Published in final edited form as:

J Med Chem. 2019 February 14; 62(3): 1690–1695. doi:10.1021/acs.jmedchem.8b01810.

Discovery of an Orally Bioavailable and Central Nervous System (CNS) Penetrant mGlu₇ Negative Allosteric Modulator (NAM) in Vivo Tool Compound: *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-(cyclopropylmethoxy)-3-methoxybenzamide (VU6012962)

Carson W. Reed^{§,⊥}, Samantha E. Yohn^{†,‡,⊥}, Jordan P. Washecheck[§], Hanna F. Roenfan^{§,†,‡}, Marc C. Quitalig[‡], Vincent B. Luscombe^{†,‡}, Matthew T. Jenkins^{†,‡}, Alice L. Rodriguez^{†,‡}, Darren W. Engers^{†,‡}, Anna L. Blobaum^{†,‡}, P. Jeffrey Conn^{†,‡,||}, Colleen M. Niswender^{*,†,‡,||}, and Craig W. Lindsley^{*,†,‡,§}

[†]Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University, Nashville, Tennessee 37232, United States

[‡]Department of Pharmacology, Vanderbilt University, Nashville, Tennessee 37232, United States

[§]Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37232, United States

^{||}Vanderbilt Kennedy Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, United States

Abstract

Herein, we report the discovery of a new, orally bioavailable and CNS-penetrant metabotropic glutamate receptor 7 (mGlu₇) negative allosteric modulator (NAM) that achieves exposure in cerebral spinal fluid (CSF) 2.5× above the in vitro IC₅₀ at minimum effective doses (MEDs) of 3 mg/kg in preclinical anxiety models.

Graphical Abstract

*Corresponding Authors C.M.N.: phone, 615-343-4303; fax, 615-343-3088; colleen.niswender@vanderbilt.edu. *C.W.L.: phone, 615-322-8700; fax, 615-343-3088; craig.lindsley@vanderbilt.edu.

⊥C.W.R. and S.E.Y. contributed equally.

The authors declare no competing financial interest.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at [10.1021/acs.jmed-chem.8b01810](https://doi.org/10.1021/acs.jmed-chem.8b01810).

General chemistry, experimental information, and syntheses of all other compounds; in vitro and in vivo pharmacology and DMPK methods; supplementary figures and table (PDF)

Molecular formula strings (CSV)

INTRODUCTION

Metabotropic glutamate receptor subtype 7 (mGlu₇) is one of the family of eight mGlu receptors (mGlu₁₋₈). Human genetic and mouse data have associated mGlu₇ with anxiety, autism, ADHD, schizophrenia, epilepsy, depression, and Rett syndrome.¹⁻¹⁵ Early studies with first generation mGlu₇ negative allosteric modulators (NAMs), such as **1**,^{16,17} **2**,^{16,17} **3**,¹⁸ and **4**,¹⁹ (Figure 1), have been reported to result in efficacy in models of anxiety but suffer from liabilities such as off target activity, the requirement of doses in some models that are quite high to induce efficacy, and context-dependent effects, which may result in unexpected pharmacology in native tissues.¹⁶⁻²⁰ These findings indicate that the pharmacology of mGlu₇ modulation is complex and suggest that the generation of additional tool compounds would be valuable to probe receptor function. Recently, we performed a high-throughput screening campaign to identify novel mGlu₇ NAM leads, and NAM **5**, based on a new chemotype, was identified.²¹ While **5** was CNS penetrant in rodents, with total brain levels in excess of the in vitro IC₅₀, the estimated unbound brain levels based on a brain homogenate binding assay indicated that **5** would not achieve levels above the in vitro mGlu₇ IC₅₀ in terms of free brain concentrations. Despite this limitation, like **3**, mGlu₇ NAM **5** demonstrated robust efficacy in native tissues in blocking high-frequency-stimulated long-term potentiation (LTP) in the hippocampus.²¹ Here, we disclose efforts aimed at the continued optimization of mGlu₇ NAM **5** and the development of a new, highly selective mGlu₇ NAM in vivo tool compound that achieves predicted unbound CNS levels in excess of the in vitro IC₅₀ and robust efficacy across multiple rodent anxiolytic models at low doses (3 mg/kg MEDs).

