mGlu_7$ PAM activity (**9c**, $mGlu_7$ EC_{50} = 1.5 μM, and **9d**, $mGlu_7$ EC_{50} = 1.7 μM), respectively. In the case of **9c**, K_p (4.3) and $K_{p,uu}$ (1.7) improved as well relative to **5**. The addition of two fluorine atoms to the 4-methoxy phenyl moiety afforded even more favorable results. Here, the 2,6-difluoro congener **9e** ($mGlu_7$ EC_{50} = 0.60 μM, pEC_{50} = 6.22±0.11, 107±12 L-AP4 Max) and the 2,3-difluoro derivative **9f** ($mGlu_7$ EC_{50} = 0.65 μM, pEC_{50} = 6.19±0.14, 112±10 L-AP4 Max) provided submicromolar $mGlu_7$ PAM EC_{50}s and good CNS penetration (K_ps of 2.2 ($K_{p,uu}$ = 0.6) and 4.3 ($K_{p,uu}$ = 2.3) for **9e** and **9f**, respectively). The 2-chloro-5-fluoro analog **9h** was the most potent $mGlu_7$ PAM in the series ($mGlu_7$ EC_{50} = 0.48 μM, pEC_{50} = 6.32±0.06, but the efficacy was diminished (51±7 L-AP4 Max). Difluoromethyl ether congeners **9j** and **9k** were also active, but solubility concerns precluded further advancement.

The *in vitro* DMPK profiles (**Table 2**) and K_ps/$K_{p,uu}$s of **5** and analogs **9** were tightly conserved in terms of predicted hepatic clearance, protein binding, and CYP450 inhibition (note, the chemotype engenders 1A2 inhibition); thus, physiochemical properties of individual PAMs and $mGlu_7$ PAM potency/efficacy assisted in prioritization. Attention then focused on further characterization of **9e** and **9f** as potential $mGlu_7$ PAM *in vivo* tool compounds. Both PAMs **9e** and **9f** were predicted to be moderate to highly cleared in rat (CL_{hep} = 64.2 and 65.5 mL/min/kg, respectively), and both displayed good free fraction in rat plasma (fu = 0.05 and 0.02; rat brain f_us of 0.014 and 0.012) and clean CYP450 profiles (**9e**: >30 μM versus 3A4, 2D6 and 2C9, 8.7 μM at 1A2; **9f**: >30 μM versus 3A4, 2D6, 24.6 μM at 2C9 and 3.1 μM at 1A2). Based on the improved rat K_p/$K_{p,uu}$, **9f** was further advanced into a mouse PBL time-course study. Here, a 10 mg/kg IP dose of **9f** was followed out to 6 hours with non-serial sampling of both plasma and brain at each selected time point, and displayed excellent CNS penetration (Kp @ 60 min of 2.1 and K_p @ 360 min of 1.1). Thus, for a first generation *in vivo* tool compound, **9f** could be studied in both rat and mouse models.

Table 2. *In vitro* DMPK Profiles of **5** and Analogs **9**

Property	**5**	**9c**	**9e**	**9f**	**9g**
MW	321	339	357	357	357
cLogP	3.70	3.78	4.11	3.90	3.97
TPSA	43.2	37.2	37.2	37.2	37.2
In vitro PK parameters					
CL_{HEP} (mL/min/kg), rat	59.4	62.1	64.2	65.5	63.0
CL_{HEP} (mL/min/kg), human	18.8	18.5	14.2	16.6	15.0
Rat fu_{plasma}	0.021	0.030	0.046	0.022	0.046
Human fu_{plasma}	0.006	0.013	0.016	0.008	0.002
Rat fu_{brain}	0.016	0.012	0.014	0.012	0.015
Cytochrome P_{450} (IC_{50}, μM)					
1A2	0.69	0.33	8.67	3.08	2.93
2C9	>30	27	>30	24.7	>30
2D6	>30	>30	>30	>30	>30
3A4	>30	>30	>30	>30	>30

