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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S Supporting Information

ABSTRACT: Herein, we report the structure–activity relationships within a series of mGlu₇ PAMs based on a pyrazolo[1,5-*a*]pyrimidine core with excellent CNS penetration ($K_{ps} > 1$ and $K_{p,uu} > 1$). Analogues 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.

KEYWORDS: Positive allosteric modulator (PAM), metabotropic glutamate receptor 7 (mGlu₇), cognition, VU6005649, Rett syndrome

Of the eight metabotropic glutamate receptors (mGlu) and their associated three groups (Group I, mGlu_{1,5}; Group II, mGlu_{2,3}; Group III, mGlu_{4,6,7,8}), mGlu₇ and mGlu₈ remain the least explored due to a lack of selective small molecule tools.^{1,2} Of these, mGlu₇ has emerged as an attractive therapeutic target for anxiety, depression, epilepsy, and schizophrenia based on data from mGlu₇ knockout (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 mGlu₇ protein expression in the brains of patients diagnosed with Rett syndrome (RTT),²⁶ as well as mice modeling the disorder, and have shown that nonselective positive allosteric modulators (PAMs) with mGlu₇

activity can correct apneas as well as numerous impaired cognitive and social domains in mice modeling RTT.²⁶

mGlu₇ is broadly expressed in the CNS where it critically modulates synaptic transmission and neuronal function.¹ Due to a lack of mGlu₇-selective small molecule tools, recent efforts, including our own, have employed a nonselective, *pan*-Group III mGlu receptor (PAMs) in combination with synaptic localization studies, mGlu₇ negative allosteric modulators (NAM), and/or *Grm7*^{−/−} mice to “isolate” selective mGlu₇ activation.^{23,27} As shown in Figure 1, all PAM ligands reported to date are not selective for mGlu₇ (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 mGlu₇ NAM.^{1,2,26–29} Thus, our lab initiated a campaign to discover and optimize selective and CNS penetrant mGlu₇ PAMs

Received: August 3, 2017

Accepted: September 1, 2017

Published: September 1, 2017

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 ADX71743 (**4**) is a selective mGlu₇ NAM. No mGlu₇ selective or preferring PAMs have been disclosed to date. Potencies were determined in-house.

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 mGlu₇-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 mGlu₇ 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 mGlu₇ PAMs. Hits with attractive chemotypes were then evaluated against mGlu₄ and mGlu₈; HTS hit **5** (Figure 2), based on a pyrazolo[1,5-*a*]pyrimidine

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

core, proved worthy of further attention. Hit **5** was selective for mGlu₇ (mGlu₇ EC₅₀ = 3.3 μM, pEC₅₀ = 5.48 ± 0.14, 104 ± 5 L-AP4 Max mGlu₄ EC₅₀ > 10 μM, mGlu₈ EC₅₀ > 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.

Scheme 1. Synthesis of Analogues 9^a

^aReagents and conditions: (a) EtOH, mw, 110 °C, 30 min, 56–76%; (b) ArB(OR)₂, 10 mol % Pd(dppf)Cl₂, Cs₂CO₃, dioxane–water, mw, 150 °C, 30 min, 44–55% or ArSnBu₃, 5 mol % Pd(PPh₃)₄, PPh₃, CuBr, LiCl, dioxane, mw, 120 °C, 3 h, 38–42%; (c) *bis*-pinacolborate, 5 mol % PdCl₂(PPh₃)₂, KOAc, dioxane, 100 °C, 16 h, 50–58% or *n*-BuLi, Bu₃SnCl, THF, 0 °C, 30 min, 84–93%.