RESULTS AND DISCUSSION

From our initial multidimensional optimization campaign around the mGlu₇ NAM, **5**, the minimum pharmacophore was identified, as well as the key metabolic liability (Figure 2).²¹ SAR demonstrated that the only region tolerant of change was the 4-alkoxy moiety on the benzamide, and the amide carbonyl was the metabolic soft-spot, with high plasma clearance due to amide hydrolysis (not the expected O-dealkylation).²¹ Therefore, we elected to pursue two paths in parallel: (1) delete the carbonyl and survey analogs **6** for mGlu₇ NAM potency and PK and (2) explore a larger collection of diverse 4-ether analogs **7**.

With large quantities of mGlu₇ NAMs **5** and **8** in hand, the concept of *des*-carbonyl, amine-linked congeners **6** could be most readily tested by reduction of amides with LiAlH₄ (Scheme 1) to afford **6a** and **9**, respectively.²² While **5** was a moderately potent mGlu₇ NAM (IC₅₀ = 760 nM, pIC₅₀ = 6.12 ± 0.04, 15 ± 3% L-AP4 min), both **6a** (IC₅₀ = 5.9 μM, pIC₅₀

= 5.23 ± 0.12 , $17 \pm 3\%$ L-AP4 min) and **9** ($IC_{50} = 7.9 \mu M$, $pIC_{50} = 5.1 \pm 0.10$, $21 \pm 3\%$ L-AP4 min) lost considerable mGlu₇ NAM activity; however, these data warranted additional analogs to see if potency could be enhanced. To access homologated, more conformationally flexible analogs of **6a**, the four-step route highlighted in Scheme 2 was employed, starting from commercial aldehydes **10**.²² A Wittig reaction delivered **11** in high yield, followed by reduction and hydrolysis to give acid **12**. A PyCIU-mediated amide coupling with known **13** provided **14**, and reduction of the amide carbonyl with LiAlH₄ provided homologated analogs **6**. Related synthetic routes also enabled the incorporation of oxygen atoms in the homologated amine-linker (see Supporting Information).²²

Homologation of the amine linker did improve mGlu₇ NAM potency (Table 1) for representative examples to within less than 2-fold of **5**, but disposition suffered. All analogs **6** displayed predicted hepatic clearances near hepatic blood flow in rats, suggesting the site of metabolism shifted in the absence of the amide bond.²² Moreover, this modification did not positively impact plasma protein binding ($f_u = 0.01$ – 0.03) or rat brain homogenate binding ($f_u < 0.003$). Related analogs of **6b–d**, wherein the OCF₃ moiety was replaced with a chlorine atom, fared worse in terms of potency and disposition, and numerous analogs proved to be inactive ($IC_{50} > 10 \mu M$). Thus, the pursuit of an in vivo tool compound refocused on analogs **7**.

To explore a broader range of 4-alkoxy analogs **7**, commercial ester **15** was alkylated with various alkyl bromides under microwave conditions to afford analogs **16** in yields ranging from 79% to 96%.²² Ester hydrolysis smoothly afforded acid **17**, which was subsequently coupled to aniline **13** under PyCIU conditions to deliver final analogs **7** in good overall yields. Within this series, as before, SAR was steep (Table 2). As many mGlu allosteric ligands engage induced-fit pockets, SAR can be challenging, and the “right” fit may only be “found” by exploring libraries of analogs via an exercise in strategic serendipity. In the present case, lipophilic moieties such as trifluoroethyl (**7a**) and *tert*-butylmethyl (**7b**) were inactive, but isopropylmethyl (**7c**) restored mGlu₇ NAM activity, and cyclopropylmethyl (**7d**) proved optimal ($IC_{50} = 350$ nM, $pIC_{50} = 6.46 \pm 0.10$, $12.6 \pm 1.5\%$ L-AP₄ min) and the most potent within this chemotype to date. Larger moieties, such as **7e–g**, lost activity. Beyond an enhancement in mGlu₇ NAM potency, **7d** also showed a significant improvement in predicted hepatic clearance (rat $CL_{hep} = 15.9$ mL min⁻¹ kg⁻¹), generating enthusiasm for the further profiling of **7d**. While the route depicted in Scheme 3 was suitable for small-scale production of **7d**, an improved route was required to support extensive DMPK and behavioral work. For large-scale production, a two-step route was developed starting from commercial aniline **18** and intermediate **16d** (Scheme 4) via a PyCIU-mediated coupling to provide **19**. An Ullmann coupling reaction installed the requisite 1,2,4-triazole to deliver **7d** in ~500 mg scale.²²