However, broader mGlu receptor selectivity, as well as general ancillary pharmacology, needed to be assessed prior to any *in vivo* work. Gratifyingly, **9f** was inactive (EC_{50}/IC_{50}s > 10 μM) against $mGlu_{1,2,3,4,5\ and\ 6}$, but $mGlu_8$ activity was present ($mGlu_8$ EC_{50} = 2.6 μM, pEC_{50} = 5.58±0.06, 101±2 Glu Max, Table 3). Interestingly, **9f** was inactive at the other Group III mGlus ($mGlu_4$ and $mGlu_6$, see supporting data).[30] These data prompted us to examine mGlu selectivity for other analogues in this series, and, in general, as PAM potency at $mGlu_7$ increased, $mGlu_8$ PAM activity also increased (**Table 3**); however, PAM activity at $mGlu_{4/6}$ remained weak to inactive (data not shown).[30] PAM **9f** represents a missing link in the Group III receptor PAM toolbox and serves as a nice complement to the $mGlu_{4/8}$ PAM, **1**. In addition, broader ancillary pharmacology was assessed in a Eurofins Lead profiling panel[30] of 68 GPCRs, ion channels and transporters, and a single activity assessment at NK1 (K_i = 650 nM, functional antagonist IC_{50} of 3.4 μM) proved significant (all others <50% inhibition of radioligand binding at 10 μM).[31]

Table 3. Comparison of potency and % maximal agonist response across the three widely expressed CNS group III mGlu receptors for **5**, **9f** and related analogs.

Entry	$mGlu_4$ EC_{50} (μM) [% agonist Max ±SEM]	$mGlu_4$ pEC_{50} (±SEM)	$mGlu_7$ EC_{50} (μM) [% agonist Max ±SEM]	$mGlu_7$ pEC_{50} (±SEM)	$mGlu_8$ EC_{50} (μM) [% agonist Max ±SEM]	$mGlu_8$ pEC_{50} (±SEM)
5	>10 [48±3]	<5	3.3 [104±5]	5.48±0.14	>10 [86±5]	<5
9c	>30	<4.5	1.5 [78±2]	5.82±0.06	2.0 [70±6]	5.69±0.13
9e	>10 [69±3]	<5	0.60 [107±12]	6.22±0.11	2.9 [101±1]	5.54±0.18
9f	>10 [45±3]	<5	0.65 [112±10]	6.19±0.14	2.6 [101±2]	5.58±0.06
9h	>30	<4.5	0.48 [51±7]	6.32±0.06	0.49 [41±10]	6.31±0.06
9m	2.1 [53±6]	5.67±0.07	0.74 [102±4]	6.13±0.08	0.89 [85±6]	6.05±0.09

With the first $mGlu_7$-preferring, and highly CNS penetrant, PAM in hand, we assessed the activity of **9f** in a standard rat preclinical model predictive of antipsychotic activity, amphetamine-induced hyperlocomotion (AHL),[32] as human genetic association studies have identified *GRM7* polymorphisms linked to schizophrenia.[13,19] In this study, **9f** was dosed at 30 mg/kg IP in 10% Tween 80/H_2O (0.75 mg/kg. s.c. amphetamine), and there was no efficacy observed in this assay (data not shown).[30] Terminal (t = 2 hr) plasma and brain samples were taken, and **9f** displayed a terminal K_p of 2.43 with total brain levels ~9x above the $mGlu_7$ PAM *in vitro* EC_{50}. These data represent the first evaluation of an $mGlu_{7/8}$ PAM in rat AHL, but the lack of efficacy here does not rule out potential efficacy with NMDA antagonist challenge[33] or in other antipsychotic models (prepulse inhibition, conditioned avoidance responding, etc. or other genetic models (all of which are under pursuit)). As cognition deficits are another major, and

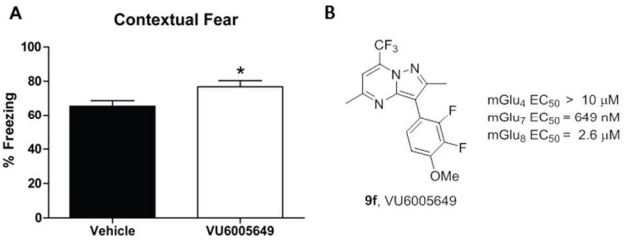


Figure 4. 9f, VU6005649, administration has pro-cognitive effects on associative learning in wild type mice. A) Contextual Fear Conditioning. Vehicle (10% Tween 80, n = 11) or **9f** (VU6005649, 50 mg/kg, n = 11) was administered (i.p.) to mice 15 minutes prior to training. On test day, VU6005649-treated mice froze significantly more than vehicle-treated mice (vehicle: 65.5 ± 3.3% vs. VU6005649: 76.8 ± 3.6%, p = 0.03) indicative of procognitive compound effects on associative learning. Two tailed Students t-test. Values presented as mean ± SEM.

unmet, symptom cluster in schizophrenia, we next evaluated **9f** in a standard mouse contextual fear conditioning (CFC) model at 50 mg/kg IP.[30] Here, **9f** showed modest but significant pro-cognitive effects on associative learning in wild type mice (**Figure 4**), and the first example of efficacy of an $mGlu_{7/8}$ PAM in this model. We would note that the compound did induce some level of sedation, which was still present when the compound was evaluated in $mGlu_7$ knockout mice (data not shown). As sedation during training would be predicted to diminish the capacity for associative learning, it is likely that the full efficacy of PAM **9f** in this cognitive assay was masked by off-target effects (such as NK1). Such results suggest that the utility of this tool may be limited in certain *in vivo* assays. Moreover, these exciting data warrant further optimization of this and other HTS hits to develop a highly selective $mGlu_7$ PAM for further *in vivo* target validation studies.

