VU6010608, a Novel mGlu₇ NAM from a Series of *N*-(2-(1*H*-1,2,4-Triazol-1-yl)-5-(trifluoromethoxy)phenyl)benzamides

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S Supporting Information

ABSTRACT: Herein, we report the structure–activity relationships within a series of mGlu₇ NAMs based on an *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)benzamide core with excellent CNS penetration (*K*_p 1.9–5.8 and *K*_{p,uu} 0.4–1.4). Analogues in this series displayed steep SAR. Of these, VU6010608 (**11a**) emerged with robust efficacy in blocking high frequency stimulated long-term potentiation in electrophysiology studies.

KEYWORDS: negative allosteric modulator, metabotropic glutamate receptor 7, long-term potentiation, VU6010608

Of the eight subtypes of metabotropic glutamate receptors (mGlu_{1–8}), the physiology and therapeutic potential of mGlu₇ is one of the least developed due to the absence of selective tool compounds.^{1,2} Despite this deficit, mouse and human genetics have associated mGlu₇ with anxiety, depression, epilepsy, schizophrenia, ADHD, and autism.^{3–11} Recent efforts from our laboratories, using nonselective Group III mGlu receptor positive allosteric modulators (PAMs) in combination with an mGlu₇ negative allosteric modulator (NAM), synaptic localization, and/or mGlu₇ knockout mice to isolate effects of selective mGlu₇ activation, have identified a role for mGlu₇ in a mouse model of Rett syndrome and cognition.^{12,13} Recent efforts to develop a first-in-class mGlu₇ PAM to further validate the Rett connection resulted in an mGlu₇-preferring PAM **1**, VU6005649, that demonstrated pro-cognitive effects on associative learning in wild type mice.¹⁴ There have been several mGlu₇ NAM tools reported in the literature (Figure 1),^{15–17} and the therapeutic potential of mGlu₇ has been reviewed.¹⁸ In 2007, Tsukuba researchers reported on the first mGlu₇ NAMs **2** (MDIP) and **3** (MMPiP);¹⁵ however, subsequent work with **2** and **3** demonstrated context-dependent pharmacology in cells and a lack of activity in electrophysiological studies.^{17,19} Six years later, Addex Ther-

apeutics reported on the discovery of a structurally related chemotype, **4** (ADX71743), with anxiolytic activity in several rodent models.¹⁷ However, **4** possesses an electrophilic ketone moiety and has weak activity (in our cell lines) at mGlu₂.²⁰ Here, we describe the identification of a novel mGlu₇ NAM chemotype, devoid of electrophilic character, with potency comparable to **4** and high mGlu selectivity. Moreover, this new series was brain penetrant and efficacious in electrophysiology studies.

As reported recently, we initiated a high-throughput screening (HTS) campaign on a 63000-membered library, utilizing a triple-addition protocol, to simultaneously identify mGlu₇ agonists, PAMs, and NAMs.¹⁴ This screen was very successful in identifying mGlu₇ PAM leads, which ultimately led to the development of **1**. This campaign also identified 156 mGlu₇ antagonists/NAMs; 75 of these compounds were validated after counter-screening against untransfected HEK cells and full concentration response curve (CRC) confirma-

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Figure 1. Structures of the recently reported mGlu₇-preferring PAM (1) and mGlu₇ NAMs (2–4), with a conserved 5,6-bicyclic chemotype.

tion. Attractive chemotypes were evaluated against mGlu₄ and mGlu₈, and selective hits were then optimized via iterative parallel synthesis. Of these, HTS hit **5** (Figure 2), based on an

Figure 2. Structure of HTS hit **5** (VU6009748), and the multiple regions to be surveyed in the lead optimization campaign.

N-(2-(1*H*-1,2,4-triazol-1-yl)phenyl)benzamide core, proved to be structurally distinct from the known mGlu₇ NAM chemotypes (2–4)^{15–17} and was prioritized for optimization. Hit **5** was selective for mGlu₇ (mGlu₇ IC₅₀ = 5.8 μM, pIC₅₀ = 5.24 ± 0.05, 19 ± 3 L-AP4 min (reduced an EC₈₀ level of activation to an EC₁₉) and >30 μM versus mGlu₄ and mGlu₈), but interestingly, appeared to be a partial antagonist as it did not completely block the agonist response. ADX71743 is more potent (mGlu₇ IC₅₀ = 676 nM, pIC₅₀ = 6.17 ± 0.06, 8 ± 1 L-AP4 min) than **5** but also a partial antagonist in our in vitro assay.^{17,20} From an optimization standpoint, **5** was attractive in that multiple regions could be evaluated in parallel.