NAM **7d** possessed an acceptable molecular weight (448 g/mol), cLogP (3.8), and polar surface area ($84.7 \text{ \AA}^2$). In addition to low predicted hepatic clearance in rat ($CL_{hep} = 15.9$ mL min⁻¹ kg⁻¹), **7d** also showed moderate predicted hepatic clearance in mouse ($CL_{hep} = 44.0$ mL min⁻¹ kg⁻¹) and good free fraction in rat and mouse plasma ($f_u = 0.028$ (rat) and 0.026 (mouse)).²² In a rat plasma:brain level (PBI) IV cassette study,^{22,23} NAM **7d**

displayed high brain penetration (rat $K_p = 2.35$ ($[\text{brain}]_{\text{tot}} = 375 \text{ nM}$), $K_{p,\text{uu}} = 0.75$ ($[\text{brain}]_{\text{unbound}} = 3.4 \text{ nM}$), the latter diminished due to high rat brain homogenate binding ($f_u = 0.009$)). These data prompted an evaluation of discrete iv and po pharmacokinetics in rat, wherein **7d** showed moderate clearance ($\text{CL}_p = 31.5 \text{ mL min}^{-1} \text{ kg}^{-1}$), moderate volume ($V_{ss} = 1.9 \text{ L/kg}$), and a short half-life ($t_{1/2} = 40 \text{ min}$); however, at a po dose of 10 mg/kg , **7d** displayed high oral bioavailability ($F = 74.9\%$).²² Finally, NAM **7d** was selective for mGlu₇ versus the other seven mGlu receptors ($>10 \mu\text{M}$ versus mGlu_{1-6,8}) and largely devoid of ancillary pharmacology (compound activity at only one target, 5-HT_{2B} receptor, that was greater than 50% at $10 \mu\text{M}$) in a Eurofins lead profiling panel of 68 GPCRs, ion channels, and transporters.²² On the basis of this profile, if in vivo efficacy was driven by total brain levels, **7d** was suitable to advance, but we were aware that for a number of lipophilic allosteric GPCR ligands, the results from brain homogenate binding assays (and thus the estimate of unbound brain concentrations) are occasionally unreliable. For such compounds, drug levels in the cerebral spinal fluid (CSF) could be significantly higher and afford a better correlation with in vivo efficacy.^{24,25} Thus, we performed a 30 mg/kg (ip) tissue distribution study in rat and assessed levels of **7d** in plasma, brain, and CSF. Here, we noted a brain:plasma K_p of 1.24 ($[\text{plasma}]_{\text{tot}} = 598 \text{ nM}$, $[\text{brain}]_{\text{tot}} = 745 \text{ nM}$) and a $K_{p,\text{uu}}$ of 0.38 ($[\text{plasma}]_{\text{unbound}} = 16.7 \text{ nM}$, $[\text{brain}]_{\text{unbound}} = 6.4 \text{ nM}$); however, the CSF:plasma K_p was 2.15, with levels of **7d** in CSF of $1.3 \mu\text{M}$, or ~ 3.8 -fold above the in vitro IC_{50} . As with recent mGlu₄ PAMs, assessing CSF exposure was critical, as opposed to simply estimating free brain levels (i.e., $K_{p,\text{uu}}$).^{24,25}

On the basis of the potency, efficacy, disposition, and high concentrations in CSF of **7d**, we progressed NAM **7d** as an in vivo rodent tool molecule. First generation mGlu₇ NAMs **3** and **4** have been previously evaluated in rodent models of anxiety but required relatively high doses to observe efficacy.^{18,19} We first assessed the activity of **7d** in an elevated zero maze (EZM) task in mice after intraperitoneal administration (Figure 3).²² **7d** increased total time spent in the open arms at a dose of 3 mg/kg (Figure 3A). This minimum effective dose of 3 mg/kg is a 20- to 50-fold improvement in in vivo potency over the first generation tool compounds. It should be noted, however, that 10 mg/kg did cause a decrease in overall locomotion (Figure 3B).