PAM **9f**, an $mGlu_{7/8}$ PAM, and the first reported $mGlu_7$ preferring PAM, complements existing Group III mGlu receptor PAM tool compounds **1-4**, and represents a major advance in the field. This novel series of pyrazolo[1,5-*a*]pyrimidines possess good free fraction and CNS penetration, lending utility as both *in vitro* and *in vivo* tool compounds alone or in combination studies with **1-4** and *Grm7*$^{-/-}$ mice. Additional behavioral pharmacology with **9f**, and optimization of additional hits from the HTS screen, are underway and will be reported in due course.

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Author Contributions

CWL, CMN, PJC, and RGG drafted/corrected the manuscript. MA, MS, KAB and DWE performed the chemical synthesis. CWL, PJC, CMN, CJK, and ALR oversaw the target selection and interpreted the biological data. ML and ALR performed the *in vitro* molecular pharmacology studies. ALB and SC performed the *in vitro* and *in vivo* DMPK studies. CKJ, MB and RGG performed the *in vivo* experiments. All authors have approved the manuscript.

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ASSOCIATED CONTENT

Supporting Information. General methods for the synthesis and characterization of all compounds, and methods for the *in vitro* and *in vivo* DMPK protocols and supplemental figures and detailed explantion of the sedation studies. This material is available free of charge via the Internet at http://pubs.acs.org.

ABBREVIATIONS

AHL, amphetamine-induced hyperlocomotion; metabotropic glutamate receptor (mGlu); PAM, positive allosteric modulator; NAM, negative allosteric modulator; HTS, high-throughput screen; CFC, contextual fear conditioning; PBL, plasma:brain level; RTT, Rett Syndrome; CNS, central nervous system; mGlu7, metabotropic glutamate receptor subtype 7; ADHD, attention deficit hyperactivity disorder

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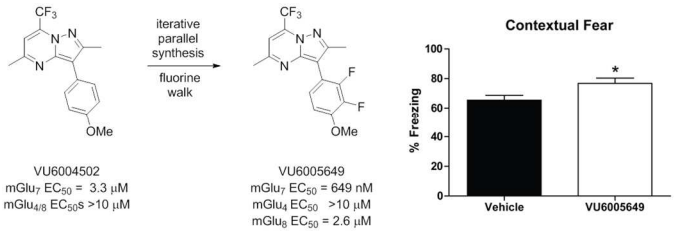
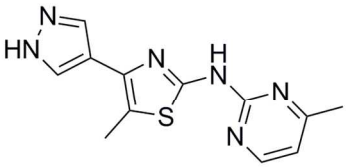
Table of Contents Graphic





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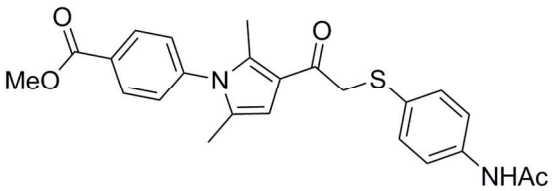

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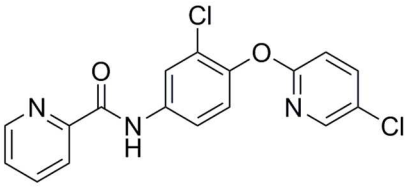

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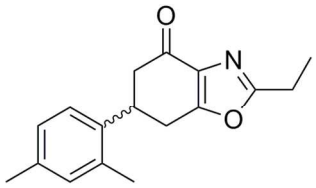