The synthesis of **5** and related analogues **9** was straightforward (retaining the CF₃ 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 crystallography³⁰ confirmed the correct regioisomer was obtained (insert). Subsequent Suzuki or Stille cross-coupling of **8**, with partners **11**, generated analogs **9** in moderate yields. To explore alternatives for the CF₃ moiety, additional chemistry was required (Scheme 2).³⁰ Here, a related condensation between **12** and **7** provided a single regioisomer **13**,

Scheme 2. Synthesis of Non-CF₃ Analogues 16, 17, 18, and 19^a

^aReagents and conditions: (a) 4-bromo-3-methyl-1H-pyrazol-5-amine, AcOH, mw, 110 °C, 30 min, 68%; (b) ArB(OR)₂, 10 mol % Pd(dppf)Cl₂, Cs₂CO₃, dioxane–water, mw, 150 °C, 30 min, 26–38%; (c) POCl₃, reflux, 30 min, 61–70%; (d) HNR₁R₂, EtOH, rt, 2 h, 46–56%, or NaOR, ROH, rt, 30 min, 86–97%, or Zn(CN)₂, 5 mol % Pd(PPh₃)₄, NMP, 110 °C, 5 h, 53–75%, or (cycloalkyl)ZnCl, 5 mol % Pd(PPh₃)₄, THF, reflux, 3 h, 74–83%.

Figure 3. Substituents explored in analogues **9**, **16**, **17**, **18**, and **19**, which were inactive as mGlu₇ PAMs.

which smoothly underwent cross-coupling reactions to deliver **14**. Treatment with POCl₃ 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 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 analogues in our functional mGlu₇ assay highlighted extremely steep structure–activity relationships (SARs), with the vast majority of the 100 analogues prepared devoid of mGlu₇ PAM activity (Figure 3). Both the 7-CF₃ and 5-CH₃ moieties in **5** proved essential for mGlu₇ PAM activity, as all other substituents were inactive (mGlu₇ EC₅₀s > 10 μM). The 2-CH₃ could be replaced with an ethyl moiety in **5**, but all other substituents lost mGlu₇ PAM activity. Thus, the only productive SAR resulted from functionalized aryl moieties in analogues **9**, where once again the “fluorine walk” strategy² for allosteric modulator optimization proved fruitful (Table 1). Moreover, all analogues **9** evaluated for CNS penetration in the context of the broader SAR displayed favorable brain penetration (K_p s > 1, $K_{p,uu}$ s > 0.6) in our high-throughput rat plasma–brain level (PBL) cassette paradigm.²⁸

As discussed previously, HTS hit **5** exhibited improved selectivity for mGlu₇ over previous compounds VU0155094 and VU0422288 (mGlu₇ EC₅₀ for **5** = 3.3 μM, pEC₅₀ = 5.48 ± 0.14, 104 ± 5 L-AP4 Max, mGlu₄ EC₅₀ > 10 μM, mGlu₈ EC₅₀ > 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 analogues, 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” strategy³¹ 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-methoxy phenyl moiety increased mGlu₇ PAM activity (**9c**, mGlu₇ EC₅₀ = 1.5 μM, and **9d**, mGlu₇ EC₅₀ = 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** (mGlu₇ EC₅₀ = 0.60 μM, pEC₅₀ = 6.22 ± 0.11, 107 ± 12 L-AP4 Max) and the 2,3-difluoro derivative **9f** (mGlu₇ EC₅₀ = 0.65 μM,

pEC₅₀ = 6.19 ± 0.14, 112 ± 10 L-AP4 Max) provided submicromolar mGlu₇ PAM EC₅₀s and good CNS penetration (K_p s 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 analogue **9h** was the most potent mGlu₇ PAM in the series (mGlu₇ EC₅₀ = 0.48 μM, pEC₅₀ = 6.32 ± 0.06, but the efficacy was diminished (51 ± 7 L-AP4Max). Difluoromethyl ether congeners **9j** and **9k** were also active, but solubility concerns precluded further advancement.

The *in vitro* DMPK profiles (Table 2) and K_p s/ $K_{p,uu}$ s of **5** and analogues **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 mGlu₇ PAM potency/efficacy assisted in prioritization. Attention then focused on further characterization of **9e** and **9f** as potential mGlu₇ 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 (f_u = 0.05 and 0.02; rat brain f_u = 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 h with nonserial sampling of both plasma and brain at each selected time point and displayed excellent CNS penetration (K_p @ 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.