Having established a 3 mg/kg MED in the EZM assay, we then evaluated **7d** in two other mouse anxiety models: the light/dark box and marble burying assay. The effects of 3 mg/kg **7d** were compared to the selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLX, Figure 4).²² Results from the light/dark box assay showed that administration of either **7d** (3 mg/kg ip) or fluoxetine (15 mg/kg ip) increased total time spent in the light side of the chamber compare to vehicle (VEH) controls (Figure 4A). Similarly, both **7d** and fluoxetine decreased the number of marbles buried in a mouse marble-burying assay, consistent with an anxiolytic effect, compared to vehicle-control conditions (Figure 4B). The observation of efficacy at the 3 mg/kg dose prompted us to perform a pharmacokinetic assessment at this dose and the 1 h time point used for treatment in mice. These studies revealed values of $[\text{plasma}]_{\text{tot}} = 303 \text{ nM}$ and $[\text{CSF}] = 883 \text{ nM}$; this CSF level is $2.5\times$ higher than the in vitro IC_{50} of 350 nM . Taken together, mGlu₇ NAM **7d** decreases anxiety responses in three distinct preclinical models and displays a low of MED of 3 mg/kg , highlighting

improvements of this tool compound in the realms of potency, physiochemical properties, disposition, and unbound CSF/brain levels.

■ CONCLUSION

In summary, we have reported on the discovery of a new, structurally distinct mGlu₇ NAM in vivo tool compound, **7d** (VU6012962), suitable for robust target validation studies. NAM **7d** is potent, orally bioavailable, highly CNS penetrant and at modest doses achieves predicted unbound levels (CSF concentrations) ~4-fold above the in vitro IC₅₀. Moreover, NAM **7d** is highly selective for mGlu₇ versus the other seven mGlu receptor subtypes and across large ancillary pharmacology panels. Like first generation mGlu₇ NAMs, **7d** was efficacious in multiple preclinical models of anxiety but with a MED ~20- to 35-fold lower than earlier tool compounds. Further in vivo target validation studies with **7d** are in progress and will be reported in due course.

■ EXPERIMENTAL SECTION

Chemistry.

All compounds were purified to 95% as determined by analytical LCMS (214 nm, 254 nm, and ELSD), ¹H and ¹³C NMR, and high-resolution MS.

***N*-(2-(1*H*-1,2,4-Triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-(cyclopropylmethoxy)-3-methoxybenzamide (7d) (VU6012962).**

To a suspension of **19** (1.15 g, 2.50 mmol), 1*H*-1,2,4-triazole (173 mg, 2.50 mmol), potassium phosphate tribasic (1.34 g, 6.25 mmol), and copper(I) iodide (23.8 mg, 0.125 mmol) in DMF (10 mL) was added *trans*-*N,N'*-dimethylcyclohexane-1,2-diamine (39.4 μL, 0.250 mmol). The resulting suspension was degassed by vigorously bubbling argon through the mixture for 5 min. The reaction was then heated to 100 °C for 16 h, whereupon LCMS indicated complete consumption of starting material and formation of the desired product. The reaction was diluted with EtOAc and filtered over a pad of Celite. The combined organic material was washed with sat. NH₄Cl × 2, brine, dried over MgSO₄, filtered, concentrated, and purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to afford the desired product as a beige solid (785 mg, 70% yield). ¹H NMR (400 MHz, CDCl₃) δ 10.52 (bs, 1H), 8.75 (d, *J* = 2.2 Hz, 1H), 8.51 (s, 1H), 8.28 (s, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.39 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.07 (dd, *J* = 8.7, 1.7 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 3.95 (s, 3h), 3.92 (d, 2H), 1.39–1.32 (m, 1H), 0.69–0.65 (m, 2H), 0.40–0.36 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ = 165.1, 153.1, 152.3, 149.7 (d, *J*_{CF} = 2.0 Hz), 149.6, 143.9, 133.7, 126.3, 123.8, 123.6, 120.5 (q, *J*_{CF} = 257.1 Hz), 120.0, 115.9, 115.5, 112.1, 111.0, 74.1, 56.2, 10.2, 3.6 ppm. HRMS (TOF, ES+) calcd for C₂₁H₁₉F₃N₄O₄, 448.1358; found, 448.1365.