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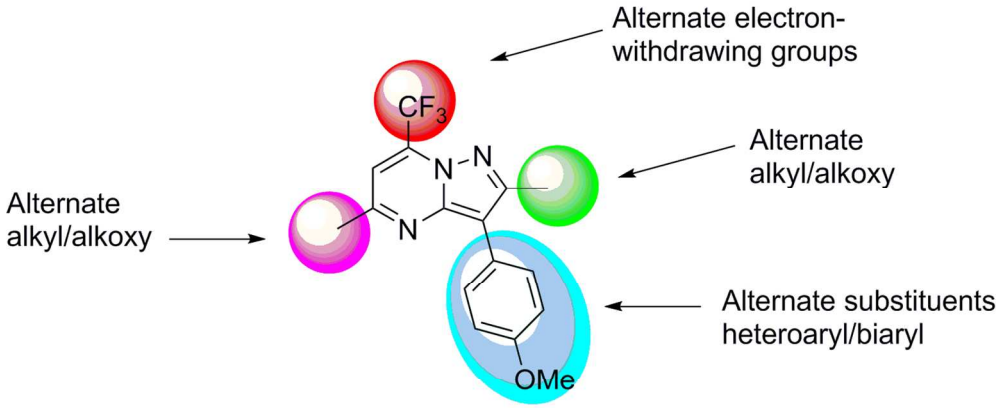

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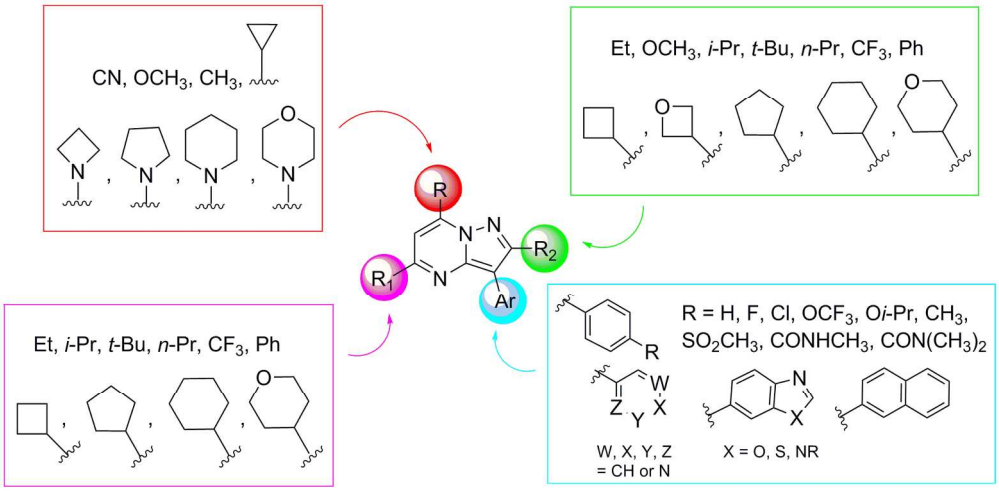

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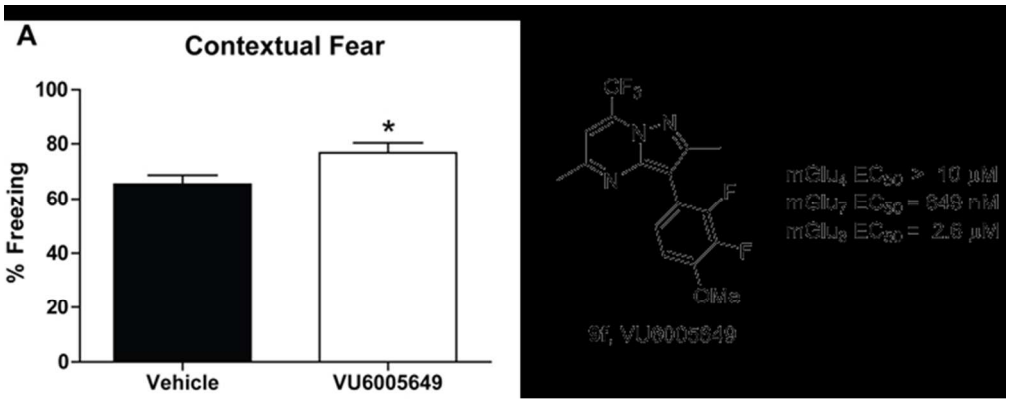