The resynthesis of **5** and the rapid synthesis of novel analogues **11** was straightforward, requiring only four steps from commercial materials (Scheme 1).²⁰ Methyl 4-hydroxy-3-methoxybenzoate **6** was alkylated under standard conditions to afford analogues **7** (diverse R₁), followed by ester hydrolysis to deliver benzoic acids **8**. A variety of 5-substituted (R₂) 2-bromoanilines **9** were coupled to acids **8** under PyCIU conditions to give amides **10**. Finally, a copper-mediated Ullman coupling provided analogues **11**. Overall, yields were good, and a broad range of diversity was tolerated. In addition, **10** also was a competent partner for Suzuki and Stille couplings,

Scheme 1. Synthesis of Analogues **11**^a

^aReagents and conditions: (a) R₁X, K₂CO₃, DMF, 100 °C, 3 h, 94–97%; (b) LiOH, THF:H₂O (1:1), 60 °C, 2 h, quantitative; (c) PyCIU, DIEA, CH₂Cl₂, 100 °C, mw, 20 min, 47–82%; azaheterocycle, *N,N*-dimethylcyclohexane-1,2-diamine, K₃PO₄, CuI, DMF, 100 °C, 16 h, 51–68%.

under standard conditions, where aryl and heteroaryl congeners were also surveyed.

Evaluation of analogues **11** in our functional mGlu₇ assay^{12–14,20} demonstrated steep SAR (akin to the SAR noted with the mGlu₇ PAM **1**), and compound **5** is included for comparison (R₂ = Cl).¹⁴ Here, the majority of ~100 analogues prepared were inactive (IC₅₀s > 10 μM) at mGlu₇ (Figure 3). The 1,2,4-triazole proved essential for mGlu₇ NAM activity, with other regioisomeric triazoles, pyrazoles, pyrroles, and imidazoles devoid of activity. Ring expansions to 6-membered azaheterocycles, accessed via Suzuki couplings, were also inactive. Similarly, the 5-position (R₂) was largely intolerant of change, with only an OCF₃ moiety proving superior to chlorine. While alternate ethers in the 4-position (R₁) were active, increasing steric bulk led to a diminution in potency (e.g., **11f**, mGlu₇ IC₅₀ = 1.4 μM). Importantly, the 3-methoxy moiety was also essential and intolerant to any modifications. Reverse amides, sulfonamides, and α-trifluoromethyl amines were not effective as linker replacements, and additional substituents to the ether aryl ring (even fluorine) led to a complete loss of mGlu₇ NAM activity, as did the incorporation of heterocyclic systems. However, the analogues **11a–f** with mGlu₇ NAM activity (Table 1) generally displayed favorable brain penetration (K_p > 1, K_{p,uu} > 0.4) in our high throughput rat plasma/brain level (PBL) cassette paradigm,²¹ and all proved to be partial antagonists (mGlu₇ Glu Min 10–19%).²¹ The steep (all active analogues possessed IC₅₀s in the ~700 nM range) and flat (minor structural changes led to loss of mGlu₇ NAM activity) SAR profile of analogs **11** left five analogues (**11a–e**) for DMPK profiling to assess potential as in vivo tools.^{14,20,21}

As in vitro potency and CNS penetration were generally conserved and favorable for **11a–e**, in vitro DMPK profiles (Table 2) were employed to prioritize advanced characterization toward a tool compound. In terms of cLogP (2.86), molecular weight (408), and fraction unbound in both mouse (*f*_u = 0.178) and rat (*f*_u = 0.244) plasma and rat brain (*f*_u = 0.038), **11a** (VU6010608) was superior among the new analogues **11**. In addition, **11a** displayed low predicted hepatic clearance in rat (CL_{hep} = 9.5 mL/min/kg) and moderate in mouse (CL_{hep} = 48.5 mL/min/kg). **11e**, the cyclopropyl congener, was the closest competitor, with low predicted hepatic clearance in rat (CL_{hep} = 1.6 mL/min/kg), but a higher

Figure 3. Substituents explored in analogs **11** that were inactive as mGlu₇ NAMs (IC_{50} s > 10 μ M).