***N*-(2-Bromo-5-(trifluoromethoxy)phenyl)-4-(cyclopropylmethoxy)-3-methoxybenzamide (19).**

To a solution of aniline **18** (1.70 g, 6.64 mmol) in CH₂Cl₂ (15 mL) in a Biotage microwave vial were added **17d** (1.48 g, 6.64 mmol), *N,N*-diisopropylethylamine (3.47 mL, 19.9 mmol), and chlorodipyrrolidinocarbenium hexafluoro-phosphate (PyClU) (2.21 g, 6.64

mmol) at room temperature. The vial was sealed and heated to 100 °C using a Biotage microwave reactor for 30 min, whereupon LCMS showed formation of the desired product. The reaction mixture was diluted with DCM and quenched with the addition of saturated NH₄Cl. The layers were separated, and the aqueous layer was washed with DCM × 3. The combined organic layer was passed through a phase separator, concentrated, and purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to afford the desired product as a white solid (1.86 g, 61% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.61 (d, *J* = 2.3 Hz, 1H), 8.47 (bs, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 2.1 Hz, 1H), 7.43 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.91–6.87 (m, 1H), 3.97 (s, 3H), 3.94 (d, 2H), 1.40–1.33 (m, 1H), 0.71–0.66 (m, 2H), 0.42–0.38 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ = 165.0, 152.4, 149.9, 149.1, 137.3, 132.9, 126.6, 120.5 (q, *J*_{CF} = 256.6 Hz), 119.6, 117.1, 114.2, 112.2, 111.2, 110.7, 74.1, 56.3, 10.2, 3.6 ppm. HRMS (TOF, ES+) calcd for C₁₉H₁₇BrF₃NO₄, 459.0293; found 459.0296.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

■ ACKNOWLEDGMENTS

We thank the Warren Family and Foundation for establishing the William K. Warren, Jr. Chair in Medicine (C.W.L.). The authors also acknowledge funding by CDMRP Grant W81XWH-17-1-0266 (to C.M.N.).

■ ABBREVIATIONS USED

mGlu₇	metabotropic glutamate receptor subtype 7
CRC	concentration–response curve
NAM	negative allosteric modulator
PBL	plasma:brain level
SAR	structure–activity–relationship
CSF	cerebral spinal fluid
EZM	elevated Z maze

■ REFERENCES

- (1). Niswender CM; Conn PJ Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* 2010, 50, 295–322. [PubMed: 20055706]
- (2). Lindsley CW; Emmitte KA; Hopkins CR; Bridges TM; Gregory KJ; Niswender CM; Conn PJ Practical strategies and concepts in GPCR allosteric modulator discovery: Recent advances with metabotropic glutamate receptors. *Chem. Rev.* 2016, 116, 6707–6741. [PubMed: 26882314]
- (3). Fisher NM; Seto M; Lindsley CW; Niswender CM Metabotropic Glutamate Receptor 7: A new therapeutic target in neurodevelopmental disorders. *Front. Mol. Neurosci.* 2018, 11, 387. [PubMed: 30405350]
- (4). Sansig G; Bushell TJ; Clarke VR; Rozov A; Burnashev N; Portet C; Gasparini F; Schmutz M; Klebs K; Shigemoto R; Flor PJ; Kuhn R; Knoepfel T; Schroeder M; Hampson DR; Collett VJ; Zhang C; Duvoisin RM; Collingridge GL; van Der Putten H Increased seizure susceptibility in

mice lacking metabotropic glutamate receptor 7. *J. Neurosci.* 2001, 21, 8734–8745. [PubMed: 11698585]