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 (EC₅₀/IC₅₀s > 10 μM) against mGlu_{1,2,3,4,5,6}, but mGlu₈ activity was present (mGlu₈ EC₅₀ = 2.6 μM, pEC₅₀ = 5.58 ± 0.06, 101 ± 2 Glu Max, Table 3). Interestingly, **9f** was inactive at the other Group III mGlu (mGlu₄ and mGlu₆, see Supporting Information).³⁰ These data prompted us to examine mGlu selectivity for other analogues in this series, and in general, as PAM potency at mGlu₇ increased, mGlu₈ PAM activity also increased (Table 3); however, PAM activity at mGlu_{4/6} remained weak to inactive.³⁰ PAM **9f** represents a missing link in the Group III receptor

Table 1. Structures and Activities of Analogues 9^a

Entry	Ar (Het)	R	mGlu ₇ EC ₅₀ (μM) ^a [% L-AP4 Max ±SEM]	mGlu ₇ pEC ₅₀ (±SEM)	Rat K _p (K _{p,0.01}) ^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

^aCalcium mobilization assays with rat mGlu₇/G_{q15}-HEK cells performed in the presence of an EC₂₀ fixed concentration of L-AP4; values represent means from three (*n* = 3) independent experiments performed in triplicate. ^bTotal and calculated unbound brain–plasma partition coefficients determined at 0.25 h postadministration 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.

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 panel³⁰ of 68 GPCRs, ion channels, and transporters, and a single activity assessment at

Table 2. *In Vitro* DMPK Profiles of 5 and Analogues 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
<i>in vitro</i> 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 <i>f</i> _{u,plasma}	0.021	0.030	0.046	0.022	0.046
human <i>f</i> _{u,plasma}	0.006	0.013	0.016	0.008	0.002
rat <i>f</i> _{u,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

NK1 (*K_i* = 650 nM, functional antagonist IC₅₀ of 3.4 μM) proved significant (all others <50% inhibition of radioligand binding at 10 μM).³¹

With the first mGlu₇-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/H₂O (0.75 mg/kg. s.c. amphetamine), and there was no efficacy observed in this assay (data not shown).³⁰ Terminal (*t* = 2 h) plasma and brain samples were taken, and 9f displayed a terminal *K_p* of 2.43 with total brain levels ~9× above the mGlu₇ PAM *in vitro* EC₅₀. 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 challenges 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 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 mGlu₇ knockout mice. 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 mGlu₇ PAM for further *in vivo* target validation studies.

PAM 9f, an mGlu_{7/8} PAM, and the first reported mGlu₇-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

Table 3. Comparison of Potency and % Maximal Agonist Response Across the Three Widely Expressed CNS Group III mGlu Receptors for 5, 9f, and Related Analogues

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

Figure 4. VU6005649, **9f**, 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 min prior to training. On test day, VU6005649-treated mice froze significantly more than vehicle-treated mice (vehicle, $65.5 \pm 3.3\%$, vs VU6005649, $76.8 \pm 3.6\%$, $p = 0.03$) indicative of procognitive compound effects on associative learning. Two-tailed students t test. Values presented as mean $\pm$ SEM.

of additional hits from the HTS screen are underway and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00317.

General methods for the synthesis and characterization of all compounds, methods for the *in vitro* and *in vivo* DMPK protocols, supplemental figures, and detailed explanation of the sedation studies (PDF)

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[†]These three authors contributed equally. C.W.L., C.M.N., P.J.C., and R.G.G. drafted/corrected the manuscript. M.A., M.S., K.A.B., and D.W.E. performed the chemical synthesis. C.W.L., P.J.C., C.M.N., C.J.K., and A.L.R. oversaw the target selection and interpreted the biological data. M.L. and A.L.R. performed the *in vitro* molecular pharmacology studies. A.L.B. and S.C. performed the *in vitro* and *in vivo* DMPK studies. C.K.J., M.B., and R.G.G. performed the *in vivo* experiments. All authors have approved the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank the NIH/NIMH MH102548 (to C.M.N.) and MH113543 (to C.M.N. and C.W.L.) and the William K. Warren, Jr. and the William K. Warren Foundation who funded the William K. Warren, Jr. Chair in Medicine (to C.W.L.). We would like to thank the Vanderbilt High Throughput Screening Facility for their assistance with primary screening.

■ ABBREVIATIONS

AHL, amphetamine-induced hyperlocomotion; mGlu, metabotropic glutamate receptor; 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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