Table 1. Structures and Activities of Compound **5** and Analogues **11**

Compound	R ₁	rmGlu ₇ IC ₅₀ (μ M) ^a [% L-AP4 Min $\pm$ SEM]	rmGlu ₇ pIC ₅₀ ($\pm$ SEM)	Rat K _p (K _{p,uu}) ^b
5	Me	5.8 [19 $\pm$ 3]	5.24 $\pm$ 0.05	ND
11a	Me	0.76 [15 $\pm$ 3]	6.12 $\pm$ 0.04	3.4 (0.48)
11b	Et	0.51 [11 $\pm$ 2]	6.29 $\pm$ 0.08	3.5 (0.83)
11c	ⁿ Pr	0.78 [19 $\pm$ 5]	6.11 $\pm$ 0.05	1.9 (1.0)
11d	ⁱ Pr	0.78 [14 $\pm$ 4]	6.11 $\pm$ 0.07	4.4 (0.89)
11e		0.71 [11 $\pm$ 3]	6.15 $\pm$ 0.11	5.8 (1.5)
11f		1.4 [10 $\pm$ 3]	5.87 $\pm$ 0.07	ND

^aCalcium mobilization assays with rat mGlu₇/G_{α15}/HEK cells performed in the presence of an EC₈₀ 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.

cLogP and lower absolute plasma and brain levels in the rat PBL cassette study (data not shown) diverted all attention to **11a**.²¹

In vivo, **11a** displayed a poor in vitro:in vivo correlation (IVIVC), displaying high clearance in rat ($CL_p = 64.2$ mL/min/kg), with a 1.73 h half-life and high volume ($V_{ss} = 6.2$ L/kg), yet with acceptable oral bioavailability (18.9%F). From previous experience, we assumed that CYP-mediated oxidative dealkylation of one or both methoxy moieties in **11a** might also be a contributor to the high in vivo clearance. Thus, we prepared

Table 2. In Vitro DMPK Profiles of Analogues **11**

property	11a	11b	11c	11d	11e
MW	408	422	436	436	434
cLogP	2.86	3.41	4.41	4.32	3.96
TPSA	84.7	84.5	84.5	84.5	84.5
in vitro PK parameters					
CL _{HEP} (mL/min/kg) , rat	9.50	26.2	36.9	31.4	1.66
CL _{HEP} (mL/min/kg) , mouse	48.3	68.4	72.9	65.1	46.6
rat fu _{plasma}	0.244	0.072	0.017	0.065	0.052
mouse fu _{plasma}	0.178	0.086	0.019	0.063	0.039
rat fu _{brain}	0.034	0.017	0.009	0.013	0.013

both the 3- and 4-OCD₃ congeners as well as the 3,4-di-OCD₃ analogue and found that these modifications had no impact on clearance. A metabolite identification study in rat hepatic S9 proved informative, as the major metabolite was determined to be amide hydrolysis.²⁰ Future efforts within this series would have to focus on the modification of the amide linker.

As many of our rodent behavioral models related to mGlu₇ are performed in mice,^{12–14,22} we evaluated the CNS penetration of **11a** in an intraperitoneal (IP) mouse PBL study. At a standard dose of 10 mg/kg IP, a K_p of 2.01 was observed, with a K_{p,uu} of 0.44, comparable to the rat data. However, both total brain (380 nM) and free brain levels (14.3 nM) were below the in vitro mGlu₇ IC₅₀. Thus, we performed a mouse IP PBL dose response at 100 mg/kg to assess if **11a** achieved sufficient exposure to serve as an in vivo tool compound (Table 3). At 100 mg/kg, total brain levels (1.55 μ M) were 2-fold above the in vitro mGlu₇ IC₅₀, but free brain levels peaked at only 59 nM. Thus, if efficacy was driven by total brain, **11a** is a reasonable in vivo tool, but not if efficacy requires free brain concentrations at or above the in vitro IC₅₀. We would note that, when dosed at 100 mg/kg, neither **11a** nor **4** showed any adverse effects in a standard CNS mouse study.²³