- (5). Goddyn H; Callaerts-Vegh Z; Stroobants S; Dirikx T; Vansteenwegen D; Hermans D; van der Putten H; D’Hooge R Deficits in acquisition and extinction of conditioned responses in mGluR7 knockout mice. *Neurobiol Learn. Mem.* 2008, 90, 103–111. [PubMed: 18289889]
- (6). Palucha A; Klak K; Branski P; van der Putten H; Flor PJ; Pilc A Activation of the mGlu7 receptor elicits antidepressant-like effects in mice. *Psychopharmacology* 2007, 194, 555–562. [PubMed: 17622518]
- (7). Callaerts-Vegh Z; Beckers T; Ball SM; Baeyens F; Callaerts PF; Cryan JF; Molnar E; D’Hooge R Concomitant deficits in working memory and fear extinction are functionally dissociated from reduced anxiety in metabotropic glutamate receptor 7-deficient mice. *J. Neurosci.* 2006, 26, 6573–6582. [PubMed: 16775145]
- (8). Mitsukawa K; Mombereau C; Lötscher E; Uzunov DP; van der Putten H; Flor PJ; Cryan JF Metabotropic glutamate receptor subtype 7 ablation causes dysregulation of the HPA axis and increases hippocampal BDNF protein levels: implications for stress-related psychiatric disorders. *Neuropsychopharmacology* 2006, 31, 1112–1122. [PubMed: 16237391]
- (9). Hölscher C; Schmid S; Pilz PK; Sansig G; van der Putten H; Plappert CF Lack of the metabotropic glutamate receptor subtype 7 selectively modulates Theta rhythm and working memory. *Learn. Mem.* 2005, 12, 450–455. [PubMed: 16204199]
- (10). Holscher C; Schmid S; Pilz PK; Sansig G; van der Putten H; Plappert CF Lack of the metabotropic glutamate receptor subtype 7 selectively impairs short-term working memory but not long-term memory. *Behav. Brain Res.* 2004, 154, 473–481. [PubMed: 15313036]
- (11). Bushell TJ; Sansig G; Collett VJ; van der Putten H; Collingridge GL Altered short-term synaptic plasticity in mice lacking the metabotropic glutamate receptor mGlu7. *Sci. World J.* 2002, 2, 730–737.
- (12). Masugi M; Yokoi M; Shigemoto R; Muguruma K; Watanabe Y; Sansig G; van der Putten H; Nakanishi S Metabotropic glutamate receptor subtype 7 ablation causes deficit in fear response and conditioned taste aversion. *J. Neurosci.* 1999, 19, 955–963. [PubMed: 9920659]
- (13). Gogliotti RG; Senter RK; Fisher N; Adams J; Zamorano R; Walker AG; Blobaum AL; Engers DW; Hopkins CR; Daniels JS; Jones CK; Lindsley CW; Xiang Z; Conn PJ; Niswender CM mGlu7 potentiation rescues cognitive, social, and respiratory phenotypes in a mouse model of Rett syndrome. *Sci. Transl. Med.* 2017, 9, No. eaai7459.
- (14). Jalan-Sakrikar N; Field JR; Klar R; Mattmann ME; Gregory KJ; Zamorano R; Engers DW; Bollinger SR; Weaver CD; Days EL; Lewis LM; Utley TJ; Hurtado M; Rigault D; Acher F; Walker AG; Melancon BJ; Wood MR; Lindsley CW; Conn PJ; Xiang Z; Hopkins CR; Niswender CM Identification of positive allosteric modulators VU0155094 (ML397) and VU0422288 (ML396) reveals new insights into the biology of metabotropic glutamate receptor 7. *ACS Chem. Neurosci.* 2014, 5, 1221–1237. [PubMed: 25225882]
- (15). Abe M; Seto M; Gogliotti RG; Loch MT; Bollinger KA; Chang S; Engelberg EM; Luscombe VB; Harp JM; Bubser M; Engers DW; Jones CK; Rodriguez AL; Blobaum AL; Conn PJ; Niswender CM; Lindsley CW Discovery of VU6005649, a CNS penetrant mGlu_{7/8} receptor PAM derived from a series of pyrazolo[1,5-*a*]pyrimidines. *ACS Med. Chem. Lett.* 2017, 8, 1110–1115. [PubMed: 29057060]
- (16). Suzuki G; Tsukamoto N; Fushiki H; Kawagishi A; Nakamura M; Kurihara H; Mitsuya M; Ohkubo M; Ohta H In vitro pharmacological characterization of novel isoxazolopyridone derivatives as allosteric metabotropic glutamate receptor 7 antagonists. *J. Pharmacol. Exp. Ther.* 2007, 323, 147–156. [PubMed: 17609420]
- (17). Nakamura M; Kurihara H; Suzuki G; Mitsuya M; Ohkubo M; Ohta H Isoxazolopyridone derivatives as allosteric metabotropic glutamate receptor 7 antagonists. *Bioorg. Med. Chem. Lett.* 2010, 20, 726–729. [PubMed: 20005101]
- (18). Kalinichev M; Rouillier M; Girard F; Royer-Urios I; Bournique B; Finn T; Charvin D; Campo B; Le Poul E; Mutel V; Poli S; Neale SA; Salt TE; Lutjens R ADX71743, a potent and selective negative allosteric modulator of metabotropic glutamate receptor 7: in vitro and in vivo characterization. *J. Pharmacol. Exp. Ther.* 2013, 344, 624–636. [PubMed: 23257312]