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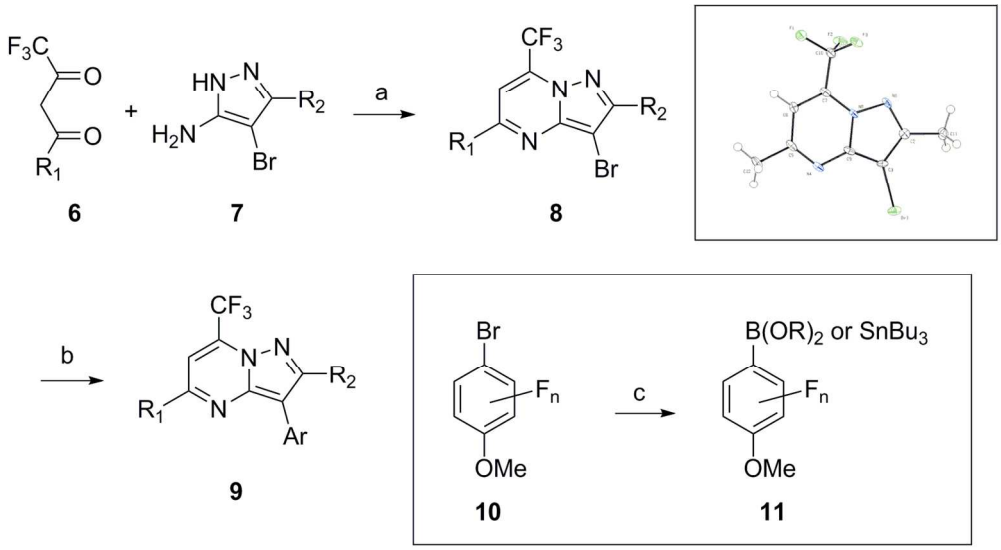

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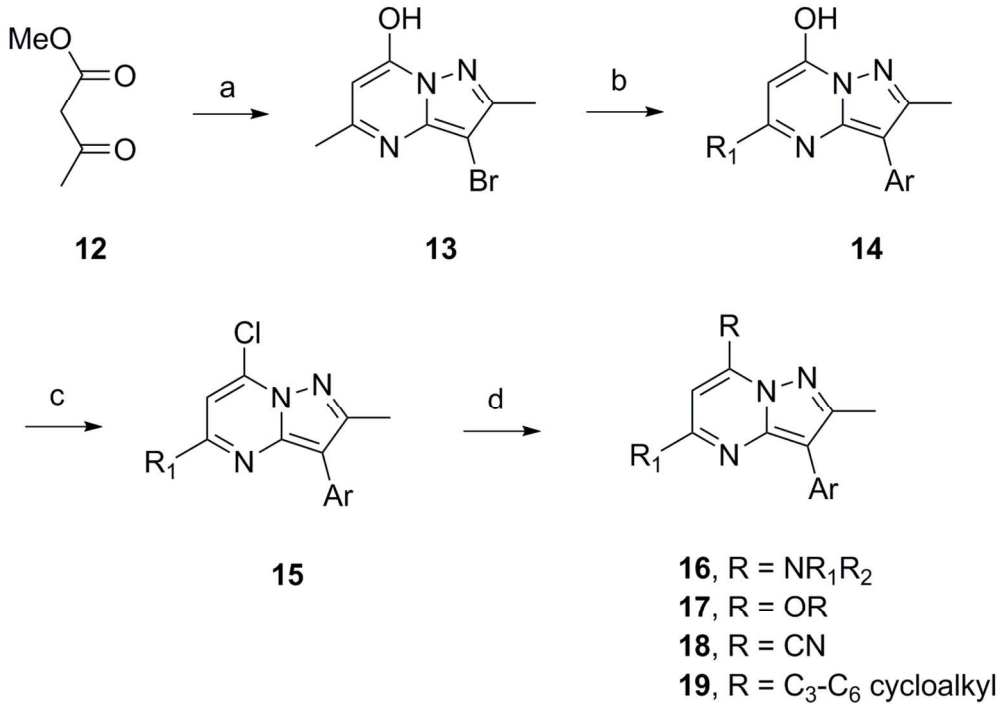

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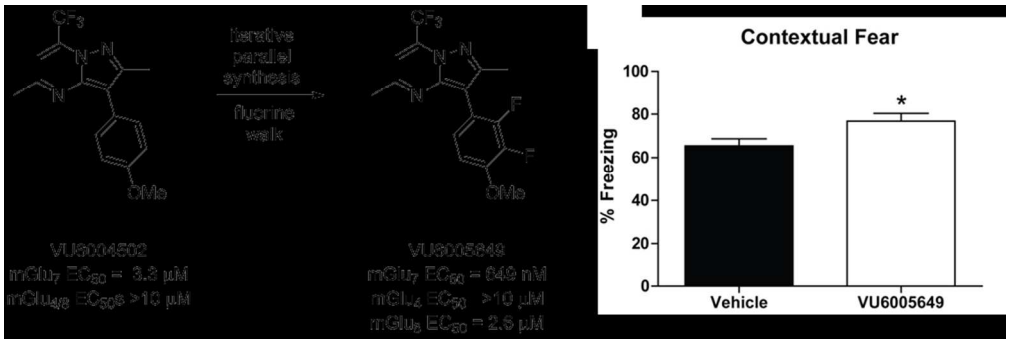

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