Thus, while **11a** would not be suitable as an in vivo tool for robust target validation studies, we were still excited about its potential as an in vitro tool and for electrophysiology studies. First, however, we needed to assess mGlu receptor selectivity and broader ancillary pharmacology for **11a** prior to any further studies. Fortunately, **11a** was inactive (EC_{50}/IC_{50} s > 10 μ M) against mGlu_{1,2,3,4,5,6,8} and was a highly selective mGlu₇ NAM (as opposed to **4**, which in our in-house mGlu selectivity panel, shows weak mGlu₂ activity).¹⁹ Furthermore, in a broader ancillary pharmacology (a Eurofins Lead profiling panel of 68

Table 3. Mouse IP PBL Exposure for 11a

dose (mg/kg)	plasma (total, nM)	plasma (free, nM)	brain (total, nM)	brain (free, nM)	K_p	$K_{p,uu}$
10	180	32.5	380	14.3	2.01	0.44
30	402	71.5	952	36.2	2.36	0.51
100	772	137	1550	59.0	2.00	0.43

Figure 4. HFS-induced LTP is blocked by the mGlu₇ NAM VU6010608 (**11a**). (A) Time course showing the effects of vehicle or mGlu₇ NAM (VU6010608, 10 μ M, gray symbols) followed by high-frequency stimulation (HFS; 2 $\times$ 100 Hz, 20 s ISI) on fEPSP slope. (B) Comparison between drug treatment groups based on average slope from last 5 min of recording. (** p < 0.01 compared to vehicle; Student's t test, n = 4 per group). Data are expressed as mean $\pm$ SEM.

GPCRs, ion channels and transporters), no significant activities were noted at 10 μ M.²⁰

mGlu₇ is broadly distributed within the mammalian CNS where it plays a key role in neuronal function and synaptic plasticity.^{1,2} We have previously shown that mGlu₇ regulates long-term potentiation (LTP) and that antagonism of mGlu₇ using ADX71743 completely blocks LTP at Schaffer Collateral (SC) SC-CA1 synapses in brain slices in wild-type mice.¹² Thus, we were pleased to see that the mGlu₇ NAM **11a** could block LTP at SC-CA1 in brain slices induced by high frequency stimulation (Figure 4), further highlighting the utility of **11a** as an in vitro probe.²⁰

Compound **11a**, a highly selective mGlu₇ NAM based on a novel chemotype (and devoid of reactive functionality), provides the field with a much needed in vitro tool compound to further dissect the physiology and therapeutic potential of mGlu₇. While this series, and **11a** in particular, possesses good free fraction and CNS penetration, total and free brain levels limit its translational utility as an in vivo probe. However, **11a** afforded robust efficacy in blocking high frequency stimulated LTP and has broad utility in other in vitro molecular pharmacology studies. Additional optimization of **11a** is underway toward the goal of developing a robust in vivo mGlu₇ NAM probe, as well as the optimization of other mGlu₇ NAM hits from our HTS campaign, which 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/acsmchemlett.7b00429.

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

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C.W.L., C.M.N., P.J.C., J.M.R., and B.J.S. drafted/corrected the manuscript. C.W.R., K.M.M., P.K.S., H.F.R., and D.W.E. performed the chemical synthesis. C.W.L., P.J.C., C.M.N., J.K.R., and A.L.R. oversaw the target selection and interpreted the biological data. E.E., V.B.L., M.T.L., and A.L.R. performed the in vitro molecular pharmacology studies and the HTS. A.L.B. performed the in vitro and in vivo DMPK studies. J.M.R. oversaw the in vivo experiments. D.H.R. performed the in vivo studies. B.J.S. performed the electrophysiology studies. All authors have given approval to the final version of the manuscript.

Author Contributions

||C.W.R. and K.M.M. contributed equally.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

LTP, long-term potentiation; HFS, high-frequency stimulation; mGlu, metabotropic glutamate receptor; PAM, positive

allosteric modulator; NAM, negative allosteric modulator; HTS, high-throughput screen; PBL, plasma/brain level

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