- (19). Gee CE; Peterlik D; Neuhäuser C; Bouhelal R; Kaupmann K; Laue G; Uschold-Schmidt N; Feuerbach D; Zimmermann K; Ofner S; Cryan JF; van der Putten H; Fendt M; Vranesic I; Glatthar R; Flor PJ Blocking metabotropic glutamate receptor subtype 7 (mGlu7) via the Venus fly trap domain (VFTD) inhibits amygdala plasticity, stress, and anxiety-related behaviors. *J. Biol. Chem.* 2014, 289, 10975–10987. [PubMed: 24596089]
- (20). Niswender CM; Johnson KA; Miller NR; Ayala JE; Luo Q; Williams R; Saleh S; Orton D; Weaver CD; Conn PJ Context-dependent pharmacology exhibited by negative allosteric modulators of metabotropic glutamate receptor 7. *Mol. Pharmacol.* 2010, 77, 459–468. [PubMed: 20026717]
- (21). Reed CW; McGowan KM; Spearing PK; Stansley BJ; Roenfaniz HF; Engers DW; Rodriguez AL; Engelberg EM; Luscombe VB; Loch MT; Remke DH; Rook JM; Blobaum AL; Conn PJ; Niswender CM; Lindsley CW VU6010608, a novel mGlu7 NAM from a series of N-(2-(1H-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)benzamides. *ACS Med. Chem. Lett.* 2017, 8, 1326–1330. [PubMed: 29259756]
- (22). See Supporting Information for full experimental details.
- (23). Bubser M; Bridges TM; Dencker D; Gould RW; Grannan M; Noetzel MJ; Lamsal A; Niswender CM; Daniels JS; Poslusney MS; Melancon BJ; Tarr JC; Byers FW; Wess J; Duggan ME; Dunlop J; Wood MW; Brandon NJ; Wood MR; Lindsley CW; Conn PJ; Jones CK Selective activation of M₄ muscarinic acetylcholine receptors reverses MK-801-induced behavioral impairments and enhances associative learning in rodents. *ACS Chem. Neurosci* 2014, 5, 920–942. [PubMed: 25137629]
- (24). Engers DW; Blobaum AL; Gogliotti RD; Cheung Y-Y; Salovich JM; Garcia-Barrantes PM; Daniels JS; Morrison R; Jones CK; Soars MG; Zhuo X; Hurley J; Macor JE; Bronson JJ; Conn PJ; Lindsley CW; Niswender CM; Hopkins CR Discovery, synthesis and preclinical characterization of N-(3-chloro-4-fluorophenyl)-1H-pyrazolo[4,3-b]pyridin-3-amine (VU0418506), a novel positive allosteric modulator of the metabotropic glutamate receptor 4 (mGlu₄). *ACS Chem. Neurosci.* 2016, 7, 1192–1200. [PubMed: 27075300]
- (25). Panarese JD; Engers DW; Wu Y-J; Bronson JJ; Macor JE; Chun A; Rodriguez AL; Felts AS; Engers JL; Loch MT; Emmitte KA; Castelano AL; Kates MJ; Nader MA; Jones CK; Blobaum AL; Conn PJ; Niswender CM; Hopkins CR; Lindsley CW Discovery of VU2957 (Valiglurax): An mGlu₄ positive allosteric modulator evaluated as a preclinical candidate for the treatment of Parkinson's disease. *ACS Med. Chem. Lett.* 2018, DOI: 10.1021/acsmedchemlett.8b00426.

Figure 1. Structures of reported mGlu₇ NAMs 1–5, yet the field still requires a robust in vivo tool compound for target validation studies.

Figure 2. Overview of mGlu₇ NAM **5** SAR and metabolic liabilities, suggesting a path forward toward a new, structurally distinct, in vivo rodent tool compound. The minimum/essential pharmacophore of **5** is in blue.

Figure 3.

NAM **7d** decreases anxiety in the elevated zero maze (EZM) assay in mice. (A) Intraperitoneal administration of **7d** increases time spent in the open arms (MED = 3 mg/kg): one-way ANOVA ($F[3,39] = 3.112$, $p = 0.0382$), Bonferroni's post hoc test, VEH vs 3 mg/kg, $p < 0.05$. (B). The 10 mg/kg dose decreased distance traveled: one-way ANOVA ($F[3,39] = 4.613$, $p = 0.0079$), Bonferroni's post hoc test, 3 mg/kg vs 10 mg/kg, $p < 0.05$.

Figure 4.

NAM **7d** is efficacious at 3 mg/kg in the light/dark box and marble burying assays and is comparable to fluoxetine. (A) Administration of 3 mg/kg **7d** and 15 mg/kg fluoxetine (FLX) increased total time spent in the light side of the chamber compared to vehicle (VEH) controls. One-way ANOVA ($F[2,20] = 6.160$, $p = 0.0092$), Dunnett's post hoc test, VEH vs **7d**, $p < 0.01$; VEH vs FLX, $p < 0.05$. (B) Administration of 3 mg/kg of **7d** reduced the number of marbles buried (one-way ANOVA ($F[2,31] = 6.376$, $p = 0.0051$)), Dunnett's post hoc test, VEH vs **7d**, $p < 0.05$; VEH vs FLX, $p < 0.01$. All data points were run with Grubb's outlier test.

Scheme 1. Synthesis of Amine-Linked Congeners 6a and 9^a

^aReagents and conditions: (a) LiAlH₄ (3.5 equiv, 1.0 M in THF), THF, 0 °C, 2 h, 51–55%.

Scheme 2. Synthesis of Extended Amine-Linked Congeners 6^a

^aReagents and conditions: (a) (carbethoxymethylene)triphenyl-phosphorane, CH₂Cl₂, rt, 16h, 90–94%; (b) (i) NaBH₄, AcOH, Pd/C, toluene, rt, 1 h, (ii) LiOH, THF:H₂O (1:1), rt, 3h, 86–90%; (c) **13**, PyClU, DIEA, CH₂Cl₂, microwave 100 °C, 30 min, 38–53%; (d) LiAlH₄ (3.5 equiv, 1.0 M in THF), THF, 0 °C, 2 h, 45–60%.

Scheme 3. Synthesis of 4-Alkoxy Congeners **7^a**

^aReagents and conditions: (a) R-Br , K_2CO_3 , MeCN, microwave, 170°C , 1 h, 79–96%; (b) LiOH , THF:H₂O (1:1), rt, 3h, 88–97%; (c) **13**, PyClU, DIEA, CH_2Cl_2 , microwave 100°C , 30 min, 38–53%.

Scheme 4. Scale-Up Route To Access 7d^a

^aReagents and conditions: (a) PyClU, DIEA, CH₂Cl₂, microwave 100 °C, 30 min, 53%; (b) *H*-1,2,4-triazole, *trans*-*N,N'*-dimethylcyclohexane-1,2-diamine, K₃PO₄, CuI, DMF, 100 °C, 16 h, 56%.

Table 1.Structures and Rat mGlu₇ Activities of Analogs **6**^a

6					
compd	W	Y	mGlu ₇ IC ₅₀ (μM) ^a (pIC ₅₀ ± SEM)	% L-AP ₄ min ± SEM	rat CL _{hep} (mL min ⁻¹ kg ⁻¹)
6a			5.9 (5.23 ± 0.12)	17.7 ± 3.3	ND
6b	CH ₂	H	1.6 (5.79 ± 0.08)	9.5 ± 1.4	68.1
6c	CH ₂	F	1.3 (5.88 ± 0.10)	10.2 ± 1.2	66.4
6d	O	F	1.2 (5.93 ± 0.09)	5.9 ± 0.6	66.4

^aCalcium mobilization assay with rat mGlu₇/Gα15/HEK cells performed in the presence of an EC₈₀ fixed concentration of L-AP₄ (a more potent agonist at mGlu₇ compared to glutamate); values represent the mean from three (*n* = 3) independent experiments in triplicate. ND, not determined. CL_{hep}, predicted hepatic clearance.

Table 2.Structures and Rat mGlu₇ Activities of Analogs 7^a

7				
Entry	R	mGlu ₇ IC ₅₀ (μM) ^a (pIC ₅₀ ±SEM)	%L-AP ₄ Min±SEM	Rat CL _{hep} (mL/min/kg)
7a		>30000 (<4.5)	-----	ND
7b		>10000 (<5)	25.1±7.9	ND
7c		1.2 5.91±0.01	35.4±3.7	38.4
7d		0.35 6.46±0.10	12.6±1.5	15.9
7e		1.4 5.84±0.11	15.5±3.8	ND
7f		2.8 5.55±0.08	26.7±7.9	ND
7g		3.1 5.51±0.18	19.2±5.24	ND

^aCalcium mobilization assay with rat mGlu₇/Gα15/HEK cells performed in the presence of an EC₈₀ fixed concentration of L-AP₄ (a more potent agonist at mGlu₇ compared to glutamate); values represent the mean from three (*n* = 3) independent experiments in triplicate. ND, not determined. CL_{hep}, predicted hepatic clearance.